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Effects of light- vs. heavy-load squat training on velocity, strength, power, and total mechanical work in recreationally trained men and women

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ABSTRACT: The purpose of this study was to investigate the effects of light and heavy loads in the squat exercise on kinematics and mechanical variables in recreationally trained men and women. Twenty-two men and sixteen women were assigned to 4 groups: 40% and 80% one-repetition maximum (1RM) male (M40 and M80) and female (F40 and F80). Over 6 weeks, participants performed twice a week the full back-squat (SQ) exercise with initially equated relative volume load (Sets*Repetitions/Set*%1RM). All groups performed different amounts of work ($p < 0.05$), while relative work (work/1RM) only differed between load groups ($p < 0.001$). There was no significant Time*Sex*Load interaction. Based on the magnitude of effect sizes: M80 achieved small improvements in the SQ maximum isometric force (MIF; ES = 0.43, 95% CI [0.16, 0.81]); small gains in squat estimated 1RM strength were observed in the 80%-1RM groups (M80: 0.42 [0.18, 0.77]; F80: 0.44 [0.26, 0.76]) and the F40 group (0.42 [0.17, 0.81]); all groups made moderate to large gains in the average velocity attained against heavy loads (> 60%1RM; F40: 1.20 [0.52, 2.27]; F80: 2.20 [1.23, 3.93]; M40: 0.85 [0.29, 1.59]; M80: 1.03 [0.55, 1.77]), as well as small to moderate improvements in the average velocity against light loads (< 60%1RM; F40: 0.49[-0.24, 1.68]; F80: 1.10 [0.06, 3.16]; M40: 0.80 [0.41, 1.35]; M80: 0.93 [0.25, 1.84]). Lastly, only the F40 group showed small improvements in countermovement jump (CMJ) height (ES = 0.65 [0.14, 1.37]). In conclusion, light and heavy loads produced similar strength gains in men and women when initially equated by relative volume load, although the standardized mean differences suggest nuances depending on the sample and task.

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INTRODUCTION

Given the benefits of resistance training (RT) in the general population, it is recommended for both men and women in health guidelines [1]. The loading magnitude (intensity) is considered one of the most important variables when designing RT programmes [2]. The use of heavy over light loads is recommended when the goal is to develop muscular strength [3]. This assumption is supported by the idea of a "strength-endurance continuum" where a heavy-load low-repetition RT scheme promotes greater gains in strength performance, while a light-load high-repetition routine benefits endurance-related capacities more [3, 4]. This recommendation is mainly based on the variations observed in the one-repetition maximum (1RM) value. However, this approach may bias results towards heavier loads, underestimating the benefits potentially generated by lighter loads due to the fewer similarities between the training load and the exercise tested [3]. In this regard, it can be expected that training-induced adaptations would be greater for lifting conditions similar to those experienced during training [2]. Despite this, the use of light loads (< 50% of 1RM) can also be a suitable method for developing muscle strength and hypertrophy, with the additional advantage of reducing articular stress and requiring no specific facilities compared to heavy-load training [5].

The quantification and monitoring of training volume are also relevant factors in RT interventions [6], as they influence strength adaptations [4]. From a scientific standpoint, volume quantification is important not only when training volume is the independent variable, but also when the goal is to elucidate the effect of another variable, such as training load [7]. This control allows researchers to isolate the effects of the parameter under study (i.e., dependent variable). Among the variety of methods used to determine RT volume [6], volume load (number of repetitions × load) is prominent, especially

because it is easy to implement [6] and is strongly associated with strength changes in both men and women [8]. Indeed, this parameter has been used in an attempt to match the volume accumulated with different loads [7]. However, this approach does not account for different parameters such as the body mass lifted in exercises such as squats, the total force applied, and the load displacement [6]. For these reasons, quantifying volume as the total amount of work performed (i.e., force [N] × displacement [m]) may be a suitable method for determining exercise volume, as it considers the total force produced and the load displacement [6].

Overall, there is a lack of studies comparing the effects of different RT interventions between men and women [9]. The same holds true for studies examining the effects of different training loads in both sexes simultaneously. Specifically, volume-matched RT protocols with multi-joint lower-limbs exercises, such as the squat, conducted with men, resulted in greater strength gains for heavy loads compared to light loads [4, 7, 10, 11]. However, studies including only women showed that both heavy and light loads were equally effective to improve strength performance [12–14]. When pooled results from a mixed-sex sample (both sexes together) were reported, again heavy and light loads provided substantial improvements without significant differences between them, although groups were not matched by volume load [15, 16]. It has already been suggested that researchers should consider expressing the results separately for both sexes for a more comprehensive analysis of the differences and similarities between the sexes [17]. Some studies have compared the effect of the same RT intervention on men and women without comparing different training loads within the study. In this regard, both sexes responded equally to heavy-load [18], light-load [19], and combined light- and heavy-load protocols [20]. To our knowledge, the potential interaction between sexes and training loads remains unknown. A better understanding of this interaction could provide deeper comprehension regarding the feasibility of light and heavy loads in men and women as minimum effective training doses [21]. In light of these considerations, the purpose of this study was to analyse the effects of different RT loads (40 vs. 80% 1RM) with an initially equated relative volume load on the physical performance of recreationally trained men and women.

MATERIALS AND METHODS

Procedures

An experimental study was developed to compare the effects of two loading magnitudes during full back-squat (SQ) training on men's and women's physical performance. Subjects were divided by sex, and then men and women were matched according to their estimated 1RM in the Smith machine SQ exercise and allocated following a counterbalanced sequence (AB-BA) among two load groups (40% and 80% 1RM), thus generating the following groups: females: F40 and F80; males: M40 and M80, respectively. All groups trained with, initially, the same relative volume load (sets × repetitions × %1RM) using the SQ exercise for 6 weeks, twice a week, for a total of 11 sessions. Only one session was performed in the last week, so there was a rest period of 4–5 days between the last session and the post-test, similar to the pre-test and the first session. Although the external load (kg) is usually changed daily or weekly in order to meet the principle of progressive overload, it was kept constant in this study throughout the intervention, even though it goes against that principle, and the relative load (% 1RM) that the same absolute load represents would decrease as their performance increases [2], which is why we are talking about "initially equated relative volume load", since keeping the absolute external load constant would eventually lead to a different relative volume load for each subject, depending on their changes in performance during the study. The reason behind that decision was to simulate an environment where a pre-test is performed and a certain relative load is selected, with no modifications after that to see the different adaptations provided by those initial loads over time. Moreover, although training with the same absolute load for a long period might not be the most optimal regarding short-term adaptations, it could be beneficial, especially in this kind of population (recreationally trained subjects), in the medium and long term, since they could make the most out of each load, getting all (or at least more of) the positive adaptations that come from the use of that load before changing it. Participants were tested on two occasions, before and after the RT intervention, according to the following sequence: countermovement jump (CMJ); maximal isometric SQ test; and progressive loading SQ test. The same testing protocol was carried out during both pre- and post-training sessions in the same order. Testing and training sessions took place at the same time of day (± 2 h), under the same environmental conditions (20°C and 60% humidity).

Participants

Twenty-two men (age: 23.8 ± 3.8 years; height: 1.78 ± 0.06 m; BM: 76.8 ± 10.2 kg; SQ estimated 1RM: 92.5 ± 15.1 kg; ratio 1RM to BM: 1.21 ± 0.20) and sixteen women (age: 23.0 ± 4.3 years; height: 1.62 ± 0.05 m; body mass (BM): 57.4 ± 9.8 kg; SQ estimated 1RM: 52.2 ± 12.9 kg; ratio 1RM to BM: 0.90 ± 0.17) were voluntarily recruited to participate in this study (RT experience = 2.2 ± 1.3 years, considered "recreational" based on Rhea's classification [22]), most of them being sport science students from the university campus and some of them already familiar with lifting at maximum intended velocity, who were randomly assigned to groups. Male and female participants (age range 18–32 years) were required to be healthy and physically active individuals, regularly participating in sports or physical activities, with no physical impairments that could hinder their ability to engage in a RT programme or pose a risk to their health. No lower-body strength training or exhausting exercise was allowed outside of the study; nor was the use of any recovery treatment or anti-inflammatory supplement. This was controlled in an informal daily check on "how they had been doing" or "what kind of activities they had performed between sessions", to make sure they were properly following the protocol. Subjects were informed

about the experimental procedure and risks associated with the study, whereupon informed consent was signed. From the 41 participants who initially entered the study (number of participants per group: M40 = 12, M80 = 12, F40 = 9, F80 = 8), 3 subjects (2 from the M80 and 1 from the F40 group) dropped out due to discomfort (popliteus and low-back pain); the rest completed all programmed sessions and only their data were considerer for analysis. The study was approved by two hospitals' ethics committees (0398-N-17) and conducted according to the Declaration of Helsinki.

Testing Procedures

Countermovement Jump

Jump height was calculated through flight time measured with an infrared timing system (OptojumpNext, Microgate, Bolzano, Italy), with an accuracy of 0.001 s. Starting position: feet flat on the ground hip-width apart, hands on the hips, and looking ahead. Then, a triple flexion-extension (about 90° knee angle) was performed. Subjects were encouraged to jump as high as possible. During landing, legs were kept straight, and toes were pointing down when they touched the ground. Five jumps were performed with 20 s of rest between them, with the best and worst values discarded, and the average of the remaining 3 jumps was used for further analyses. This protocol was employed because the jump height was estimated throughout the flight time, which depends on the subject's technique during the airborne phase. In this regard, slight modifications in the execution of the jump could inflate the real value, for example: performing ankle dorsiflexion increases the flight time and, therefore, estimates a higher jump height. Reliability was as follows: coefficient of variation (CV) = 1.8% and intraclass correlation coefficient (ICC) = 0.996, 95% CI [0.977, 0.998].

Maximum Isometric Squat Test

Subjects started in an upright position, with hips and knees fully extended, stance approximately shoulder-width apart, feet flat on the platform parallel or externally rotated up to 15°, bar placed on the upper part of the trapezius, looking forward, neutral spine and a closed pronated grip. Then, they went down in a controlled manner until an approximately 90° knee angle was achieved, where two spotters helped them to rotate and hook the bar in the rack pins. The knee flexion angle was visually controlled by a researcher, the height of the bar being individualized for every subject and kept constant at pre- and post-training assessments. After the cue "ready, set, go!" (under a light pretension), subjects were verbally encouraged to push "as hard and fast as possible", during a 4-s maximal effort. Two attempts with a 1-min rest were performed. The average value of the maximal isometric force (MIF) attained in each attempt was recorded. An 80 × 80 cm dynamometric platform (F-500, Ergotech, Murcia, Spain) was placed in the middle of the vertical projection of the Smith machine bar (Multipower Fitness Line, Peroga, Murcia, Spain). The force signal was sampled at 1,000 Hz and smooth with a 4th-order low-pass Butterworth filter with no phase shift and 200 Hz cut-off frequency. The CV for test-retest reliability was 9.2%, and the ICC was 0.950, 95% CI [0.905, 0.974].

Progressive Loading Test in the Full Back-Squat Exercise

All subjects started with the empty bar (20 kg), in the same starting position as described in the previous isometric test. Participants were instructed to perform a controlled and continuous descent as low as possible, to immediately reverse the motion and ascend back to the upright position at the maximal intended velocity without jumping. The number of repetitions with each load and the load increments were individually adjusted as follows: 3 repetitions and 10 kg increments if the mean propulsive velocity (MPV) > 1.00 $m \cdot s^{-1}$, 2 repetitions and 5 kg if 0.80 < MPV < 1.00 $m \cdot s^{-1}$, 1 repetition and 2.5 kg if MPV < 0.8 $m \cdot s^{-1}$. When the MPV was lower than or equal to 0.50 $m \cdot s^{-1}$ (approximately ≥ 90% estimated 1RM) the test was concluded to ensure a proper technique and safety considering the subjects' experience. The 1RM value was estimated from the MPV attained against the heaviest load lifted; first, the %1RM that that absolute load (kg) represents for a certain subject was estimated through the equation: Load (%1RM) = $-5.961 \cdot MPV^2 - 50.71 \cdot MPV + 117$ [23]. Once the absolute load and its associated %1RM are known, the load in kg corresponding to their 100% of the 1RM can be calculated by a cross-multiplication (e.g., if 90 kg is estimated to be the 90% 1RM, the estimated 1RM would be 100 kg). A 3-min rest was allowed between sets. The fastest repetition for each load was considered for subsequent analysis. Velocity was measured through a linear velocity transducer (T-Force System, Ergotech, Murcia, Spain). The velocity signal was time-synchronized with the force signal coming from the previously mentioned force platform through specific software (T-Force System Version 3.60, Ergotech, Murcia, Spain). Vertical bar velocity was sampled at 1,000 Hz and smoothed with a fourth-order low-pass Butterworth filter with no phase shift and 10 Hz cut-off frequency. During the post-test, subjects followed the same loading scheme as in the pre-test, i.e., they lifted the same absolute loads, adding or removing loads depending on whether their performance was enhanced or worsened, that is, if they were able to lift more than or fewer loads before reaching the cut-off velocity (≤ 0.50 $m \cdot s^{-1}$). Once pre- and post-training testing had been carried out, in addition to the estimated 1RM load and to further investigate possible adaptations against different loading magnitudes, the absolute loads common to pre- and post-training were evaluated and divided into light loads (those loads lifted faster than 1.00 $m \cdot s^{-1}$ during the pre-test, i.e., loads lighter than the 60% of the pre-test estimated 1RM based on the general equation [23]), and heavy loads (loads lifted slower than 1.00 $m \cdot s^{-1}$ during the pre-test, i.e., loads heavier than the 60% of the pre-test estimated 1RM). After identifying the absolute loads of interest for every subject and their best repetition with each load (fastest repetition), the average MPV across all those loads considered light loads was calculated (AV > 1), as well as the average mean propulsive force (MPF; AF > 1) and the average mean propulsive power (MPP; AP > 1). The same procedure

was conducted with those considered heavy loads to calculate the average MPV (AV < 1), average MPF (AF < 1), and average MPP (AP < 1). The number of repetitions across the average was calculated depending on every subject: since all subjects started with the empty bar (20 kg) to progressively warm up until their approximately 90% 1RM, the stronger the participant the higher the number of sets that were performed until the completion of the test.

Resistance Training Procedures

Participants trained twice a week for 11 sessions using the Smith machine SQ exercise, with the same technique as described in the progressive loading SQ test. The absolute load (i.e. kg) for each subject was calculated as 40% or 80% of their estimated 1RM achieved at the pre-test. The number of sets, repetitions per set, and absolute load were kept constant in all training sessions (3×12 with pre-test estimated 40% 1RM or 3×6 with pre-test estimated 80% 1RM). Inter-set recovery was 3 minutes. All groups completed a standardized warm-up in every training session to isolate the effects of the independent variable (i.e. relative load), which was as follows: i) 5 min of jogging at a self-selected easy pace; ii) 2×10 squats without external loads; iii) 6-4-2 SQ repetitions with 20%, 40%, and 60% of 1RM, respectively. Feedback and encouragement were provided in every repetition, which was recorded using a force platform synchronized with a linear position transducer, from which the MPV, MPF, MPP, propulsive time under tension (TUT), and propulsive mechanical work were extracted. The latter, given that the software did not provide that variable or the propulsive distance, was calculated as follows:

$$\begin{aligned} &Propulsive\ Mechanical\ Work\ (J) \\ &= Mean\ Propulsive\ Force\ (N) \\ &\quad \cdot Propulsive\ Distance\ (m) \\ &= Mean\ Propulsive\ Force\ (N) \\ &\quad \cdot Mean\ Propulsive\ Velocity\ (m \cdot s^{-1}) \\ &\quad \cdot Propulsive\ Time\ (s) \end{aligned}$$

The average propulsive mechanical work (W) of the training programme was used to compare the training performed during all training sessions by each group. To account for differences in absolute strength levels between subjects, the W completed by each subject was divided by their pretraining 1RM, obtaining the relative work (rW).

Statistical Analyses

Data are presented as mean ± standard deviation (SD). Normality was examined with the Shapiro-Wilk test. Homoscedasticity was determined through Levene's test. Training variables (i.e., number of repetitions, time under tension, average velocity, absolute and relative work) were analysed through a two-way ANOVA (Sex × Load). Post-training strength-related parameters (i.e., MIF, 1RM, average velocity, force and power, and CMJ) were analysed through a three-way ANCOVA (Time × Sex × Load) with Bonferroni's post hoc comparisons, after adjusting for pretraining values with MIF_{pre}/BM (for MIF analysis) and $1RM_{pre}$/BM as covariates, to make between-sex comparisons possible. Statistical analyses were performed using IBM SPSS Statistics v.26 (SPSS Inc., Chicago, IL). The significance level was accepted at $p \leq 0.05$. Intraday test-retest reliabilities of the MIF and CMJ assessments were estimated from the pre-training session using intraclass correlation coefficients (ICC; 2-way mixed-effects, absolute agreement, multiple measurements) [24]. The coefficient of variation (CV) was calculated based on the standard error of measurement (SEM) [25]. Within-group effect sizes (ESs) were calculated using Cohen's *d* for a paired design (repeated measures) and then corrected for small samples (degrees of freedom < 50), which is sometimes referred to as *d* unbiased or Hedges' g [26]. ESs were interpreted based on Rhea's classification for "recreationally trained" individuals (1–5 years of RT experience): < 0.35 "trivial", 0.35–0.80 "small", 0.80–1.50 "moderate", and > 1.5 "large" [22].

RESULTS

At pre-training, nonsignificant differences were found between load groups within the same sex for all strength-related parameters analysed ($p > 0.05$). In contrast, there were significant between-sex differences in most of them, except for their performance against the same absolute loads common to pre- and post-training (AV > 1, AV < 1, AF > 1, and AP > 1).

Description of training performed

Training features are reported in Table 1. The F40 group accumulated significantly longer TUT compared to the F80 group ($p < 0.001$) and trained at a lower average training velocity (AV) compared to the male load-peers ($p = 0.016$). Men performed a greater volume load than women ($p < 0.001$). While the W was significantly different for each group ($p < 0.05$), the 40%-1RM groups completed higher rW than the 80%-1RM groups ($p < 0.001$), with no differences being found between sexes (F40 vs. M40 $p = 0.423$; F80 vs. M80 $p = 0.605$).

Strength-related parameters

Changes in the selected performance variables from pre- to post-training for each group are reported in Table 2 and Figure 2. A significant overall *Time* effect was observed for MIF ($p = 0.011$), AV < 1 ($p = 0.001$), AF < 1 ($p = 0.034$), AP < 1 ($p = 0.011$), and AV > 1 ($p = 0.007$) variables, for which within-group time effect post hoc analyses were performed. There were no significant *Time*Sex*, *Time*Load*, or *Time*Sex*Load* interactions for any parameter analysed. Based on the magnitude of the effect sizes, M80 was the only group that achieved small and significant MIF within-group improvements after the training programme (ES = 0.43, 95% CI [0.16, 0.81]; $p = 0.002$), while both 80%-1RM groups and the F40 group showed small gains in estimated 1RM strength (M80: 0.42 [0.18, 0.77]; F80: 0.44 [0.26, 0.76]); F40: 0.42 [0.17, 0.81]). All groups

TABLE 1. Descriptive variables of the training performed during all training sessions.

	F40	F80	M40	M80
REP (n)	396 ± 0†††	190 ± 14	396 ± 0†††	194 ± 4
TUT (s)	288 ± 36††	231 ± 29	267 ± 24	260 ± 28
AV ($m \cdot s^{-1}$)	1.01 ± 0.13†††	0.63 ± 0.10	1.13 ± 0.12$^{\#\dagger\dagger\dagger}$	0.59 ± 0.06
VL (rep · kg)	8638 ± 1372	7937 ± 2082	13547 ± 1478$^{\#\#\#}$	15279 ± 2944$^{\#\#\#}$
W (kJ)	90 ± 22†	61 ± 14	151 ± 32$^{\#\#\#\ \dagger\dagger}$	120 ± 24$^{\#\#\#}$
rW ($kJ \cdot kg^{-1}$)	1.81 ± 0.42†††	1.16 ± 0.14	1.72 ± 0.23†††	1.22 ± 0.07

Data are mean ± SD. F40 = Female group training at 40% 1RM (n = 8); F80 = Female group training at 80% 1RM (n = 8); M40 = Male group training at 40% 1RM (n = 14); M80 = Male group training at 80% 1RM (n = 11); REP = Total number of repetitions performed in the training program; TUT = Total propulsive time under tension accumulated; AV = Average training velocity; VL = Absolute volume load; W = Total propulsive work; rW = Total propulsive work divided by the pretest 1RM for every subject. Between-sexes differences: # $p \leq 0.05$; ## $p < 0.01$; ### $p < 0.001$. Between-intensities differences: † $p \leq 0.05$; †† $p < 0.01$; ††† $p < 0.001$.

TABLE 2. Mean values and pre- to post- training changes for each group in the selected strength-related parameters.

	F40			F80			M40			M80		
	Pre-test	Post-test	ES ± [LL, UL]	Pre-test	Post-test	ES ± [LL, UL]	Pre-test	Post-test	ES ± [LL, UL]	Pre-test	Post-test	ES ± [LL, UL]
MIF^{t}	674 ± 211	711 ± 224	0.16 ± [-0.09, 0.47]	807 ± 248	848 ± 224	0.16 ± [-0.29, 0.68]	978 ± 216	979 ± 182	0.01 ± [-0.30, 0.31]	1096 ± 224	1192 ± 205*	0.43 ± [0.16, 0.81]
1RM	51 ± 13	56 ± 10	0.42 ± [0.17, 0.81]	53 ± 14	60 ± 14	0.44 ± [0.26, 0.76]	88 ± 10	91 ± 16	0.23 ± [-0.19, 0.70]	99 ± 18	108 ± 24	0.42 ± [0.18, 0.77]
AV < 1^{tt}	0.67 ± 0.04	0.82 ± 0.17*	1.20 ± [0.52, 2.27]	0.70 ± 0.05	0.89 ± 0.10**	2.22 ± [1.23, 3.93]	0.73 ± 0.09	0.84 ± 0.16***	0.85 ± [0.29, 1.59]	0.72 ± 0.06	0.82 ± 0.13***	1.03 ± [0.55, 1.77]
AF < 1^{t}	425 ± 133	486 ± 116**	0.46 ± [0.23, 0.84]	419 ± 108	476 ± 124**	0.46 ± [0.29, 0.79]	730 ± 92	786 ± 114***	0.53 ± [0.29, 0.88]	828 ± 176	880 ± 183***	0.28 ± [0.10, 0.54]
AP < 1^{t}	260 ± 69	351 ± 44*	1.49 ± [0.75, 2.72]	271 ± 73	372 ± 78*	1.28 ± [0.79, 2.18]	487 ± 81	606 ± 159***	0.91 ± [0.44, 1.58]	553 ± 135	668 ± 178***	0.70 ± [0.36, 1.23]
AV > 1^{tt}	1.17 ± 0.13	1.25 ± 0.16	0.49 ± [-0.24, 1.68]	1.16 ± 0.09	1.26 ± 0.07	1.10 ± [0.06, 3.16]	1.21 ± 0.08	1.34 ± 0.21**	0.80 ± [0.41, 1.35]	1.21 ± 0.09	1.33 ± 0.14***	0.93 ± [0.25, 1.84]
AF > 1	366 ± 59	398 ± 55	0.48 ± [0.28, 1.10]	365 ± 59	386 ± 88	0.25 ± [-0.09, 0.80]	545 ± 80	581 ± 85	0.42 ± [0.09, 0.84]	616 ± 164	656 ± 1668	0.23 ± [0.11, 0.41]
AP > 1	365 ± 86	420 ± 100	0.52 ± [0.17, 1.33]	335 ± 65	395 ± 77	0.73 ± [0.34, 1.77]	539 ± 95	626 ± 130	0.73 ± [0.23, 1.39]	625 ± 171	703 ± 183	0.42 ± [0.18, 0.76]
CMJ	23.6 ± 4.3	26.6 ± 4.4	0.65 ± [0.14, 1.37]	25.0 ± 3.0	26.3 ± 2.3	0.46 ± [-0.12, 1.21]	33.0 ± 3.4	33.9 ± 4.7	0.20 ± [-0.06, 0.51]	36.2 ± 7.1	37.5 ± 5.4	0.20 ± [-0.12, 0.57]

Data = mean ± SD. F40 = Female group training at 40% 1RM; F80 = Female group training at 80% 1RM; M40 = Male group training at 40% 1RM; M80 = Male group training at 80% 1RM; ES = Effect size; LL = Lower limit 95% Confidence Interval; UL = Upper limit 95% Confidence Interval; MIF = Maximum isometric force attained at 90° squat (N); 1RM = estimated one-repetition maximum in full-back squat (kg); AV < 1/AF < 1/AP < 1 = Average mean propulsive velocity ($m \cdot s^{-1}$) / force (N) / power (W) attained against absolute loads common to pre- and posttraining that were moved slower than 1 $m \cdot s^{-1}$. AV > 1/AF > 1/AP > 1 = Average mean propulsive velocity ($m \cdot s^{-1}$) / force (N) / power (W) attained against absolute loads common to pre- and post- training that were moved faster than 1 $m \cdot s^{-1}$ at pre-training; CMJ = Countermovement jump height (cm). Mean Effect of time: "^{t}" $p \leq 0.05$; "^{tt}" $p < 0.01$; "^{ttt}" $p < 0.001$. Within-group time effect: * $p \leq 0.05$; ** $p < 0.01$; *** $p < 0.001$.

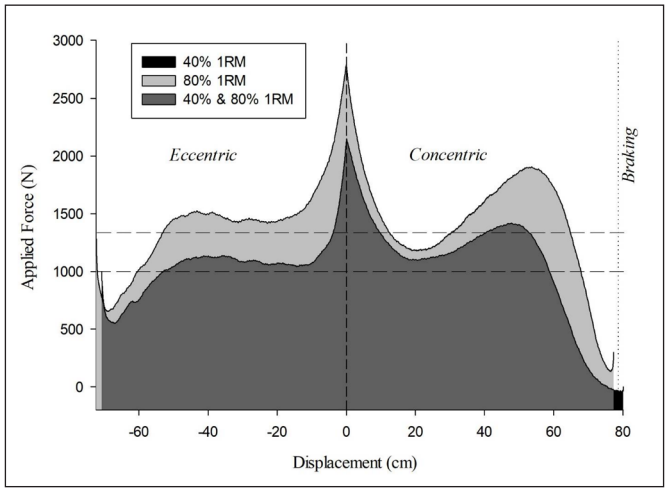


FIG. 1. Forces comparison when lifting the 40% and 80% 1RM for a representative subject. The two curves denote the applied force, as a function of displacement, with a mean value of 1116 N for the 40% 1RM load and 1429 N for the 80% 1RM load. The two horizontal dashed lines represent the system weight (external load + 88% body mass) for each load. The vertical difference between the curves and their corresponding dashed line depicts the net force (mean value 40% 1RM load: 115 N; 80% 1RM load: 59 N). The areas under the curves show the work performed to lift the respective loads (40% 1RM load: 1570 J; 80% 1RM load 2128 J). The dark grey area (40% 1RM) represents 74% of the light grey one (80% 1RM).

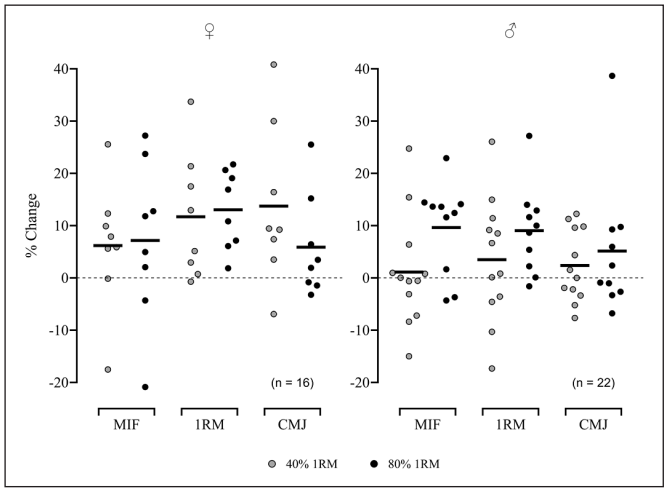


FIG. 2. Percent change pre- to post- training for every subject in three selected strength-related parameters: ♀ = Women; ♂ = Men; MIF = Maximum isometric force attained at 90° squat (N); 1RM = estimated one-repetition maximum in full-back squat (kg); CMJ = Countermovement jump height (cm).

attained improvements in performance against heavy loads: moderate to large for AV < 1, trivial to small for AF < 1, and small to moderate for AP < 1 (Table 2). Likewise, although to a lessened degree, enhancements in performance were found against light loads: small to moderate for AV > 1, trivial to small for AF < 1, and small to moderate for AP < 1 (Table 2). Lastly, only the female groups showed at least small improvements in CMJ height, F40 having a 95% confidence interval that does not contain 0 (CMJ; F40: 0.65 [0.14, 1.37]); F80: 0.46 [-0.12, 1.21]).

DISCUSSION

The present study analysed the effects of different loading magnitudes (40% vs. 80% 1RM) with initially equated relative volume load on the physical performance of recreationally trained men and women. The main findings were as follows: a) the adaptations provided by light and heavy loads were similar between men and women; b) both light and heavy loads produced small to moderate improvements in most variables studied; c) men accumulated higher total mechanical work than women; however, differences between sexes disappeared when work values were normalized to their estimated 1RM.

Although no statistically significant differences were found between sexes (men vs. women) or load groups (40% 1RM vs. 80% 1RM), the study of the within-group standardized mean differences may provide a deeper insight into the possible adaptations coming from the use of different loading magnitudes on each sex. The similarities between task requirements and training load may explain the findings observed in MIF and estimated 1RM strength in men since the 80% 1RM intervention was the only male group that obtained non-trivial gains in those maximum or near maximum force expressions. The greater effect observed in MIF for M80 in comparison to the other groups, together with being the only group where a significant within-group time effect was found ($p = 0.002$), agrees with the current literature, since men have been shown to benefit from heavy-load training [27–29], but this effect has not been observed in women [30]. Additionally, when men and women trained combining both light and heavy loads (i.e. from body weight up to 85% 1RM), greater improvements were found in men [20]. Although sex differences in MIF currently continue to be unexplained, it is plausible to consider that increases in muscle size may play a substantial role that could benefit men [20]. Unfortunately, hypertrophic responses were not evaluated in the present study. These results suggest that heavy-load training may benefit MIF production in men while the effect on women is trivial. In contrast, both the M80 and F80 groups obtained similar gains in estimated 1RM strength. In this regard, our results agree with previous studies conducted with men and matched by volume load, which showed that heavy-load training was effective to induce gains in 1RM strength [4, 7, 10, 11]. As mentioned above, the use of higher training loads may have more similar requirements and thus be more transferable to tasks with maximum or near-maximum force requirements, evoking greater adaptations to the 1RM test [31], and possibly induce larger increases in maximal neural activation and muscle thickness [29]. In

agreement with the current literature, similar improvements in women's 1RM have been observed with both light- and heavy-load RT interventions [12–14], although the use of heavier loads could be more beneficial in older adults [30]. The reason why a light-load programme was not a sufficient stimulus to achieve at least small gains in estimated 1RM strength in men, as opposed to women, may lie in the extension of the braking phase. As shown in Table 1, the average training velocity of the M40 group was significantly higher than the F40 group ($p = 0.016$). Thus, male subjects had to actively brake the movement to avoid jumping to a greater extent than their load-paired female subjects [32], affecting the potential transfer to a maximum or near-maximum test, where subjects are required to apply force throughout the entire concentric phase [23].

Force, velocity, and power outputs against light and heavy loads showed a similar pattern among them, although the within-group effect sizes in velocity values tended to be higher. As previously reported, both male groups achieved non-trivial improvements with no differences between them in the velocity-load [28, 29, 33], and power-load [33] relationships. However, the trivial improvements for the M80 group found in the present study regarding the force-load relationship contradict those seen in the literature [29], possibly because of the selected variable (mean force instead of peak force). However, it should be mentioned that the mechanisms potentially responsible for these adaptations were shown to differ depending on training intensity [29]. In concordance with a previous study [13], female groups enhanced their performance against both light (small to moderate) and heavy (moderate to large) loads, again with no differences between groups. In mixed-sample research with heavy loads, men increased power output significantly more against light-moderate loads compared to women [18], something that cannot be drawn from our results. Taken together, data from the present study suggest that both light and heavy RT loads could be equally suitable for improving performance against light and heavy loads in both men and women.

While no significant differences were found between groups regarding CMJ height, F40 was the only group that showed small improvements with a 95% confidence interval that did not contain 0. Studies with men have found light and heavy RT loads to equally improve CMJ performance; however, the "heavy-load groups" always completed a greater volume load using alternatively the squat, the jump squat, or a combination of both exercises, whereas the "light-load groups" performed only jump squats, without stopping the movement at the end of the concentric phase [27, 29, 34]. Similar results have been obtained with women [12, 13]. It is unknown how or to what extent these differences in volume load could have affected the outcomes. Additionally, as in our study, no between-sex differences were found when a light-load RT was applied to a mixed sample [19]. In a similar manner as presented in the discussion of estimated 1RM results, even considering that lighter loads could be more suitable to improve jumping performance, the higher training velocity (AV) attained by the M40 group has certainly required a longer braking phase to avoid jumping [32], which may have reduced the potential benefits of this load, since jumping was not allowed in our study. This fact was attenuated in the F40 group given its slower AV (Table 1).

Although both load interventions were initially equalized in terms of relative volume load (Sets × Repetitions/Set × %1RM), the absolute volume load was different between sexes, and the 40%-1RM groups performed more work than the 80%-1RM groups (both in absolute and relative terms). This finding is in agreement with previous studies [27, 30, 34] and indicates that volume load and actual work should not be equated at the same time. Since volume load does not account for the total force applied [6], when this force is measured during one repetition at maximal voluntary velocity, it can be observed that the force applied at 40% 1RM is greater than half that required to lift the resistance equivalent to 80% 1RM, especially when body mass is included (curves in Figure 1). Indeed, Figure 1 shows that the area under the force-distance curve (mechanical work) at 40% 1RM was 74% of the area at 80% 1RM. For this reason, 2 repetitions at 40% 1RM require performing greater work than 1 repetition at 80% 1RM. Accordingly, although lifting 80% 1RM requires a higher force to be applied than when lifting 40% 1RM (curves in Figure 1), the net force (i.e., the difference between the applied force and the force that the weight represents) is higher when lifting the 40% 1RM load (vertical difference between the force curves and their corresponding horizontal lines in Figure 1). This fact explains why the 40% 1RM load can be lifted faster than the 80% 1RM load. Given that the applied force is considerably higher than the load when lifting lighter loads compared to heavier loads, there is an underestimation of the actual work performed, especially with light loads, when equated by volume load. Furthermore, in the present study, men performed greater total work than women; however, when total work values were relativized by the subjects' estimated 1RM, these differences disappeared (Table 1). Hence, sex differences in total mechanical work seem to be due to differences in maximum strength levels.

A drawback of the present study is its statistical power due to the sample size [35]. For instance, the observed power to detect a significant ($p \leq 0.05$) Time*Sex*Load interaction was approximately 25%, which falls below the recommended 80%. These circumstances justify the use of additional analyses such as the effect sizes together with their confidence intervals to provide a deeper understanding of the effects that are taking place [35]. Subjects were recreationally trained so the results are not generalizable to other populations. On the other hand, the relative volume load was not equated during the entire duration of the study since the external load was kept constant, and for every subject that initial load would represent a different percent of their 1RM by the end of the study. Additionally, the effect of the variable *"Load"* has been investigated in isolation for just one exercise (full back-squat) in the present study; thus, the results may differ in an RT programme where multiple exercises are performed for the same muscle groups or movement patterns.

Lastly, some of the results obtained in the 40% 1RM male group could be attributed to the effect of the braking phase when a light load is intended to be lifted as fast as possible without jumping in the squat exercise. For all the aforementioned reasons, it would be interesting to further investigate how different loading magnitudes influence men's and women's performance in the future, addressing the limitations of the present study by using a larger sample size to confirm the possible differences in strength adaptations suggested by the within-group standardized mean differences that resulted from the use of different training loads in men and women, as well as recruiting participants with different training experiences, keeping the relative load constant, and/or performing multiple exercises for the same muscle group, including those where there is no braking phase, such as in ballistic exercises (e.g. jump squats at 40% and 80% 1RM).

CONCLUSIONS

In conclusion, light and heavy loads produced similar strength gains in men and women when initially equated by relative volume load. However, based on standardized mean differences, heavier loads seem to provide more maximum and near-maximum force adaptations (MIF and estimated 1RM) in men, while women could equally benefit from either low or high loading magnitudes to improve near-maximum dynamic force production (estimated 1RM). Although light loads (40% 1RM) may be more applicable to optimize force application in tasks using only the body weight as workload (e.g. jump tasks in the F40 group), this "potential benefit" may be compromised in stronger subjects (i.e. M40 group), who tend to decelerate more aggressively at the end of the lift. It is important to emphasize that greater mechanical work is performed when training with lighter loads and equalizing by relative volume load; hence, more fatigue may be induced, which might delay a complete recovery from the RT session [36].

The decision to keep the external load constant recreates a situation where the absolute training load (kg) is based on a pre-test and it remains unchanged for several weeks until another test is conducted with no adjustment during the training period, as is the case when no rate of perceived exertion RPE, jump assessments, velocity measurements or any other methods are used to adjust the training load. In this way, we could observe the adaptations caused by those initial relative loads, something that has not been thoroughly studied to the best of our knowledge. Even though it might not be the most optimal way to improve short-term performance, training with the 40% 1RM, or rather what initially was the 40% 1RM, still produced some positive adaptations, even when at the end of the training period it probably meant a lighter load. The same held true for the initial 80% 1RM load, some groups achieving greater effect sizes and then more positive adaptations in tasks with maximum or near-maximum force production requirements (maximum isometric force and estimated 1RM). These findings help to provide some insight regarding the viability and progression of certain loads when focusing on making the most out of each load, especially important when looking at the mid- and long-term development of this kind of population.

Conflict of interest

The authors declare no conflict of interest.

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Does the sequence of plyometric and dynamic stretching exercises influence subsequent sprint performance? A randomized crossover intervention study

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ABSTRACT: The objectives of this study were to evaluate the acute effects of the sequence order of drop jumps (DJ) and dynamic stretching (DS) on sprinting performances in competitive athletes and to investigate the relationships between post-activation performance enhancement (PAPE) in sprint performance and lower limb power. Thirteen male jumpers and sprinters participated in this study (19 ± 2 years; 177 ± 7 cm; 71.7 ± 5.6 kg). Through a randomized crossover design, the athletes were exposed to three different conditions after a standardized warm-up: DS+DJ, DJ+DS, and control. Sprinting performance over 40 m was analysed with consideration of initial (0 to 20 m) and final acceleration (20 to 40 m) phases. The effect of intervention was examined by two-way repeated-measures of ANOVA. Pearson's correlation test was used to determine the association between PAPE during sprinting and jump performance. There was no effect of any factor on 40-m sprint performance. Meanwhile, the performance at 20–40 m was higher after the DS+DJ condition when compared to baseline (8.79 ± 0.43 vs. 8.91 ± 0.35 m/s; $p = 0.015$). However, the initial acceleration was worsened in the DJ+DS condition when compared to baseline (6.26 ± 0.25 vs. 6.22 ± 0.26 m/s; $p = 0.002$). There was a negative correlation between CMJ height and the improvement in final acceleration ($r = -0.741$; $p = 0.004$). The use of DS prior to DJ is an effective strategy to improve performance in the final acceleration phase (20–40 m). The athletes with lower levels of lower limb power benefited the most from this PAPE strategy.

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INTRODUCTION

Warming up is an important part of athletes' routine during both training sessions and competitions, aiming to provide adequate biomechanical and physiological conditions that can influence the performance of the subsequent main activity [1]. Hence, previous investigations have sought to determine the best warm-up strategies in various sports to promote higher levels of force production [2, 3], decrease the oxygen deficit [4], increase body temperature [1, 4] and induce post-activation performance enhancement (PAPE) [5, 6].

To promote PAPE, various types of conditioning activities (CAs) have been used, including traditional strength exercises [7], eccentric loading exercises [8], ballistic exercises [9] and sled towing [10], with the possibility of combining these exercises in different sequences [11]. However, some of these activities require the use of additional equipment, including supports, bars, etc., thus making them difficult to use in a competitive environment. From this perspective, the use of ballistic exercises can be considered a viable alternative without the need for complementary equipment [9, 12, 13], therefore presenting greater applicability in competitive settings. Ballistic exercises have also shown greater concentric speed, strength and muscle activation compared to a similar traditional exercise movement [14]. Among the most commonly used ballistic exercises are plyometric exercises (PE) [10, 12, 15] and dynamic stretching (DS) [16, 17].

The use of DS exercises during the warm-up has previously shown improvements in jumping performance [18–20], peak strength [19], and linear sprinting speed [19]. In addition, PAPE has also been observed in sprint [21, 22], countermovement jump (CMJ) [23] and competitive performances in athletics [24, 25] after implementation of PE in the warm-up routine. Interestingly, the inclusion of three depth jumps after DS promoted higher performance enhancements in a 20-m sprint when compared to DS alone [22]. Moreover, the inclusion of

five DJs after DS promoted better performance in a 1000-m run in male runners [12]. In contrast, another previous study observed a better vertical jump performance after using ten minutes of DS when compared to the protocol that added three sets with three tuck jumps after DS [19]. Meanwhile, to date, there are no studies that have examined the use of DS after PE as a PAPE strategy. This information would be useful to determine whether the positive effects of these exercises on neuromuscular function and subsequent performance are additive or not depending on the exercise sequence used.

There is no consensus on the best combination or sequence of these exercises as a PAPE strategy. Meanwhile, other moderators such as strength and power levels may also influence PAPE effects [26] and should also be explored. Therefore, the aim of the current study was to evaluate the acute effects of exercise sequence (DS+DJ vs. DJ+DS) on sprint performance and determine whether the PAPE responses are related to lower limb power. We hypothesized that the combination of these stimuli could improve sprint performance after both protocols, and that athletes with the greater lower limb power would exhibit greater PAPE responses.

MATERIALS AND METHODS

Study design

A randomized crossover design was used to study the influence of DJ combined with DS as a strategy to enhance sprinting in track and field athletes. Our study was conducted at an athletics outdoor track over a 3-week period. Every subject was informed about the purpose, procedures, and risks of the study. All procedures were approved by the research ethics committee of the UFS (process number 4.890.323) and were carried out according to the Helsinki Declaration.

The subjects visited the athletics track on five different occasions: two familiarization sessions and three experimental sessions, 72 hours apart, thus allowing for an appropriate recovery period (see Figure 1). In the first familiarization session, the subjects performed the countermovement jump (CMJ) assessments, in addition to the warm-up protocols and the tests to be performed. In the second familiarization session, the optimal height for the drop jump was determined and the procedures of the first session were repeated, to minimize the learning effects for the subsequent experimental sessions. For the next experimental sessions, the subjects were randomly assigned, using Microsoft Office Excel software, to the three warm-up conditions: Dynamic Stretching + Drop Jump (DS+DJ), Drop Jumps + Dynamic Stretching (DJ+DS), and Control (C).

In each experimental session, the warm-up was performed after the baseline sprint, followed by the evaluation of the 40-m sprint performance with consideration of the 0–20-m (initial acceleration) and 20–40-m (final acceleration phase) phases. Subjects were recommended to maintain their eating and training routines throughout the experimental period, except for the 24 hours before the experimental sessions in which a resting day was recommended. The ingestion of alcohol and caffeine was not allowed before the experimental sessions.

Subjects

The sample was composed of competitive male sprint (100- and 200-m dash) and jump (triple and long jump) athletes, who participated in the under-20 and adult championships in the State of Sergipe (Brazil). All the athletes who won medals in these specific events were eligible to participate in the research. The sample size was calculated a priori based on a statistical power of 0.95, assuming an effect size of 0.66 according to the findings of Lima et al. [27] and an alpha level of $P < 0.05$. The minimum sample size of 9 subjects was obtained (G*Power software package [version 3.1.9.4], Franz, Universitat Kiel, Germany). After sending invitations to all the eligible athletes, the following inclusion criteria were used for those who accepted to participate: (1) age of 16 years or older, (2) minimum of three training sessions per week, (3) minimum experience of six months in plyometric training, and (4) absence of lower limb injuries in the last six months. Sixteen athletes were finally included in the study. All subjects were encouraged to continue their normal training routines, except 24 hours before the experimental sessions. Exclusion criteria were suffering injuries during the study period and not attending any session of the study. All subjects signed an informed consent form. The parents of the athletes under the age of 18 signed the informed consent form.

Drop jump assessment

During the second familiarization session, subjects completed a test to determine the optimal DJ height to be used in the next experimental sessions. Subjects performed three DJ from two different heights (20 and 40 cm) [28]. The optimal height was selected using the best reactive strength index (jump height/contact time). The jump was recorded and analysed using the My Jump 2 app [28], which was installed on an iPhone (v. 8, Apple Inc., Cupertino, CA, USA).

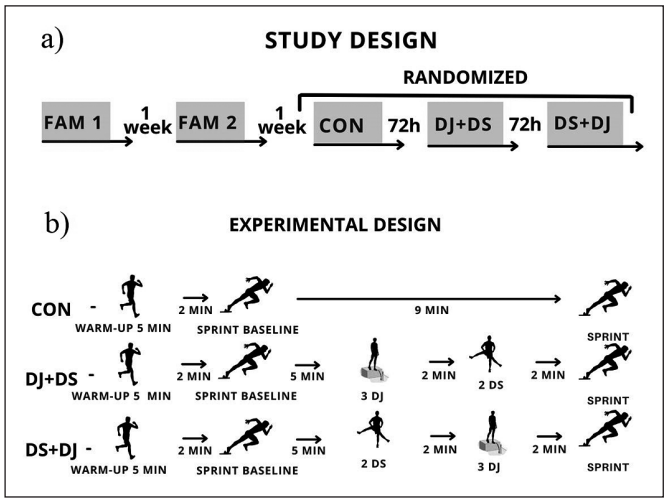


FIG. 1. a) Study design; b) Experimental design FAM = Familiarization; CON = Control; DJ+DS= Plyometrics + dynamic stretching; DS+DJ = Dynamic stretching + plyometrics.

Standard warm-up

In all conditions, the athletes completed a standardized 5-min warm-up which consisted of: 2-min × 500 m of slow running on the track, 4 running technique drills over 20 m with 20 s of active recovery by walking (totalizing 2:30 min); and a 5-m maximum acceleration. Two minutes after completing this warm-up protocol, the subjects performed the baseline sprint in all conditions.

Dynamic stretching and drop jump protocols

The subjects performed two dynamic stretching exercises (vertical and lateral leg swings), 1 set of 15 repetitions for each leg, increasing the speed progressively until the 10th repetition while maintaining the maximum amplitude and speed until the last repetition. The athletes performed three DJ with the optimal DJ height previously determined in the familiarization session. During the DJ, the subjects were instructed to keep their hands on their waist and perform the jumps as fast and high as possible.

Sprint assessment

Subjects performed two 40-m linear sprints on the track, one after the standardized warm-up which served as baseline and another sprint after the potentiation protocols or control condition. All attempts were recorded using an iPhone 8 (Apple Inc., Cupertino, CA, USA) and the MySprint app (T-Mobile Inc., Bellevue, WA, USA). The system is based on high-speed video analysis (240 fps) and has been demonstrated to be valid and reliable for assessing linear sprint performance [29]. The athletes started from a three-stand position with their preferred hand on the track. The start of the sprint was determined when the athletes' thumb took off after visual inspection. Six cones were located at 5, 10, 15, 20, 30, and 40 m to ensure that split times were recorded correctly. Two independent observers were asked to select the first frame in which the subjects' right thumb left the ground (i.e., the start of the sprint) and the frames in which the marker placed on the pelvis was aligned with the cones for each sprint [29]. For data analysis, we used the mean velocities recorded in the 40-m sprint, and from 0 to 20 m (i.e. acceleration phase) and 20 to 40 m (i.e. final acceleration phase).

Countermovement jump assessment

The evaluation of the CMJ was performed during the second familiarization session as a measure of lower limb power. CMJ performance was verified with the jump height (cm), which was estimated using a validated contact mat [30] (Chronojump, Bosco systems, Barcelona, Spain) following the flight-time method. The subject started in a standing upright position with his feet on a mat and with his hands on his hips, followed by a downward movement flexing his knees to approximately 90° to immediately jump as high as possible. Each athlete made three attempts separated by 10 s. The highest jump was used for further analyses.

Statistical analysis

The results were presented as mean ± SD. The normality assumption for each variable was verified using the Shapiro-Wilk test. The effect of intervention on sprinting was examined by two-way repeated-measures ANOVA (3 conditions × 2 time points [baseline and post-CA]). Mauchly's test of sphericity was applied, and the Greenhouse-Geisser Epsilon correction was used when the criteria for sphericity were not met. Effects sizes for main effects were calculated as partial eta squared (η_p^2) and interpreted as small (0.01), medium (0.06), and large effects (0.14) [31]. The analyses were completed with the Bonferroni post hoc test. The intraclass correlation coefficient (ICC) was calculated (95% CIs), using a two-way mixed-effect absolute agreement model [32], to verify the reliability of speed data between testers. ICC values less than 0.5 are considered poor, values between 0.5 and 0.75 are moderate, values between 0.75 and 0.9 are good, and values greater than 0.9 are excellent. Pearson's product moment correlation test was used to determine the association between the level of the athletes and the improvement in performance. A significance level of $p \leq 0.05$ was set in all analyses. The software used was SPSS (v. 25.0, IBM Corp., Chicago, IL, USA).

RESULTS

Two athletes were excluded from the analyses for not attending all the sessions and one athlete was excluded because of foot pain before starting one experimental session. Thus, the final sample was composed of 13 power track and field athletes (sprinters, n = 6; jumpers, n = 7), whose physical and anthropometric characteristics are presented in Table 1. The sprint times evaluated by the two independent observers from 10 random recordings provided excellent reliability (ICC = 0.984, 95%CI = 0.948–0.995).

Two-way repeated measures of ANOVA showed that there was no effect of any factor on the overall 40-m sprint performance. There was

TABLE 1. Physical and anthropometric characteristics

Variables	Mean ± SD
Age (years)	19.0 ± 2.0
Body mass (kg)	71.7 ± 5.6
Height (cm)	177 ± 7
CMJ height (cm)	39.0 ± 3.0
Training time (y)	3.3 ± 1.1
RSI (cm/s)	3.2 ± 0.6
100-m personal best (s)	11.4 ± 0.6
200-m personal best (s)	22.7 ± 1.1
Long jump personal best (m)	5.97 ± 0.29
Triple Jump personal best (m)	13.62 ± 1.58

Legends: CMJ = countermovement jump; RSI = reactive strength index.

TABLE 2. Mean (SD) of sprint velocity at 0–20 m, 0–40 m and 20–40 m distances between conditions (Control, DS+DJ and DJ+DS) at different times (baseline and post-intervention), and statistics of the two-way repeated measures of ANOVA (n = 13)

	Control		DS+DJ		DJ+DS		Condition		Time		Condition × Time	
	Baseline	Post	Baseline	Post-intervention	Baseline	Post-intervention	P	η_p^2	P	η_p^2	P	η_p^2
0–20 m (m/s)	6.35 (0.24)	6.26 (0.23)	6.30 (0.27)	6.25 (0.27)	6.26 (0.25)	6.22 (0.26)*	0.014	0.299	0.022	0.366	0.289	0.098
0–40 m (m/s)	7.39 (0.27)	7.33 (0.28)	7.34 (0.30)	7.34 (0.29)	7.33 (0.28)	7.31 (0.31)	0.083	0.187	0.172	0.150	0.165	0.139
20–40 m (m/s)	8.84 (0.38)	8.83 (0.39)	8.79 (0.43)	8.91 (0.35)*	8.83 (0.35)	8.86 (0.41)	0.815	0.017	0.050	0.283	0.164	0.140

ES = Effect size; DS+DJ = Dynamic stretching + Drop Jumps; DJ+DS = Drop Jumps + Dynamic stretching; * = $p < 0.05$, Bonferroni post-hoc test compared to baseline.

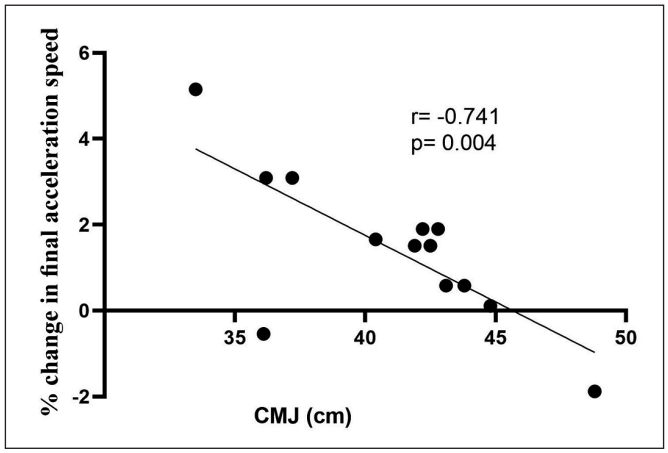


FIG. 2. Correlation between performance improvement in the fina acceleration phase after the DS+DJ protocol and CMJ performance. DS+DJ = Dynamic stretching + Drop Jump; DJ+DS = Drop Jump + Dynamic stretching; CMJ = Countermovement Jump.

an effect of time and condition on 0–20 m speed (Table 2). The Bonferroni post-hoc test showed that post-intervention sprint performance was worse than baseline in the DJ+DS condition ($p = 0.02$). For the 20–40-m interval, there was an effect of time on performance. Bonferroni's post-hoc test showed that velocity in post-intervention sprint was higher compared to baseline in the DS+DJ condition ($p = 0.015$).

A significant relationship was found between performance in the CMJ and the percentage of change in performance in the only condition in which there was an improvement in performance from baseline (see Figure 2).

DISCUSSION

The objectives of the present study were to investigate the acute effects of exercise sequence (DS+DJ vs. DJ+DS) on sprint performance and determine whether the PAPE responses are related to lower limb power. The combination of DS with DJ, independently of the sequence used, did not improve the overall performance in the 40-m sprint. However, the DS+DJ sequence promoted an increase in performance in the final acceleration phase (20–40 m). In addition, the execution of the CAs in the reverse order (i.e. DJ+DS) showed a worsening in performance in the acceleration phase. Thus, in the current study there was an indication that performing DJ after DS is effective for acute improvement of sprint performance. Although previous studies have used the combination of DS with DJ as a strategy to improve performance in the CMJ [19] and 20-m sprint [22], this is the first study comparing the influence of the order of these CAs on different sprinting phases.

The CAs used in the current study have been proven to be easy and efficient for sport settings requiring the achievement of maximum speeds after 20 m, as in the case of long and triple jumps [33]. Previously, Zimmerman et al. [34] observed an improvement in performance in 30-m sprints after a CA which consisted of continuous vertical jumps. However, no difference was found for the 10-m sprint performance. In contrast to our results, Byrne et al. [22] and Turki et al. [17] previously found performance improvements in the 20-m sprints after using DS combined with depth jumps and DS alone in team sport athletes. These discrepancies between studies may be explained by differences in samples' characteristics, CAs used and sprinting distances evaluated. While team sport players commonly execute sprints at or near to maximum velocities over distances ≤ 20-m during sport-specific activities [35], for track and field sprinters, these distances are typically used to develop their initial acceleration abilities [36]. Further studies are warranted to determine the relative influence of these factors on sprint performances in different sport settings.

We selected two exercises commonly used in training and competitive settings [12]. Previous evidence has demonstrated that plyometric jumps primarily recruit type II fibres, thus favouring the potentiation mechanisms [9] which are related to performance improvements in speed and strength tests [13]. However, DS may decrease the time to peak torque and increase the rate of torque development [37]. We hypothesized that the combination of these stimuli could increase performance after both protocols. However, we

identified no performance gains in the acceleration phase for the DS+DJ condition, perhaps because this phase requires greater activation of the leg and hip extensor muscles [38], with a more horizontal body position at the beginning of the phase [36], thus differing from the muscles and body position used in the DJs. The performance improvement in the final acceleration phase can be characterized by shorter contact times and a more vertical position of the body [36], which is more related to the execution of DJs. While these arguments are speculative, further studies should better explore the influence of the neuromuscular, kinetic and kinematic factors involved on these acute responses after exercises with different biomechanical characteristics [39], as it would seem that the change of the order between DJs and DS may influence these acute responses.

The negative correlation found between jump capacity and PAPE in final acceleration speed may indicate a greater potentiating effect in athletes with a lower level of lower limb power (see Figure 2). This is somewhat contrary to the suggestions by Wilson et al. [2], who were the first to systematically demonstrate an association between training experience and PAPE responses. In this regard, Boullosa [26] recently suggested that the two main PAPE moderating factors related to the population of athletes are strength levels and training experience; however, the consideration of other potential moderators such as sex and age has been poorly addressed in applied studies. Furthermore, previous studies indicating an influence of training background and strength levels have been conducted with traditional strength training exercises [2, 40]. Therefore, as the athletes in the current study are young power track and field athletes with limited training experience, and because we used a novel combination of ballistics exercises, the combination of these factors would explain this divergence from the current evidence. Further studies should investigate in more detail the influence of population characteristics in the PAPE response with different exercises.

Considering the performance improvement in the 20–40-m section, we may suggest the use of this PAPE strategy for long jumpers, because the approach runs are 35–40 m and thus a higher final acceleration speed could be achieved at the end of the approaching run, therefore potentially increasing the long jump distance [41]. Similarly, sprinters may use this strategy because we would expect that this effect could be maintained during the maximum speed phase of a 100-m run (50–70 m), which is a key phase for performance. However, these potential benefits should be tested in future interventions in both athletic populations.

CONCLUSIONS

In conclusion, using the combination of DS with DJ, independently of the sequence used, did not improve overall performance in the 40-m sprint. The DS+DJ sequence improved performance in the final acceleration phase (20–40 m). Athletes with lower levels of lower limb power may benefit more from this CA. When DS is preceded by DJ, there was a worsening in the initial acceleration. These findings provide coaches with a simple and practical PAPE strategy to favour better sprinting performances without the use of additional equipment and complex settings.

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Conflict of interest declaration

The authors declare no conflict of interest.

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Comparison of acute and prolonged effects of short-term foam rolling and vibration foam rolling on the properties of knee extensors

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ABSTRACT: Recently, Foam Rolling (FR) and Vibration Foam Rolling (VFR) have attracted attention in sports and rehabilitation fields. Previous studies have shown that FR and VFR acute interventions effectively increase the range of movement (ROM) and decrease tissue hardness. For application to sports and rehabilitation, it is necessary to compare the acute and prolonged effects of short duration FR and VFR. Therefore, this study aimed to compare and investigate the acute and prolonged (15 minutes) effects of short duration (30-s) FR and VFR interventions on knee extensors. The subjects were 14 male university students (22.4 ± 1.0 years old), in which the knee extensors of the dominant leg were tested. In a cross-over trial, 30-s of FR or VFR were performed with 2-s rolling of the anterior thigh (15 rolls). The frequency of VFR was 35 Hz. Measurements included knee flexion ROM, pain pressure threshold (PPT), tissue hardness, and countermovement jump height. The results of this study showed no interaction effects for all variables, but main time effects were observed for knee flexion ROM, PPT, and tissue hardness. Post-hoc tests showed that knee flexion ROM increased up to 10 minutes after the intervention. PPT significantly increased, and tissue hardness significantly decreased up to 15 minutes after intervention. This study showed that 30-s FR and VFR interventions effectively increased ROM, PPT, and decreased tissue hardness. The effects were prolonged up to 10–15 minutes after the intervention. The results of this study show no advantage of VFR over FR with acute short-term interventions.

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Range of motion
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Tissue hardness
Pain pressure threshold

INTRODUCTION

Foam rolling (FR) has recently attracted attention in sports and rehabilitation fields [1]. Previous studies on the effect of FR have reported an increase in ROM [2–4] and a decrease in tissue hardness [2, 5]. Moreover, vibration foam rolling (VFR), in which FR is provided with a vibration function, has been suggested to increase ROM [6] and decrease tissue hardness [7] to a greater extent than FR. Furthermore, a previous study [8] compared the effects of FR and VFR interventions on delayed onset muscle pain induced by exercise with induced muscle damage. They showed that VFR was more effective than FR in improving pain and hip extension ROM. In addition, VFR for older women has been reported to be effective in improving senior fitness performance when incorporated as a warm-up [9] as well as an increase in shoulder flexibility when combined with static stretching [10]. On the other hand, FR and VFR intervention for the ankle plantar flexors was reported to be effective in increasing ROM on the non-intervention side to the same extent [7]. Also, FR and VFR interventions have not been reported to decrease muscle strength or performance [11, 12]. Therefore, FR and VFR interventions that maintain muscle strength and increase ROM are currently being employed in various sports and rehabilitation fields.

A previous study suggested that FR aimed at increasing ROM requires interventions of 90-sec or longer. In a previous study investigating the effects of a 180-second FR and VFR intervention on knee extensors [2], an increase in knee flexion ROM was prolonged for at least 30 minutes after the intervention with both FR and VFR interventions. However, since static stretch interventions in sports fields have been reported to be mostly less than 20 seconds [13], it is essential information for athletes and coaches about the effect of a more

practical duration, i.e., short-duration FR and/or VFR interventions. Regarding the effect of a short-term FR intervention, a previous study investigating the acute effect of a 5-second FR intervention on hamstrings showed a significant increase in the Sit and Reach test without loss of muscle strength [14]. However, the prolonged effects of short-term FR interventions are unknown. Moreover, VFR may have a greater effect than FR [6], and may also have a greater acute effect, even for short-term interventions. Furthermore, in view of its application in sports and rehabilitation fields, it is necessary to compare the acute and prolonged effects of short-term FR and VFR interventions. Also, FR intervention has been suggested to have a dose-response relationship [14]. In this study, the duration of the intervention is set shorter than in previous studies [2], thus we expected that the prolonged effect will also be shorter than in the previous study. Hence, we investigated the prolonged effect of FR and VFR up to 15 minutes after the intervention. Thus, this study was designed to compare and examine the acute and prolonged effects of short-term FR and VFR interventions on knee extensors. Based on previous studies [6, 7], we hypothesized that the effects of increased ROM and decreased tissue hardness would be greater in VFR compared to FR because the vibration function would affect mechanoreceptors [12, 15]. Previous studies [12, 16] have reported that FR/VFR interventions do not adversely affect muscle strength or jump performance. Therefore, we hypothesized that a short-term intervention would not alter CMJ height.

MATERIALS AND METHODS

Experimental set-up

A randomized repeated measures experimental design was used to compare the acute and prolonged effects of short-term FR and VFR. The participants were instructed to visit the laboratory twice with a ≥ 48 h break. They performed the following two conditions, 30 seconds of FR and VFR (Figure 1). The FR and VFR were performed with a 2-second rolling intervention from proximal to distal to proximal and back to proximal of the target muscle. In both conditions, the 30-second intervention involved 15 rolls of the knee extensors of the dominant leg. Measurement points were pre-intervention (PRE), immediate post-intervention (POST), and 5-, 10-, and 15 minutes after the intervention. Outcome measures were tissue hardness, pain pressure threshold (PPT), knee flexion ROM, and CMJ evaluated in this order. Since dynamic activity such as a CMJ can affect ROM and stretching can impact PPT and muscle tissue hardness [17–19], the order of tests was not randomized. The reproducibility of the measured items has been confirmed in our previous study [20] with good to excellent reliability in the present study as well (see results section).

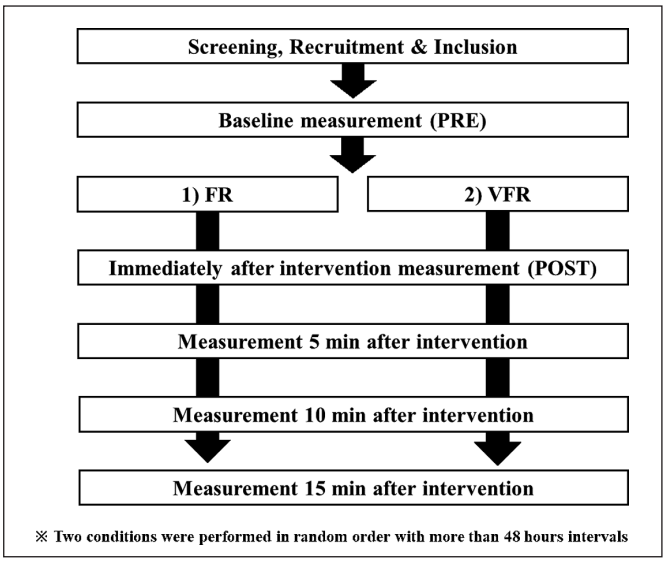


FIG. 1. The experimental set-up
FR: foam rolling, VFR: vibration foam rolling.

Participants

Fourteen healthy, recreationally active males were enrolled (mean ± SD: age, 22.4 ± 1.0 years; height, 170.7 ± 4.8 cm; weight, 67.6 ± 9.2 kg). Individuals with a history of neuromuscular disease and musculoskeletal injury involving the lower extremities were excluded. The required sample size for a repeated-measures two-way analysis of variance (ANOVA) (effect size = 0.25 [large when considering interaction effects for 2-way ANOVAs], α error = 0.05, and power = 0.95) based on our previous study's ROM results [21] using G* power 3.1 software (Heinrich Heine University, Dusseldorf, Germany) was more than 9 participants.

For the study, participants were fully informed about the procedures and aims, after which they provided written informed consent. The study complied with the requirements of the Declaration of Helsinki and was approved by the Ethics Committee of the Niigata University of Health and Welfare, Niigata, Japan (Procedure#18615).

Outcome assessments

Knee flection ROM

Each participant was placed in a side-lying position on a massage bed with the hips as well as the knee of the non-dominant leg flexed at 90° to prevent pelvic movements [22, 23]. A licensed physical therapist, the investigator, brought the dominant leg to full knee flexion with the hip joint in a neutral position. A goniometer (MMI universal goniometer Todai 300 mm, Muranaka Medical Instruments, Co., Ltd., Osaka, Japan) was used to measure knee flexion ROM. This was measured three times at each measurement point, and the average values at each measurement point were used for analysis.

Pain pressure threshold (PPT)

PPT measurements were conducted in the supine position using an algometer (NUTONE TAM-22 (BT10); TRY-ALL, Chiba, Japan). The measurement location was set at the midway of the distance between the anterior superior iliac spine and the dominant side's superior

border of the patella for the rectus femoris muscle. With continuously increasing pressure, the soft tissue in the measurement area was compressed with the metal rod of the algometer. The participants were instructed to immediately press a trigger when pain, rather than just pressure, was experienced. The value read from the device at this time point (kilograms per square centimetre) corresponded to the PPT. In each condition, PPT was measured three times at each measurement point, and the mean value at each measurement point was used for further analysis.

Tissue hardness

Tissue hardness was measured using a portable tissue hardness meter (NEUTONE TDM-N1; TRY-ALL Corp., Chiba, Japan). The participant's measurement position and posture were similar to PPT measurements. This tissue hardness meter measured the penetration distance until a 14.71 N (1.5 kgf) pressure was reached [24]. The participants were instructed to relax while tissue hardness was measured three times at each measurement point, and the mean value at each measurement point was used for further analysis.

Unilateral Countermovement Jump (CMJ) height

Unilateral CMJ height was calculated from flight time using a contact mat (Jump mat system; 4Assist, Tokyo, Japan). The participants started with the foot of the dominant leg on the mat with their arms crossed in front of their chest. The participants were instructed to dip quickly (eccentric phase) from this position, reaching a self-selected depth to jump as high as possible in the next concentric phase. Landings were performed on both feet. The knee of the non-involved leg was held at approximately 90° flexion. After three familiarization trials, three maximal unilateral CMJ were conducted at both PRE and POST in each condition, and the average of the three trials was used for further analysis [20].

Foam rolling (FR), Vibration Foam Rolling (VFR) intervention

The participants were instructed on how to use the foam roller (Stretch Roll SR-002, Dream Factory, Umeda, Japan) by a physical therapist. For familiarization, they were allowed to practice using the foam roller three to five times on the non-dominant leg (non-intervention leg) immediately before the FR intervention to verify that the participants were able to perform the FR intervention at the specified velocity and location. Based on the Behm et al. [4] recommendations, FR and VFR involved 1 set of 30-seconds with 2-second cycles (defined as one distal rolling movement followed by one proximal rolling movement). A metronome (Smart Metronome; Tomohiro Ihara, Japan) was used for control. The participants were asked to place as much body mass on the roller as tolerable.

Statistical analysis

SPSS (version 28.0; IBM Corp., Armonk, NY, USA) was used for the statistical analysis. To verify the consistency of PRE values, PRE values were tested between FR and VFR conditions using a paired t-test. For all the variables, a two-way repeated-measures ANOVA using two factors (test time [PRE vs. POST vs. 5 minutes vs. 10 minutes vs. 15 minutes] and conditions [FR vs. VFR]) was used to analyze the interaction and main effects. Classification of effect size (ES) was set where $\eta_p^2 < 0.01$ was considered small, 0.02 – 0.1 was considered medium, and more than 0.1 was considered to be a large effect size [25]. Where appropriate, post-hoc analyses were performed using multiple comparison tests with Bonferroni correction to determine differences between PRE, POST, 5, 10, and 15 minutes. Also, we calculated the effect sizes (Cohen d) on the PRE value. Cohen d of 0.00–0.19 was considered trivial, 0.20–0.49 was small, 0.50–0.79 was moderate, and ≥ 0.80 was large [26]. The significance level was set to 5%, and all the results are shown as mean ± SD.

RESULTS

Comparison between PRE values among the two conditions

There were no significant differences in all PRE variables between the two conditions. We calculated the coefficient of variation (CV) and intraclass correlation coefficient (ICC) from these data. The CVs of measurements for knee ROM, PPT, tissue hardness, and CMJ height were 0.8 ± 0.7%, 8.5 ± 6.0%, 6.6 ± 4.3%, and 2.7 ± 1.5%, respectively, and the ICC (1,2) for measurements were 0.706, 0.805, 0.737, and 0.958, respectively.

Changes in knee flexion ROM, PPT, tissue hardness, and CMJ height

There were no significant interaction effects for all variables (knee flexion ROM: $F = 0.7$, $p = 0.56$, $\eta_p^2 = 0.03$, PPT: $F = 1.0$, $p = 0.39$, $\eta_p^2 = 0.04$, tissue hardness: $F = 1.9$, $p = 0.12$, $\eta_p^2 = 0.07$, CMJ height: $F = 0.2$, $p = 0.94$, $\eta_p^2 = 0.01$) (Figure 2). There were significant ($p < 0.01$) main effects of test time with knee flexion ROM ($F = 37.4$, $p < 0.01$, $\eta_p^2 = 0.61$), PPT ($F = 7.9$, $p < 0.01$, $\eta_p^2 = 0.25$), and tissue hardness ($F = 14.6$, $p < 0.01$, $\eta_p^2 = 0.38$), but there was no significant main effect of test time for CMJ height ($F = 1.3$, $p = 0.26$, $\eta_p^2 = 0.05$). The post-hoc test results showed that knee flexion ROM was significantly ($p < 0.05$) higher at POST (FR: d = 0.64, 1.5 ± 0.8%, VFR; d = 0.67, 1.9 ± 1.5%), 5 (FR: d = 0.52, 1.2 ± 0.9%, VFR: d = 0.43, 11.1 ± 0.9%) and 10 minutes (FR: d = 0.33, 0.7 ± 0.8%, VFR: d = 0.22, 0.5 ± 0.6%) after the intervention compared to PRE. However, ROM returned to baseline values 15 minutes after the intervention (FR: d = 0.15, 0.3 ± 0.6%, VFR: d = 0.08, 0.2 ± 0.6%). Knee flexion ROM was significantly lower after 5, 10, and 15 minutes after the intervention compared with POST ($p < 0.01$) and was also lower after 10 and 15 minutes compared with 5 minutes after the intervention ($p < 0.01$). Moreover, it was significantly lower after 15 minutes after the intervention compared to 10 minutes after the intervention ($p < 0.05$). PPT was significantly ($p < 0.05$) higher at POST (FR: d = 0.59, 20.9 ± 18.8%, VFR: d = 0.45, 9.9 ± 16.3%), 5 (FR: d = 0.40, 15.2 ± 20.2%, VFR: d = 0.41, 11.7 ± 18.8%), 10 (FR: d = 0.42, 16.1 ± 2.20%, VFR: d = 0.29, 9.0 ± 16.3), and 15 minutes (FR: d = 0.42,

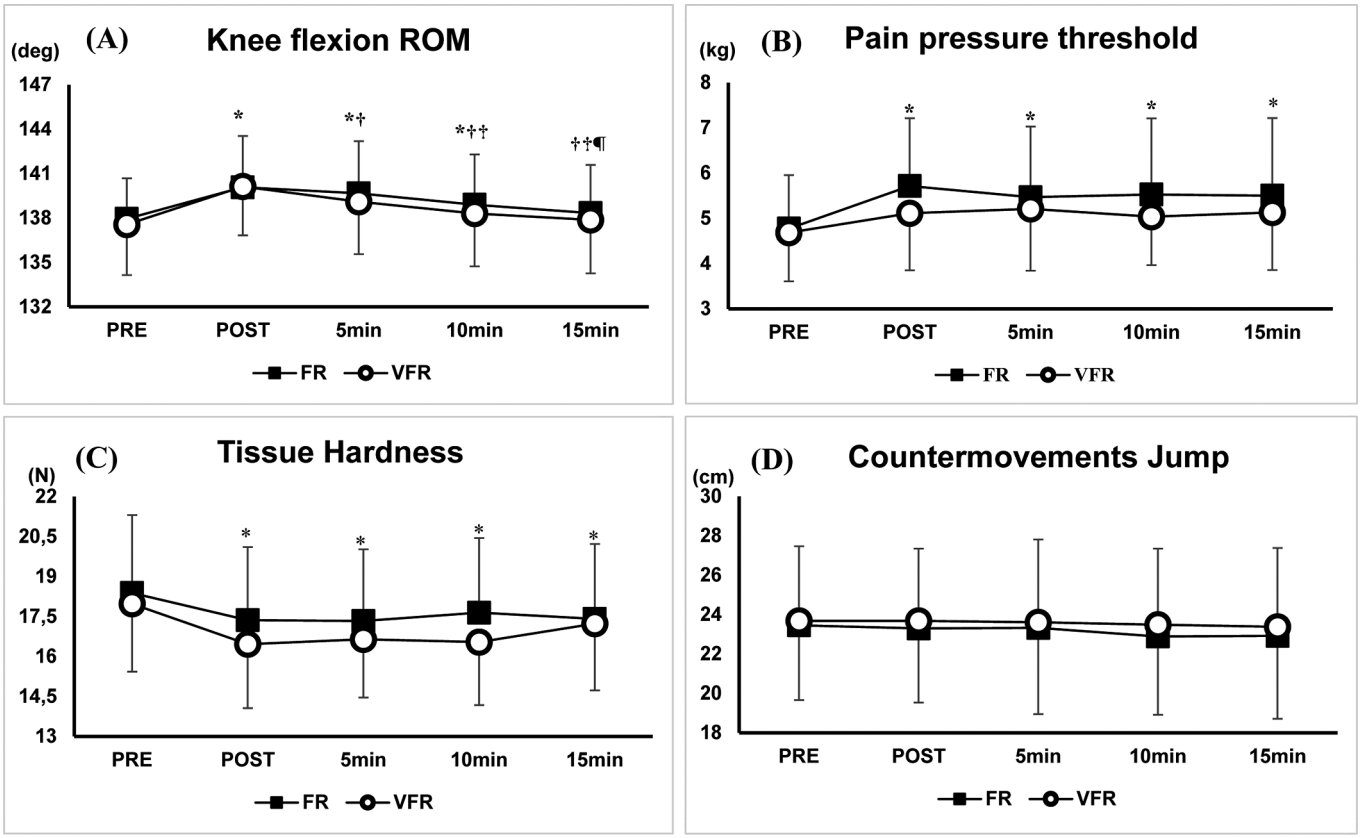


FIG. 2. Changes in knee flexion range of motion (ROM), pain pressure threshold, Tissue Hardness, and countermovement jump height after 30 seconds foam rolling (FR) and vibration foam rolling (VFR) intervention (*: Significant difference ($p < 0.01$) from PRE, †: Significant difference ($p < 0.01$) from POST, ‡: Significant difference ($p < 0.01$) from 5 minutes after the intervention, ¶: Significant difference ($p < 0.05$) from 10 minutes after the intervention).

15.0 ± 18.5%, VFR: d = 0.33, 10.1 ± 15.0%) after the intervention compared to PRE. Tissue hardness was significantly ($p < 0.05$) lower at POST (FR: d = 0.29, -5.4 ± 5.0%, VFR: d = 0.53, -8.2 ± 7.2%), 5 (FR: d = 0.33, -5.6 ± 5.1%, VFR: d = 0.54, -7.0 ± 6.9%), 10 (FR: d = 0.25, -4.0 ± 4.4%, VFR: d = 0.58, -7.9 ± 5.5%), and 15 minutes (FR: d = 0.32, -5.3 ± 4.8%, VFR d = 0.30, -4.0 ± 6.0%) after the intervention compared to PRE.

DISCUSSION

This study compared the acute and prolonged effects of short-term (i.e., 30-second) FR and VFR interventions on knee extensors. This study showed that both 30-second FR and VFR increased knee flexion ROM up to 10 minutes after the intervention. Also, PPT significantly increased, and tissue hardness significantly decreased up to 15 min after both FR and VFR interventions. However, no significant change in CMJ was observed. In addition, there were no significant differences between FR and VFR interventions for any measure. These results suggest that acute, short-duration FR and VFR interventions are recommended when the goal is to increase ROM without decreasing performance. To the best of our knowledge, this is the first study examining and comparing the acute and prolonged effects of short-term FR and VFR interventions.

The significant increases in knee flexion ROM were 2.14 ± 0.01° from PRE to POST, 1.56 ± 0.20° from PRE to 5 minutes, and 0.88 ± 0.21 from PRE to 10 minutes, respectively, after 30-sec FR interventions in this study. These increases in ROM were consistent with several previous studies that report moderate to large magnitude effect size [27–29] FR-induced knee flexion improvements immediately post-intervention ranging from 1.4–8.0° [29–32]. Only two FR studies have not reported an immediate significant knee flexion ROM increase with either 2 minutes of FR [33] or a combination of jogging and FR for 8 minutes [8]. By the present findings, a recent study has shown no significant difference in increased ROM between FR and VFR [7].

The significant knee flexion ROM increase up to 10 minutes in the present study is in agreement with MacDonald et al. [34], who found 10° and 8° knee flexion ROM improvements at 2- and 10-minutes post-FR intervention. However, the present findings are in contrast to Wilke et al. [5], who did not find a significant FR-induced knee flexion ROM increase immediately, 5-, or 10-minute post-test. The Wilke et al. [5] study used four repetitions of 45 seconds each at either a high or low rolling velocity, whereas the present study incorporated only one set of 30 seconds. A previous study from this lab showed no significant increase in ankle dorsiflexion ROM after

30 seconds but a significant increase in ankle dorsiflexion ROM after more than 90 seconds FR intervention [35]. This discrepancy between the previous study from this lab and the present results may vary depending on the targeted muscle. Nakamura et al. [35] investigated the acute effect of short-term FR intervention on ankle plantar flexors, but the current study investigated the acute effect of short-term FR intervention on knee extensors. Also, Sullivan et al. [14] showed that even a 5-second FR intervention on hamstrings increased the Sit and Reach test. Thus, the FR intervention duration required to increase ROM in the target muscle may differ. Therefore, it is necessary to examine and compare the minimum FR intervention duration required to increase ROM in various muscles.

In addition, previous studies suggested that increases in stretch (pain) tolerance are involved with the increase in ROM after FR and VFR interventions [1, 7, 35, 36]. An FR intervention may reduce pain by activating either or both neural-gating mechanisms [37, 38] or the release of endorphins and enkephalins as theorized with the diffuse noxious inhibitory control mechanism [39]. The results of this study showed that the knee flexion ROM returned to the baseline value 15 minutes after the intervention. Previous studies with a 180-second intervention period [2, 3] showed that the prolonged effect of increased ROM lasted for 20–30 minutes. FR suggests a capacity-response relationship [14]. In this study, the duration of the intervention is set shorter than in previous studies [2, 3], thus it can be assumed that the prolonged effect was shorter than in the previous studies. With this study, a significant increase in PPT after short-term FR and VFR intervention was prolonged for up to 15 minutes after both interventions. On the other hand, the significant increase in knee flexion ROM only persisted for 10 minutes after both interventions.

Tissue hardness was also significantly decreased up to 15 minutes after the intervention. A systematic review and meta-analysis examining the acute effects of FR interventions highlighted decreases in tissue hardness with the thoracolumbar fascia and quadriceps muscles [40]. The mechanism of the decrease in tissue hardness after FR intervention could be thixotropic changes (decreased tissue visco-elasticity) [15]. Moreover, previous studies showed that FR intervention increased tissue perfusion and consequently decreased tissue hardness [41, 42]. On the other hand, the previous study pointed out that VFR has a greater effect than FR due to the stimulation of mechanoreceptors by vibration [6, 15]. Kasahara et al. [2] reported that a 180-seconds VFR intervention could decrease tissue hardness longer than an FR intervention. In addition, Nakamura et al. [7] examined the effects of 180 seconds of FR and VFR interventions on the ankle plantar flexors. The results showed that only the VFR intervention decreased muscle stiffness of the medial gastrocnemius muscle, but not in the FR intervention. However, in the present study, there was no significant difference in the decrease in tissue hardness between short-term FR and VFR interventions. These results support previous studies [2, 43] that found a significant decrease in tissue hardness of the knee extensors after FR and VFR interventions. Interestingly, Kasahara et al. [2] reported that a 180-seconds VFR intervention could decrease tissue hardness longer than an FR intervention. The intervention duration in this study was only 30 seconds, suggesting that with a longer intervention time, there was a greater effect of the vibration function. The results of this study also suggest that even short-term intervention possibly induced thixotropic changes and increased blood flow, resulting in a decrease in tissue hardness.

However, there is an unclear relationship between PPT increases (stretch tolerance), and tissue hardness decreases over 15 minutes versus ROM improvements for only 10 minutes. These findings may suggest there may be other factors contributing to the increased ROM that persist for 10 minutes or less. For example, tissue hardness is affected by tissue perfusion [42], which has been implicated in increased concentrations of oxygen and carbon monoxide [44], thus tissue hardness may not directly represent muscle tissue stiffness [41]. Although decreased tissue hardness was evident for 15 minutes post-intervention, decreased muscle stiffness has been reported to be prolonged for only 5 minutes [19, 45, 46]. Whereas PPT and muscle hardness may persist for 15 minutes post-intervention, significant increases in ROM might be attributed to the contributions of a greater variety of factors such as muscle stiffness which may have shorter time courses. Future research should consider the influence and durations of the various possible ROM increasing mechanisms associated with FR and VFR.

This study showed that short-term FR and VFR interventions did not significantly change CMJ. A previous study comparing the effects of dynamic stretching and FR in tennis players reported no significant changes in CMJ [47]. On the other hand, a previous study investigating the acute effect of a 180-second FR and VFR intervention on knee extensors showed a significant increase in muscle strength after both FR and VFR interventions [43]. The acute effect of FR intervention on muscle strength and performance with FR has not been consistent, but in recent reviews, FR interventions tend to have no beneficial or harmful effect on muscle strength or performance [4, 11] or are effective when compared to stretching interventions [48]. Furthermore, a systematic review and meta-analysis [12] examining the effects of VFR interventions showed no significant effect of VFR interventions on jumping performance. Our results expanded these findings and suggested that short-term FR and VFR interventions do not change jump performance after both FR and VFR interventions.

This study has some limitations. The first is that post-intervention measurements could only be taken up to 15 minutes after the intervention, and thus, the maximum duration of these effects (increased PPT and decreased tissue hardness) are unknown. The second limitation is the frequency of FR intervention. In this study, the speed was standardized to one cycle of 2 seconds, so it is unclear whether the effect of different FR intervention velocities differs. The third limitation is the target population. This study was conducted on healthy male university students, so it is not clear whether the

same effects could be obtained on athletes or female participants. The fourth limitation is no control condition in this study. A major objective of the control condition is to ensure that the testing measures did not contribute to significant changes irrespective of the intervention. However, in our previous study [2], there were no significant changes in the control condition with the same setup. Therefore, we did not adopt a control condition in this study. Although there is no control group in this study, we believe that the results of this study demonstrate the effectiveness of FR and VFR intervention. The fifth is the intervention time. This study investigated the acute and prolonged effects of FR and VFR for 30 seconds. The previous study [14] showed an increase in sit and reach score after a 5-second FR intervention. Therefore, it is necessary to examine changes in shorter periods than the FR and VFR intervention periods used in this study (i.e., 30 s).

Practical implications

As a pre-exercise warm-up, short-term, i.e., 30-second FR or VFR intervention is recommended when the goal is to increase ROM while maintaining muscle strength. In addition, athletes and coaches need to be careful when performing the intervention because the effect of a 30-second FR/VFR intervention to increase ROM lasts for more than 10 minutes and disappears after 15 minutes. Also, in short-term interventions, vibration function does not necessarily need to be added to FR as a warm-up routine.

CONCLUSIONS

This study compared and examined the acute and prolonged effects of short-term FR and VFR interventions on knee extensors. The results showed that 30 seconds of intervention significantly increased ROM, and the effect lasted up to 10 minutes after the intervention. The results for PPT and tissue hardness were found to change up to at least 15 minutes after the intervention. However, there was no significant effect on CMJ height. No significant differences were found between FR and VFR, suggesting that VFR is not always necessary for short-term ROM increase and muscle stiffness reduction.

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Disclosure of interest

The authors report no conflicts of interest.

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Ethics Approval information

This study was approved by the Ethics Committee of the Niigata University of Health and Welfare, Niigata, Japan (Procedure #18615), and has complied with the requirements of the Declaration of Helsinki.

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Effects of ischaemic post-conditioning on eccentric exercise-induced muscle damage

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ABSTRACT: Exercise-induced muscle damage (EIMD) is a common phenomenon resulting from high-intensity exercise that impairs subsequent performance. Ischaemic post-conditioning (IPOC) is a simple intervention that has been shown to reduce muscle damage after prolonged ischaemia, a condition mechanistically similar to EIMD. The purpose of this study was to determine whether IPOC could alleviate muscle damage after eccentric exercise. Thirty-two young male participants were randomized into either a sham (n = 16) or an IPOC (n = 16) intervention group. Biceps brachii muscle damage was induced by eccentric exercise, with IPOC or sham intervention applied on the dominant arm following exercise (3 cycles of 30 s ischaemia). Visual analogue scale (VAS) pain, arm circumference, muscle thickness, echo-intensity, and microvascular function (using near-infrared spectroscopy) were measured bilaterally at baseline, 24, 48, and 72 hours after eccentric exercise. Biceps curl one repetition maximum (1RM) was also measured. 1RM was higher for the IPOC group at 48 and 72 hours (both $p < 0.05$). On the dominant arm, VAS pain was lower at 72 hours for the IPOC group ($p = 0.039$). Muscle thickness was lower at all post-exercise time points for the IPOC group (all $p < 0.05$). VAS pain, echo-intensity, and arm circumference were elevated on the non-dominant arm in the sham group at 72 hours (all $p < 0.05$). These parameters all returned to the baseline level for the IPOC group at 72 hours (all $p > 0.05$IPOC could attenuate the decrease in strength, and alleviate EIMD with both local and remote effects after high-intensity exercise.

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INTRODUCTION

Exercise-induced muscle damage (EIMD) usually occurs after unaccustomed exercise, especially when there is a prominent eccentric component in the exercise performed [1]. The resultant damage is characterized by both immediate and prolonged symptoms following the exercise [2], and often manifests as delayed onset muscle soreness (DOMS), swelling and stiffness that could last for several days. Although EIMD is considered a mild injury, and is often regarded as a necessary process for post-exercise recovery [3], it may also greatly impair subsequent sports performance [4, 5]. Thus, when performance in subsequent tasks is important (as is the case with athletes or recreationally active individuals), prescribing effective and appropriate recovery techniques is crucial to preserve performance and avoid accumulative injury.

Previously studied recovery methods, including massage, cold water immersion, electrical stimulation and compression garments, have demonstrated effectiveness with the goal of alleviating EIMD and DOMS [6–8]. However, these methods are less feasible for athletes as they require assistance, additional equipment and/or space. Given the challenges associated with the previously mentioned methods, it is necessary to explore effective, affordable, and time-efficient methods of recovery that can be performed by a single individual.

Recent research using multiple short cycles of ischaemia and reperfusion as an intervention has gained considerable focus and is now highly valued in the medical and sports fields [9, 10]. Ischaemic preconditioning (IPC), which was originally applied before heart surgery to reduce cardiac muscle damage after long-term ischaemia [11], has been applied to athletic populations to alleviate fatigue and improve performance [12, 13]. Importantly, recent studies have shown that both acute and repetitive IPC are effective for reducing EIMD and DOMS [14, 15]. Similar in concept, ischaemic post-conditioning (IPOC) is applied acutely after tissue-damaging physiological conditions, and also uses multiple short-term ischaemia-reperfusion cycles. IPOC has been shown to produce protective effects on prolonged ischaemia-induced muscle damage through similar mechanisms to that of IPC [16]. IPOC may also have effects on ischaemic limbs that have not directly received IPOC treatment, otherwise known as remote effects [17]. However, the application of IPOC in sports has been scarce, and whether IPOC could reduce EIMD and DOMS remains to be elucidated.

There are two noteworthy differences between IPOC and IPC. The first is that IPOC is applied in the early stages of recovery rather than

prior to ischaemia, and the second is that it takes much less time to perform (~3 minutes for IPOC vs. 25–35 minutes for IPC) than IPC. The application of IPOC can be achieved using a simple sphygmomanometer and does not include active exercise or additional energy consumption, making it a passive strategy for sport implementation. From a mechanistic perspective, EIMD and ischaemia-reperfusion injury influence ion homeostasis, cell acidosis, and immune responses similarly [14]. For this reason, it is plausible to suggest that IPOC may also alleviate EIMD and DOMS. However, to the best of our knowledge, no human studies have directly examined the influence of IPOC on EIMD and DOMS.

Therefore, the first aim of this study was to evaluate whether IPOC has protective effects against EIMD and DOMS, and whether a remote effect exists. Additionally, because IPOC is known to have vascular protective effects [18], and EIMD is often associated with impaired vascular function [19], the second aim of the study was to determine whether the potential beneficial effects of IPOC against EIMD and DOMS might be mediated through the improvement of vascular function.

MATERIALS AND METHODS

Participants

Thirty-two young healthy males, without any known cardiovascular or musculoskeletal disease and injury, between the ages of 18 and 30, were recruited for this study. The inclusion criteria were 1) not having performed any resistance training routine for the past six months, and 2) not actively engaged in any other sports for the past six months. We targeted this group to avoid the potential influence of gender and training status on the study variables. All participants reported being right-hand dominant. The participants were randomly assigned to either an IPOC intervention group (n = 16) or a sham group (n = 16).

Ethics

This study was approved by an institutional review board (approval No. CCNU-IRB-202212006) and was conducted in accordance with the Declaration of Helsinki. All participants received an explanation of the study aims and methods, and they signed a consent form prior to data collection.

Experimental design and procedure

This study used a parallel group design, with the between-group factor being intervention type (IPOC vs. sham) and the within-group factor being time (baseline, 24, 48 and 72 hours after exercise). The experiment was conducted over four days. Each participant completed the same procedures at the same time of day to remove any circadian influences on exercise effort, vascular function, and subjective feelings. Muscle damage was induced by performing eccentric exercise using a barbell and plates. Immediately after eccentric exercise, the ischaemic intervention or sham control intervention was applied to participants. Before and 24, 48 and 72 hours after eccentric exercise and intervention, EIMD markers including the visual analogue scale (VAS) for pain, muscle thickness, arm circumference, and echo intensity of the biceps brachii were measured bilaterally. Additionally, bilateral microvascular function by near-infrared spectroscopy (NIRS) and elbow flexion 1RM were measured (Figure 1).

Eccentric exercise

The biceps curl exercise was used to induce muscle damage and DOMS bilaterally on the biceps brachii. Resistance was applied using a barbell and plates at 80% of each participant's one repetition maximum (1RM) as the exercise intensity. The exercise consisted of three sets of ten repetitions with a one-minute rest period between sets. Participants were instructed to assume and maintain an erect position, with backs against a wall and elbows at their sides throughout the repetitions. Participants completed a 4 second eccentric (lowering portion) curl through a full range of motion (from 50° to 180°), while two assistants lifted the barbell back to 180° (from 50°) in a coordinated fashion [14]. This was adhered to as a way to ensure that only eccentric repetitions contributed to EIMD.

The ischaemic and sham control interventions were applied to the right biceps brachii muscle of each participant. All tests were performed bilaterally, with the exception of 1RM. All tests were conducted in the same laboratory throughout the experiment, with a laboratory temperature set at 24–25 degrees Celsius. The same tester conducted all the tests throughout the experiment to eliminate inter-tester variance.

IPOC or sham intervention

The post-exercise intervention was applied 30 s after finishing the last set using a tourniquet. The tourniquet was placed on the proximal humerus of the dominant arm. The non-dominant arm was also tested to determine whether a remote effect for IPOC exists. For the IPOC intervention group, the tourniquet was inflated to 220 mmHg for 30 s to achieve ischaemia. The 30 s inflation and deflation was repeated for 3 cycles, which has been previously performed [18]. For the sham group, the pressure was inflated to 20 mmHg for 30 s and the cycle was repeated for 3 cycles. By applying a minimal pressure tourniquet in the sham condition, we hoped to control the potential placebo effect that may influence the experimental results. Since all participants were right-side dominant, the intervention was performed on the right arm of participants. The total intervention time was 3 minutes.

VAS pain

Perceived pain intensity was measured using a 100 mm VAS scale with 0 indicating no pain and 100 indicating the most excruciating pain imaginable. Participants were instructed to draw a vertical mark on this scale representing their current level of pain.

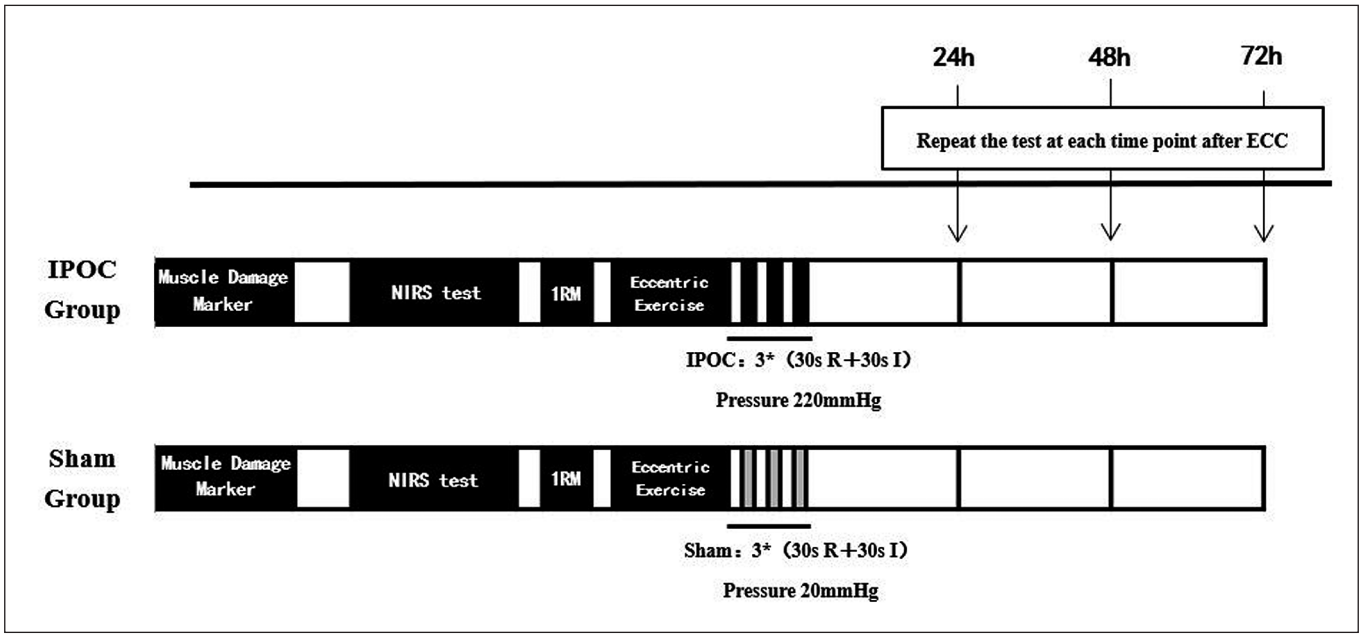


FIG. 1. Experimental design. IPOC, ischemic postconditioning; EIMD, exercise-induced muscle damage; R, tourniquet release; I, tourniquet inflation.

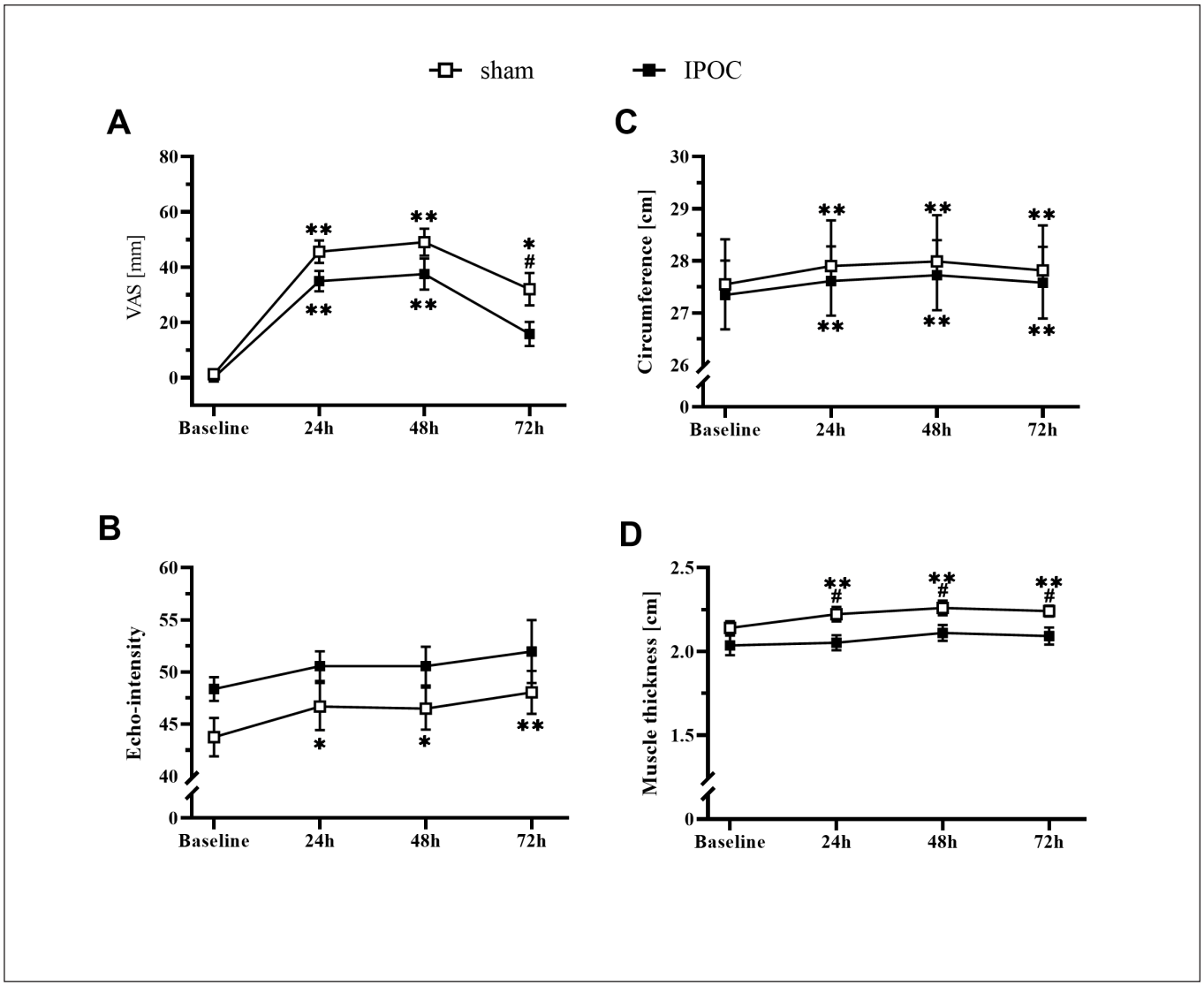


FIG. 2. Results for the dominant arm for visual analog scale (VAS) pain, arm circumference, echo-intensity and muscle thickness at baseline and 24, 48 and 72 hours after eccentric exercise. *p <0.05, **p<0.01 compared to baseline; # significant between groups difference.

Arm circumference

Arm circumference was taken as a surrogate for muscle swelling, and measured using a flexible tape [14]. The assessment was conducted at 60% of the length between the acromial process and the lateral epicondyle (closer to the distal end of the upper arm). The locations of the measurement were marked using a permanent marker to ensure consistent measurement location throughout the experiment. Each participant's arm was measured three times, and the average measure of the three was recorded as arm circumference.

Muscle thickness and echo-intensity

Muscle thickness and echo-intensity were measured using a B-mode ultrasound (Logiq E, GE Healthcare China, Wuhan, China). The musculoskeletal ultrasound preset was used throughout the test while all time gains were set to a neutral position. An 8–12 MHz multi-frequency linear transducer was used. Scan depth was kept at 4 cm. Gains were set to 58 db while transducer frequency was set to 8 MHz. Other settings remained unaltered [20]. The location of the ultrasound measurement was consistent with the previously described location of the assessment of arm circumference (60% of the distance between the acromial process and the lateral epicondyle) and were marked as previously described to ensure consistent measurement. The measurement was taken longitudinally and repeated twice. The obtained images were then processed using ImageJ (Version 1.37, National Institute of Health, Bethesda, MD, USA) software. Muscle thickness was first determined at the centre of the image, and echo-intensity was then determined by manually tracing the biceps brachii area, before calculating the mean pixel intensity.

1RM test

1RM was assessed by testing elbow flexion strength with a barbell weighing 5 kg, and additional weight was added with 0.5 kg accuracy. The participant performed warm-up exercise consisting of 4–5 bilateral elbow flexions with the barbell. For the actual 1RM test, the participant performed elbow flexion from 180° (elbow fully extended) to 50° with the initial weight set to an estimated 80% 1RM. Weight was then adjusted after consulting the participant. Sufficient rest time was given between two consecutive attempts. The maximal weight that the participant was able to lift throughout the 180° to 50° range of motion was considered as 1RM [14]. All 1RM trials were completed within 5 attempts.

Microvascular function

Microvascular function was measured using near infrared spectroscopy (NIRS) [21]. A continuous wavelength NIRS probe (PortaLite, Artinis Medical Systems B.V., Einsteinweg, The Netherlands) was placed on the muscle belly and secured using double-sided tape and pre-wrap. The location of the NIRS probe placement was consistent with the previously described location of the assessment of arm circumference (60% of the distance between the acromial process and the lateral epicondyle). The location of the probe was traced using a permanent marker to ensure a consistent measurement location throughout the experiment. A tourniquet was placed proximally to the NIRS probe and connected to a custom-made rapid cuff inflation device, which can inflate a cuff to a pre-determined pressure within 0.5 s. The participant lay quietly on a bed for at least 5 minutes before the test started. The tourniquet was then rapidly inflated to 220–250 mmHg for 5 minutes, and then rapidly deflated. The NIRS signal was continuously monitored throughout the test and for at least 2 minutes following tourniquet release.

NIRS sensors use spatially resolved spectroscopy method to obtain the tissue saturation index (TSI) for local muscle oxygenation measurement. This reflects the dynamic balance between oxygen delivery and oxygen extraction. The TSI slope of change within the first 10 s of tourniquet release (TSI10) was calculated as an indicator for skeletal muscle microvascular function [22, 23]. The acquired data were processed using a custom-written MATLAB (version 2020b, MathWorks, Natick, MA, USA) script.

Statistics

All data were analysed using SPSS 27.0 software (IBM Corp., Armonk, NY, USA). Results were expressed as means ± standard deviations (mean ± SD). The Shapiro-Wilk method was used to test the distribution of data. Data that followed a normal distribution were analysed using a two-way ANOVA with group (IPOC or sham) representing the between-group factor while time (baseline, 24, 48 and 72 hours after exercise) represented the within-group repeated factor.

Data that violated the assumption of normality were analysed using nonparametric tests (independent samples and related samples) to assess between-group and within-group differences. All significance levels were set at the 0.05 level. Where appropriate, Cohen's d was calculated to determine effect size, with 0.2, 0.5 and 0.8 representing small, medium and large effects [24].

RESULTS

Table 1 summarizes physical characteristics for all participants. No significant between-group differences were detected (all $p > 0.05$). Baseline 1 RM was similar between the IPOC and sham groups ($p = 0.119$). Furthermore, no significant differences in VAS pain, echo-intensity, and arm circumference were observed between the IPOC and sham groups for either arm (all $p > 0.05$). However, participants in the sham group exhibited greater muscle thickness in the non-dominant arm compared to those in the IPOC group ($p = 0.041$). Due to this identified difference, baseline non-dominant arm muscle thickness was used as a covariate in further analysis.

The IPOC group demonstrated better 1RM performances at 48 and 72 hours ($p = 0.032, 0.047$; $d = 0.527, 0.506$) following eccentric exercise when compared to the sham group.

Pain was lower in the dominant arm at 72 hours in the IPOC group compared to the sham group ($p = 0.039$; $d = 0.790$). No differences were observed in echo-intensity for the IPOC group at all post-exercise time points when compared to baseline ($p = 0.318$ for

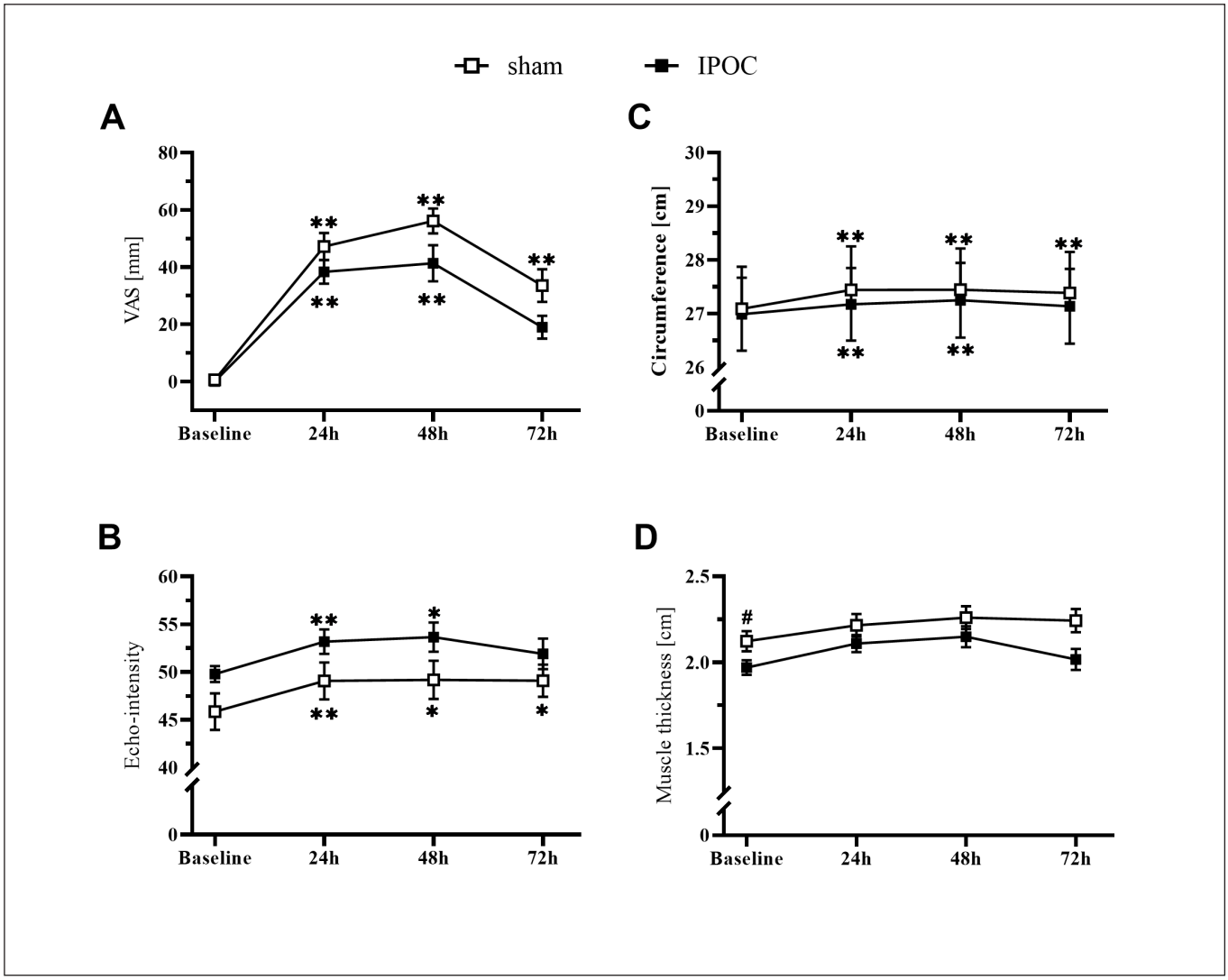


FIG. 3. Results for the non-dominant arm for visual analog scale (VAS) pain, arm circumference, echo-intensity and muscle thickness at baseline and 24, 48 and 72 hours after eccentric exercise. *p <0.05, **p<0.01 compared to baseline; # significant between groups difference.

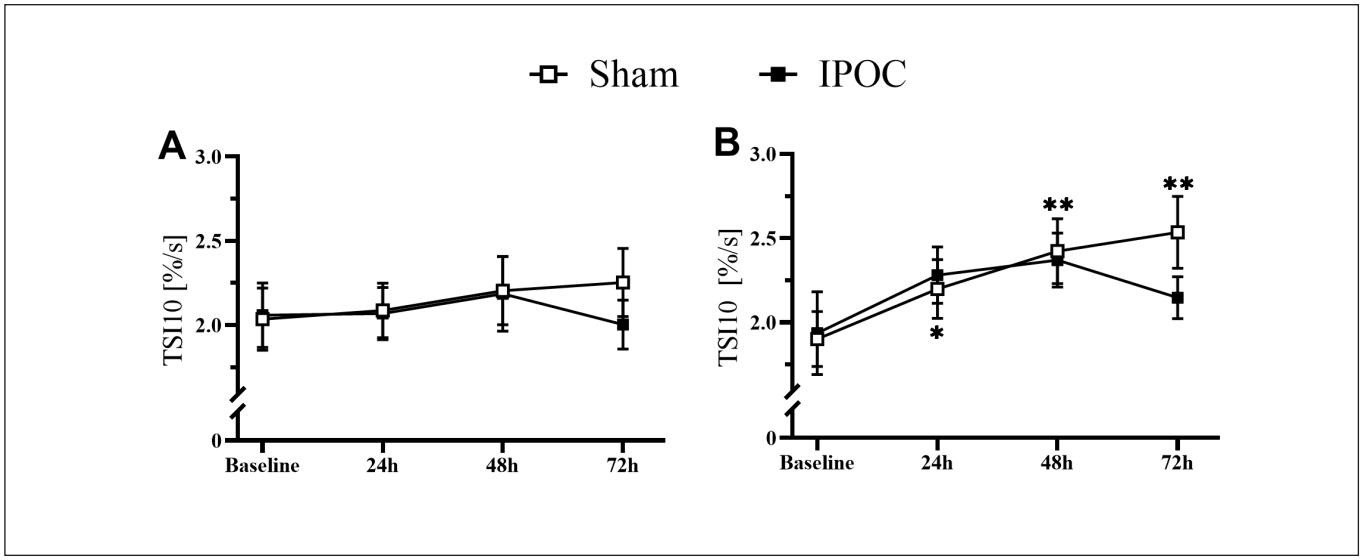


FIG. 4. Microvascular function changes as measured by tissue saturation index (TSI) 10 seconds slope following tourniquet release (TSI10) on the dominant (A) and non-dominant side (B) for both groups. *p <0.05, **p<0.01 compared to baseline.

TABLE 1. Physical characteristics for participants. Sham, group that received sham treatment; IPOC, group that received ischemic postconditioning treatment; ATT, adipose tissue thickness.

	Sham (N = 16)	**IPOC (N = 16)**
Age (years)	21.8 ± 2.8	21.4 ± 2.9
Height (cm)	174.5 ± 4.4	173.9 ± 7.2
Weight (kg)	65.4 ± 8.16	66.3 ± 10.4
Right biceps ATT (cm)	0.44 ± 0.14	0.40 ± 0.12
Left biceps ATT (cm)	0.42 ± 0.15	0.43 ± 0.11

repeated ANOVA) (Figure 2). Echo-intensity was higher compared to baseline at all post-exercise time points for the sham group ($p = 0.018$, 0.044 and < 0.001; $d = 0.356$, 0.352 and 0.549, respectively for 24, 48 and 72 hours). Muscle thickness was greater in the sham group compared to the IPOC group at each post-exercise time point ($p = 0.012$, 0.027 and 0.021; $d = 0.944$, 0.277 and 0.859, respectively for 24, 48 and 72 hours). There was a trend for TSI10 to increase following eccentric exercise for both the IPOC and the sham groups. At 72 hours, the IPOC group experienced a decrease in TSI10, while the sham group remained elevated; however, none of these between-group or within-group measures reached statistical significance (Figure 4A).

Non-dominant arm pain, echo-intensity, and arm circumference were elevated in the sham group at 72 hours compared to baseline ($p = 0.006$, 0.014 and 0.003; $d = 2.096$, 0.461 and 0.100, respectively). These markers for muscle damage all returned to the baseline level for the IPOC group at 72 hours ($p = 0.100$, 0.230 and 0.097; $d = 1.722$, 0.420 and 0.056, respectively; Figure 3). The sham group showed increased values in TSI10 compared to baseline at every post-exercise time point ($p = 0.024$, < 0.001 and 0.004; $d = 0.482$, 0.707 and 0.914, respectively for 24, 48 and 72 hours). No change in TSI10 was detected in the IPOC group compared to baseline ($p = 0.161$, 0.091 and 0.345; $d = 0.346$, 0.508 and 0.222, respectively for 24, 48 and 72 hours; Figure 4B).

DISCUSSION

Muscle damage after high-intensity exercise is a common and well-documented phenomenon [1, 2]. This study adds to the literature by exploring the effectiveness of IPOC in alleviating muscle damage and associated symptoms after eccentric exercise. We found that IPOC can accelerate the recovery of EIMD markers with both local and remote effects, and also mitigate the strength decline following eccentric exercise. These results corroborate findings from previously published studies that examined the effects of IPC on EIMD [14, 15]. While the protective effects of IPOC seen in the current study seem to be smaller than those of IPC, the shorter intervention time and the ability to apply intervention immediately after exercise make IPOC a viable, efficient alternative for reducing DOMS and EIMD.

The mechanisms through which IPOC improves EIMD markers are complex and remain to be fully elucidated. Previous studies investigating muscle damage caused by ischaemia-reperfusion injury suggest that IPOC may act on adenosine and ATP-sensitive potassium channels in the post-processing phase after ischaemia [25, 26]. An increase in adenosine levels and opening of ATP-sensitive potassium channels can promote vasodilation, thus regulating the balance of oxygen and metabolic substrate transport during exercise [27]. Consequently, one can infer that the alleviation of muscle damage following eccentric exercise might also be related to these mechanisms. On the other hand, the increased production of nitric oxide (NO) synthase may also contribute to the protective effects of IPOC against EIMD [28]. NO release as a result of IPOC application activates muscle fibre satellite cells, which then enter the proliferation cycle [29, 30]. NO also has anti-inflammatory functions, which can help alleviate the inflammatory response and reduce the muscle damage response after high-intensity training [31]. Additionally, the remote effect of IPOC has been confirmed in various tissue organs. For instance, remote IPOC of the femoral artery can alleviate brain damage resulting from focal cerebral ischaemia [32], an observation similar to the remote protective effect documented in this study. Previous studies have demonstrated that the mechanisms underlying IPOC improvements in physiological conditions could be related to factors such as neural plasticity and synapse formation, and may be regulated by the hypoxia-inducible factor 1α signal, as well as through the activation of adenosine receptors [33, 34].

We sought to investigate the role of vascular function in explaining the protective effect of IPOC against EIMD. Many studies have shown that microcirculatory function is impaired after eccentric exercise [35, 36]. In this study, the increase in TSI10 after muscle damage caused by eccentric exercise does not necessarily indicate an improvement in microcirculation function. Our results are consistent with previous studies which indicate that microvascular perfusion is increased after EIMD [37, 38]. The mechanism for this change

is not completely understood, but human studies on arterial function may shed some light. Previous studies have shown that, while flow-mediated dilation decreased after eccentric exercise, resting baseline diameter may actually increase [19, 39]. This is likely the result of an increase in the surface area for exchange of substrates that needs to pass through the basement membrane after eccentric exercise [40], causing macrophages, neutrophils, and other repair cytokines to accumulate around the damaged area, leading to an increase in blood flow [38]. In animal models, capillary diameter was increased following eccentric exercise [35]. As a result, the elevation in TSI10 could stem from an increased vessel diameter due to muscle damage, rather than enhanced microvascular reactivity, an inference requiring further study for confirmation. The trend for TSI10 to increase following eccentric exercise is more evident in the non-dominant arm, and IPOC seems to exert a better vascular protective effect in this arm as well. This finding could be related to the present study design. The same eccentric exercise load was applied to both upper limbs of the subjects in our biceps curl paradigm, while the intervention was only done on the dominant arm. It is possible that the non-dominant arm showed more significant vascular damage under the same eccentric exercise load intensity due to weaker strength. Therefore, the effects of IPOC intervention are more prominent than on the dominant arm. However, this inference remains to be confirmed with further studies.

There are practical implications of this study. The results provide scientific evidence to support the use of an affordable and time-efficient means to reduce EIMD and DOMS after high-intensity exercise. Since exercisers can apply IPOC with just a simple sphygmomanometer without the need for additional assistance, it could serve as an ideal recovery agent. Additionally, since IPOC is applied at the early recovery phase rather than before exercise (like IPC), IPOC could be used in situations where EIMD is not anticipated prior to the workout session. However, when compared to the effects of IPC reported in the literature [14], the magnitude of protection seems to be less for IPOC. Important to note, this finding was not directly demonstrated in this study. Therefore, individuals who intend to utilize IPOC and reap its purported benefits should be aware of this potential limitation of the technique.

The limitations of this study must be addressed. Firstly, the study participants were without recent resistance training experience; thus it is uncertain whether IPOC would exert similar protective effects on individuals who frequently engage in resistance training. This is certainly a topic warranting further examination. Second, as gender has previously been shown to influence the EIMD response [41], the results of this study cannot be applied to women without a dedicated study design. Thirdly, while we have proposed potential explanations, the cause of the post-exercise increased TSI10 in the non-dominant arm remains elusive, necessitating future mechanistic research to shed light on the underlying mechanisms. Fourthly, multiple IPOC protocols exist for consideration. The current study employed the three 30-second cycles of IPOC, a protocol commonly mentioned in the literature [18, 42, 43]. Previous studies have investigated the effects of different IPOC protocols on ischaemic-reperfusion injury [44]. Changing the number of cycles or ischaemic duration may alter the protective effects of IPOC against EIMD, a possibility warranting further research.

CONCLUSIONS

In conclusion, IPOC could attenuate the decrease in strength, and alleviate EIMD with both local and remote effects after high-intensity exercise. The protective effect might potentially be associated with improvements in vascular function. Nonetheless, additional research is required to elucidate the underlying mechanisms. Further investigations are necessary to determine whether IPOC confers similar protective effects in females and seasoned exercisers. Furthermore, the influence of different IPOC protocols on EIMD necessitates more in-depth exploration.

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Conflict of interest declaration

The authors declare no conflict of interest.

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The effect of normobaric hypoxia on acute exercise-induced changes in blood sphingoid base-1-phosphates metabolism in cyclists

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ABSTRACT: Extracellular sphingosine-1-phosphate (S1P) emerged as an important regulator of muscle function. We previously found that plasma S1P concentration is elevated in response to acute exercise and training. Interestingly, hypoxia, which is commonly utilized in training programs, induces a similar effect. Therefore, the aim of the current study was to determine the effect of normobaric hypoxia on exercise-induced changes in blood sphingolipid metabolism. Fifteen male competitive cyclists performed a graded cycling exercise until exhaustion (GE) and a simulated 30 km individual time trial (TT) in either normoxic or hypoxic (FiO_2 = 16.5%) conditions. Blood samples were taken before the exercise, following its cessation, and after 30 min of recovery. We found that TT increased dihydrosphingosine-1-phosphate (dhS1P) concentration in plasma (both HDL- and albumin-bound) and blood cells, as well as the rate of dhS1P release from erythrocytes, regardless of oxygen availability. Plasma concentration of S1P was, however, reduced during the recovery phase, and this trend was augmented by hypoxia. On the other hand, GE in normoxia induced a selective increase in HDL-bound S1P. This effect disappeared when the exercise was performed in hypoxia, and it was associated with reduced S1P level in platelets and erythrocytes. We conclude that submaximal exercise elevates total plasma dhS1P concentration via increased availability of dihydrosphingosine resulting in enhanced dhS1P synthesis and release by blood cells. Maximal exercise, on the other hand, induces a selective increase in HDL-bound S1P, which is a consequence of mechanisms not related to blood cells. We also conclude that hypoxia reduces post-exercise plasma S1P concentration.

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INTRODUCTION

Sphingoid base-1-phosphates represent a subgroup of sphingolipids known for their high biological activity. They are produced via phosphorylation of free sphingoid bases by the enzyme sphingosine kinase (SphK). The two best known members of this subgroup are sphingosine-1-phosphate (S1P) and dihydrosphingosine-1-phosphate (dhS1P), which are synthesized from sphingosine and dihydrosphingosine, respectively. Sphingosine is a product of ceramide degradation by ceramidase, whereas dihydrosphingosine is an intermediate in the de novo sphingolipid synthesis pathway. S1P and dhS1P are irreversibly degraded by S1P lyase, or dephosphorylated back to free sphingoid bases by S1P phosphatase or nonspecific lipid phosphate phosphohydrolase [1].

Sphingoid base-1-phosphates can be exported to the extracellular space by facilitator superfamily transporter 2b, spinster homolog 2, and several members of the ATP-binding cassette transporter family [2–4). It is generally accepted that most of their biological effects are induced extracellularly via binding to a family of five specific membrane G-protein coupled receptors (S1PRs) [5].

Sphingoid base-1-phosphates are found in relatively high concentrations in the human plasma, where they are transported mainly by high-density lipoprotein (HDL) and albumin. In addition, large quantities of S1P and dhS1P are stored in red blood cells (RBCs) and platelets, which are characterized by high SphK activity and low expression of enzymes responsible for their degradation. RBCs release sphingoid base-1-phosphates constitutively, and were found to be the major source of circulating S1P and dhS1P. Platelets, on the other hand, secrete them only upon activation. Significant amounts of sphingoid base-1-phosphates are also constitutively released to the circulation by vascular endothelial cells [6].

There is an increasing body of evidence indicating that extracellular S1P is an important regulator of muscle function, likely involved in its adaptation to exercise. It affects the excitation-contraction coupling in a way resulting in an anti-fatigue effect, stimulates muscle regeneration via activation of satellite cells, enhances mitochondrial respiration, and induces angiogenesis [7].

We have previously found that concentration of sphingoid base-1-phosphates in the plasma is elevated in response to both acute exercise and training [8, 9]. Interestingly, hypoxia, which is commonly utilized in athletes' training programs due to its performance benefits, was recently shown to induce a similar effect on circulating S1P and dhS1P at rest [10]. Stimulation of A2B adenosine receptor in RBCs was found to induce activation of SphK and accumulation of S1P, indicating that adenosine may mediate the effect of hypoxia on circulating sphingoid base-1-phosphates metabolism [11]. Accumulation of S1P and dhS1P in response to hypoxia was also observed in human cerebral endothelial cell line, which was a consequence of reduced S1P lyase activity [12].

Altitude training has become a standard training protocol in many sports disciplines, including cycling, to increase exercise capacity at sea level, to acclimatize prior to competitions at altitude, or before ascending to altitude [13]. A sudden exposure to the hypoxic environment, or a longer stay at elevated altitude, induces numerous adaptations which can lead to improved athletes' performance at sea level. These mechanisms are generally attributed to either hematological, cardiovascular, or ventilatory effects of altitude training [14–16]. In addition, acute and chronic exposure to hypoxia induces serval metabolic consequences in the body and combined with exercise presents an enormous challenge for athletes [17]. The process of adaptation to hypoxia is important not only for professional athletes. Due to the growing popularity and availability of high mountain tourism and mountain-related forms of recreation, issues related to the adaptation of the human body to hypoxia are becoming important to a growing number of people.

Therefore, considering the above facts, the aim of the current study was to determine the effect of normobaric hypoxia on acute changes in blood sphingolipid metabolism induced by exercise. Our hypothesis was that the increase in plasma sphingoid base-1-phosphates concentration in response to acute exercise is augmented by a concomitant exposure to hypoxia, which could mediate the beneficial effects of hypoxic training. In the present study we used the exercise protocols, namely graded exercise until exhaustion and 60 min of submaximal exercise, which we previously found to induce acute changes in the metabolism of circulating sphingoid base-1-phosphates [8, 18].

MATERIALS AND METHODS

Experimental design

The investigation conforms to the ethical norms and standards in the Declaration of Helsinki and was approved by the Bioethics Committee at the Medical University of Bialystok (approval no. R-I-002/325/2019). Each subject signed an informed consent before inclusion in the study.

Fifteen highly-trained male competitive cyclists (age 25.4 ± 8.4 years, BMI 21.6 ± 1.8 kg/m^2, body fat content 9.2 ± 2.1%, VO_{2max} 61.4 ± 3.1 mL/kg/min) with an average training experience of 6.3 ± 2 years participated in the study. All subjects possessed a valid medical certificate confirming the absence of contraindications to the practice of competitive sport activity.

The athletes were instructed to maintain their regular diet and supplementation throughout the experiment, and were asked to abstain from caffeine intake during 24 h preceding each test. All participants arrived at the facility one day before the start of each test series and consumed the same meals throughout their stay (40 kcal/kg/d, 50% carbohydrates, 20% proteins, and 30% fats).

The subjects were tested during two sessions, separated by two weeks, in either normoxic or hypoxic (FiO_2 = 16.5%, equivalent to 2,000 m asl) conditions applied in a random order. The athletes were blinded to exercise conditions. The tests were performed in a room equipped with a normobaric hypoxia system (AirZone 25, Air Sport, Międzyzdroje, Poland) allowing to manipulate oxygen concentration in the air. Temperature (19°C), humidity (50%), and CO_2 concentration (700–800 ppm) were controlled and held constant.

In the morning of the first day of each session, two hours after a light breakfast, the subjects performed graded cycling exercise until volitional exhaustion (GE). Volitional exhaustion was defined as the participants' inability to continue exercising, despite strong encouragement by the testing staff. The exercise started with a workload of 120 W, which was then increased by 40 W every 3 min. The total duration of exposure to hypoxia during this test was ~35 min.

On the next day, following 24 h of rest (two hours after a light breakfast), the athletes performed a simulated 30 km individual time trial (TT) in a mountainous terrain. The TT was preceded by a 15 min warm-up, carried out according to the athletes' individual preferences, under oxygen concentration corresponding to the main exercise. The total duration of exposure to hypoxia during this test was ~90 min. Both tests were performed on subjects' personal bicycles connected to an electromagnetic bicycle trainer (Cyclus 2, RBM Elektronik-Automation GmbH, Leipzig, Germany). The athletes were allowed to consume water ad libitum during each exercise. The oxygen saturation of arterial blood (SpO_2) and heart rate were measured using the WristOx2 pulse oximeter (Nonin Medical Inc., Plymouth, MN).

Blood fractionation

During each test day blood samples were taken from the antecubital vein at three time points: before the onset of exercise, within 5 min following its cessation, and after 30 min of recovery in normoxia. Blood was put on ice immediately after sampling into 4 mL EDTA tubes. Erythrocytes, platelets, and platelet-free plasma were isolated by sequential centrifugation. Temperature was maintained at 4°C throughout whole procedure to prevent sphingoid base-1-phosphates

release from erythrocytes [19]. Separated blood cells were resuspended in cold PBS and flash frozen in liquid nitrogen.

HDL were isolated from the platelet-free plasma by sequential flotation ultracentrifugation in NaBr solution in a Sorvall RC M120 GX ultracentrifuge (Thermo Fisher Scientific, Waltham, MA) equipped with a S120-AT2 rotor as described by Havel et al. [20] with some modifications. Briefly, plasma was centrifuged at 120.000 rpm for 85 min at 8°C at a density of 1.006 g/mL to remove very low-density lipoprotein, for 155 min at a density of 1.063 g/mL to remove low-density lipoprotein, and for 260 min at a density of 1.21 g/mL to obtain HDL. After the last centrifugation step lipoprotein-depleted plasma containing albumin was recovered from the bottom of the tube. To avoid oxidation of lipoproteins, 0.3 mM EDTA was present in all preparation steps. Densities of NaBr solutions used for density adjustments were checked with a digital densitometer (Densito 30P, Mettler Toledo, Columbus, OH). All samples were stored at (–80°C) until analysis.

Sphingoid base-1-phosphate release from erythrocytes

Cold erythrocyte concentrate was resuspended in precooled plasma isolated from the same blood sample to obtain hematocrit of ~45%. To determine the baseline sphingoid base-1-phosphate concentration in the medium, an aliquot of the erythrocyte suspension was immediately transferred to a fresh tube and centrifuged at 4°C. The supernatant was then flash frozen in liquid nitrogen. The remaining portion of the erythrocyte suspension was incubated for 20 min at 37°C in 5% CO_2. Samples were then immediately cooled on ice and the medium was separated by centrifugation at 4°C. The rate of S1P and dhS1P release was calculated by subtracting their basal concentration in the medium from the value measured after the incubation.

Sphingolipid analysis

The content of S1P, dhS1P, sphingosine, dihydrosphingosine, ceramide, and dihydroceramide was determined as previously described in detail [21]. Briefly, lipids were extracted from samples in the presence of internal standards (C17-sphingosine and C17-S1P, Avanti Polar Lipids, Alabaster, AL). An aliquot of the lipid extract was transferred to a fresh tube with pre-added N-palmitoyl-D-erythro-sphingosine (C17 base, Avanti Polar Lipids) as an internal standard, and then subjected to alkaline hydrolysis to deacylate ceramide and dihydroceramide to sphingosine and dihydrosphingosine, respectively. The amounts of S1P and dhS1P were determined indirectly after dephosphorylation to sphingosine and dihydrosphingosine, respectively, with the use of alkaline phosphatase. Free sphingosine and dihydrosphingosine, dephosphorylated sphingoid base-1-phosphates, and sphingoid bases released from ceramide and dihydroceramide were then converted to their o-phthalaldehyde derivatives and analyzed using a UPLC system (Nexera, Shimadzu Corp., Kioto, Japan) equipped with a fluorescence detector (RF-20Axs) and a C18 reversed-phase column (Reproshell ODS-1, 2.7 μm, 125 × 3 mm, Dr Maisch, Ammerbuch, Germany). The isocratic eluent composition of acetonitrile:water (84:16, v/v) and a flow rate of 0.4 mL/min were used. Column temperature was maintained at 30°C.

For sphingomyelin analysis, lipids were extracted from erythrocytes and platelets by the method of Folch. Next, sphingomyelin was separated by thin-layer chromatography using the method described by Mahadevappa et al. [22]. The gel bands corresponding to the sphingomyelin standard were scrapped off the plates and transferred into fresh tubes containing pentadecanoic acid as an internal standard. Sphingomyelin fatty acids were then transmethylated in the presence of 14% boron trifluoride in methanol at 100°C for 90 min. The resulting methyl esters of palmitic, stearic, arachidic, behenic and lignoceric acid were analyzed by means of gas-liquid chromatography using a Hewlett-Packard 5890 Series II system equipped with a double flame ionization detector and Agilent (Santa Clara, CA) CP-Sil 88 capillary column (100 m, 0.25 mm i.d.). The content of sphingomyelin is presented as the sum of all analyzed individual fatty acid methyl esters.

Hemoglobin concentration was determined using Drabkin's reagent kit (Sigma, Schnelldorf, Germany). Protein concentration was measured with the BCA protein assay kit (Sigma). Bovine serum albumin (fatty acid free, Sigma) was used as a standard.

Statistical analysis

All data are presented as means ± SD. Statistical comparisons were made by using two-way ANOVA (with one factor being oxygen concentration during exercise, and the other factor being time) followed by Student's t-test for paired samples. Correction for multiple comparisons was performed using Benjamini-Hochberg procedure. $P < 0.05$ was considered statistically significant.

RESULTS

General exercise and subjects characteristics

The average duration of the GE in normoxia and hypoxia was 20.2 ± 1.9 and 18.0 ± 1.9 min, respectively. The maximal work rate during the test performed under normoxic conditions was 5.1 ± 0.3 W/kg of body weight, whereas in hypoxia it amounted to 4.6 ± 0.4 W/kg. The heart rate at the point of volitional exhaustion was 193 ± 8 and 190 ± 9 bpm in normoxia and hypoxia, respectively. The resting SpO_2 under normoxia was 98.0 ± 0.8, and during the final stage of the GE it was reduced to 91.9 ± 3.0%. In hypoxia, the SpO_2 amounted to 93.3 ± 3.4 at rest, and 84.3 ± 5.4% at the point of exhaustion.

The duration of the TT in normoxia was 61.1 ± 5.4 min, whereas in hypoxia it increased to 63.7 ± 3.0 min. The average work rate during this test amounted to 3.6 ± 0.3 and 3.3 ± 0.2 W/kg of body weight under normoxic and hypoxic conditions, respectively. The heart rate was 176 ± 9, 177 ± 8, and 179 ± 10 bpm at 10, 20, and 30 km of the TT in normoxia, respectively. During the test performed under hypoxic conditions the corresponding heart rates were 174 ± 11, 176 ± 13, and 180 ± 12 bpm. In the normoxic TT, the SpO_2 values at rest, and at 10, 20, and 30 km of the test were

TABLE 1. The content of sphingolipids in erythrocytes and platelets sampled before, immediately after, and following 30 min of recovery from a graded cycling exercise until volitional exhaustion (GE) performed either in normoxia or at a simulated altitude of 2,000 m asl (n = 15).

	Normoxic GE			Hypoxic GE		
	Basal	**Post-ex.**	**Recovery**	**Basal**	**Post-ex.**	**Recovery**
			Erythrocytes (pmol/mg Hb)			
Sphingosine	0.29 ± 0.1	0.294 ± 0.123	0.274 ± 0.1	0.307 ± 0.066	0.299 ± 0.061	0.326 ± 0.062
dhSph	0.024 ± 0.008	0.034 ± 0.017	0.033 ± 0.018	0.034 ± 0.012	0.035 ± 0.01	0.039 ± 0.011
S1P	4.29 ± 1.05	4.51 ± 1.23	4.08 ± 1.11	5.38 ± 1.15	4.57 ± 0.97*	5.04 ± 1.27#
dhS1P	1.92 ± 0.66	2.25 ± 0.94	2.23 ± 0.76	2.71 ± 0.95	2.2 ± 0.46	2.85 ± 1.17
Ceramide	35.1 ± 8.5	37.6 ± 10.3	28.1 ± 8.2*#	41.8 ± 10.6	42.9 ± 9.5	43.7 ± 10.7
dhCer	2.02 ± 0.97	2.06 ± 0.82	1.57 ± 0.67*#	2.34 ± 0.71	2.53 ± 1.03	2.63 ± 1.14
Sphingomyelin	1260 ± 140	1224 ± 133	1202 ± 121	1390 ± 172	1295 ± 128*	1429 ± 224#
			Platelets (pmol/mg protein)			
Sphingosine	51.5 ± 34	51.7 ± 42.2	60.2 ± 50	60.5 ± 29	51.9 ± 40.2	51.9 ± 37.2
dhSph	11.5 ± 6.5	11.9 ± 9.2	13.6 ± 10.5	13.7 ± 6.6	12.8 ± 9.8	13.6 ± 8.7
S1P	536 ± 209	493 ± 217	560 ± 223	720 ± 181	565 ± 235*	564 ± 212*
dhS1P	214 ± 96	211 ± 100	254 ± 106#	309 ± 69	272 ± 128	277 ± 112
Ceramide	944 ± 538	784 ± 241	771 ± 256	967 ± 220	890 ± 404	973 ± 381
dhCer	516 ± 274	429 ± 174	444 ± 198	576 ± 165	507 ± 204	543 ± 199
Sphingomyelin	19912 ± 4912	17553 ± 4329	18655 ± 3843	22607 ± 3755	19782 ± 5444	22564 ± 4900

The results are means ± SD. * – $p < 0.05$ vs. the basal value, # – $p < 0.05$ vs. the post-exercise value. S1P – sphingosine-1-phosphate, dhCer – dihydroceramide, dhS1P – dihydrosphingosine-1-phosphate, dhSph – dihydrosphingosine, Hb – hemoglobin.

97.8 ± 2.0, 94.5 ± 2.4, 94.1 ± 1.7 and 93.6 ± 2.3%, respectively. Under hypoxic conditions the corresponding SpO_2 values were 93.3 ± 3.8, 85.6 ± 3.8, 86 ± 4.2 and 86.5 ± 2.7%.

Graded exercise until volitional exhaustion

The total plasma S1P concentration increased in response to GE performed under normoxic conditions, which resulted entirely from the rise in the content of the HDL-bound pool. This effect was, however, transient and disappeared following 30 min of rest (Fig. 1). In addition, compared to the basal values, the content of the HDL-bound dihydrosphingosine was reduced (Fig. 1), whereas that of dhS1P in platelets (Table 1) was increased after 30 min of recovery from the exercise. In erythrocytes statistically significant changes induced by normoxic GE were observed only for ceramide and dihydroceramide levels, which were reduced below the basal values following 30 min of recovery (Table 1). The rate of sphingoid base-1-phosphates release from RBCs was not affected by the exercise (Fig. 3).

In contrast to normoxic conditions, GE performed in hypoxia did not induce statistically significant changes in the concentration of any of the measured sphingolipids in either plasma or its fractions (Fig. 1). On the other hand, in erythrocytes the content of S1P and sphingomyelin decreased at the end of the exercise (Table 1). This effect, however, disappeared following 30 min of rest. A similar reduction in S1P level was observed in thrombocytes, but in contrast to RBCs, it persisted during the recovery period (Table 1). Hypoxic GE did not induce statistically significant changes in the rate of sphingoid base-1-phosphates release from erythrocytes (Fig. 3).

GE, either under normoxic or hypoxic conditions, did not induce statistically significant changes in the concentration of ceramide and dihydroceramide in plasma or its fractions (data not shown).

Simulated 30 km individual time trial

The TT performed under normoxic conditions induced an increase in the total plasma concentration of dihydrosphingosine and dhS1P that persisted during the recovery period. The same pattern of changes in the dhS1P level was observed also in the case of HDL- and albumin-bound fractions of this sphingolipid. On the other hand, the total S1P concentration in the plasma was reduced below the basal level following 30 min of recovery. This effect resulted from a decrease in the level of the albumin-bound S1P, since its content in the HDL-bound fraction did not show a significant change (Fig. 2).

Similarly to plasma, the level of dihydrosphingosine and dhS1P in RBCs and thrombocytes increased in response to normoxic TT, and remained elevated during the recovery period (Table 2). The same pattern of changes was observed for the rate of dhS1P release from erythrocytes (Fig. 3). In addition, compared to the basal level,

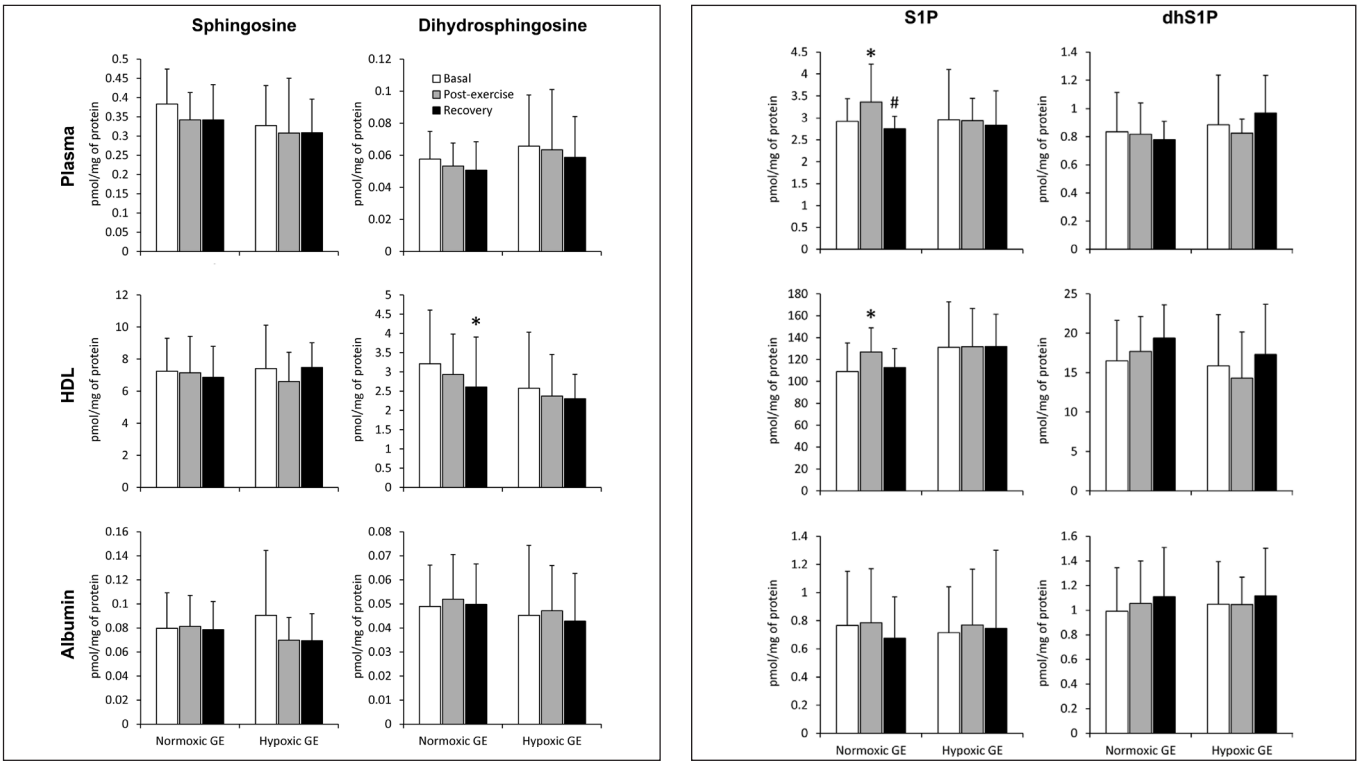


FIG. 1. The content of sphingolipids in the plasma, as well as in its high-density lipoprotein (HDL) and albumin fractions sampled before, immediately after, and following 30 min of recovery from a graded cycling exercise until volitional exhaustion (GE) performed either in normoxia or at a simulated altitude of 2,000m asl (n=15). The results are means ± SD.
Note: * – $p<0.05$ vs. the basal value, # – $p<0.05$ vs. the post-exercise value. HDL – high-density lipoprotein, dhS1P – dihydrosphingosine-1-phosphate, S1P – sphingosine-1-phosphate.

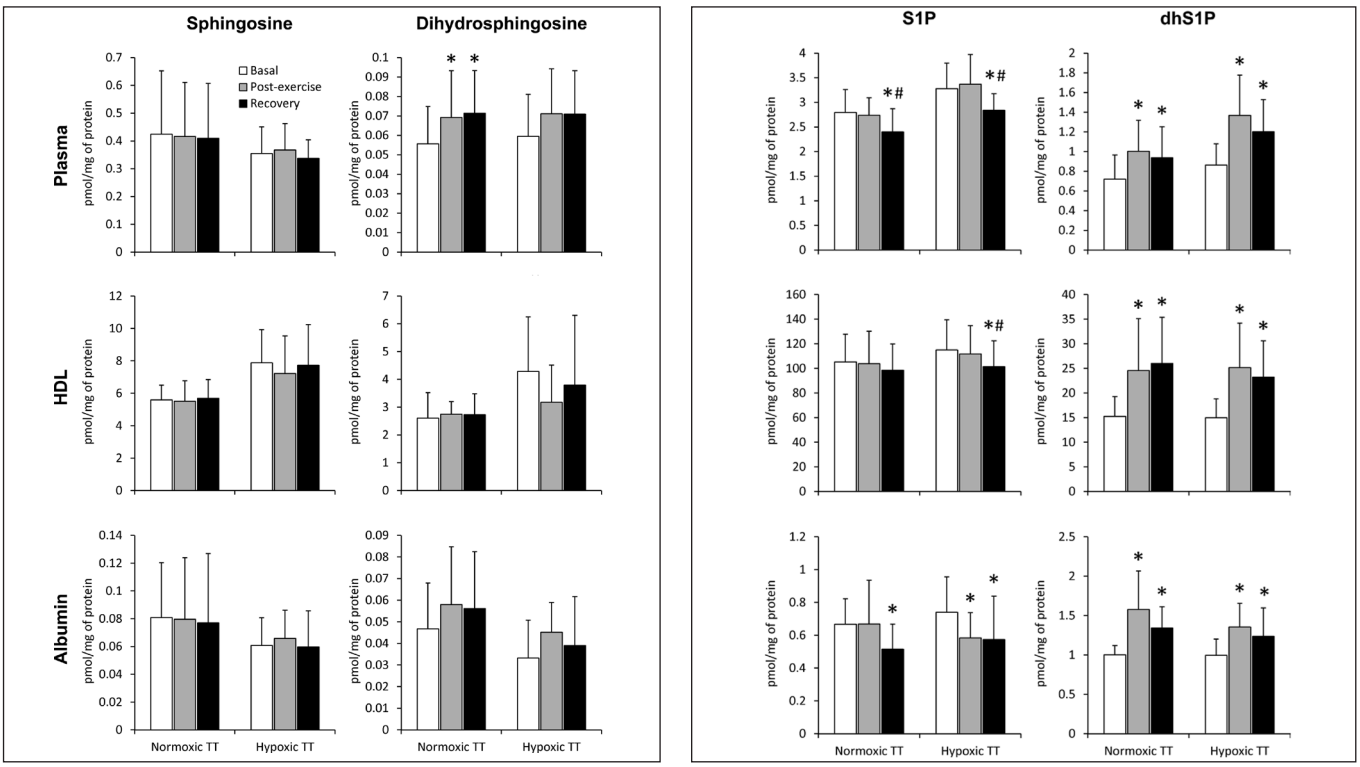


FIG. 2. The content of sphingolipids in the plasma, as well as in its high-density lipoprotein (HDL) and albumin fractions sampled before, immediately after, and following 30 min of recovery from a simulated 30km individual cycling time trial (TT) performed either in normoxia or at a simulated altitude of 2,000m asl (n=15). The results are means ± SD. * – $p<0.05$ vs. the basal value, # – $p<0.05$ vs. the post-exercise value. HDL – high-density lipoprotein, dhS1P – dihydrosphingosine-1-phosphate, S1P – sphingosine-1-phosphate.

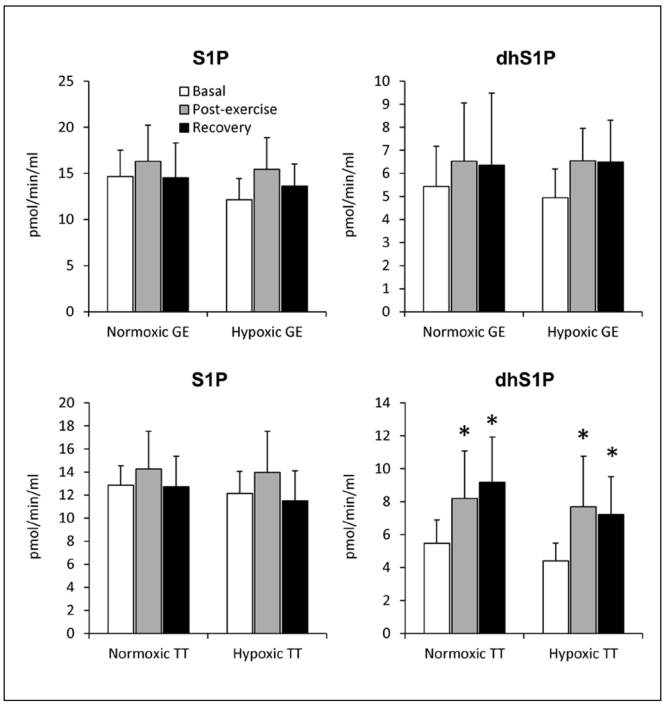


FIG. 3. The rate of sphingosine-1-phosphate (S1P) and dihydrosphingosine-1-phosphate (dhS1P) release from erythrocytes sampled before, immediately after, and following 30 min of recovery from a graded cycling exercise until volitional exhaustion (GE) or a simulated 30km individual cycling time trial (TT) performed either in normoxia or at a simulated altitude of 2,000m asl (n=15). Notes: The results are means ± SD. * – $p<0.05$ vs. the basal value.

the erythrocyte S1P content was reduced, and platelet dihydroceramide was increased following 30 min of rest (Table 2).

The TT performed in hypoxia induced an increase in the concentration of dhS1P in the plasma, as well as in HDL and albumin fractions. The level of this sphingolipid remained elevated during the recovery period. On the other hand, the total plasma concentration of S1P at this time point, as well as its content in both HDL and albumin fractions, was reduced below the basal value. In the case of the albumin-bound S1P, the reduction was observed also immediately after the exercise (Fig. 2).

Similarly to plasma, the level of dhS1P in erythrocytes and platelets increased in response to hypoxic TT, and remained elevated during the recovery period. In RBCs the same pattern of changes was observed also for the content of dihydrosphingosine (Table 2). The rate of dhS1P release from erythrocytes was increased by the TT performed in hypoxia, and remained elevated following 30 min of recovery (Fig. 3).

TT, either under normoxic or hypoxic conditions, did not induce statistically significant changes in the concentration of ceramide and dihydroceramide in plasma or its fractions (data not shown).

DISCUSSION

In line with our previous results [8, 18], we observed that metabolism of circulating S1P and dhS1P was markedly affected by acute exercise in normoxia. However, in the present study these observations were, for the first time, extended by determination of sphingoid

TABLE 2. The content of sphingolipids in erythrocytes and platelets sampled before, immediately after, and following 30 min of recovery from a simulated 30 km individual cycling time trial (TT) performed either in normoxia or at a simulated altitude of 2,000 m asl (n = 15).

	Normoxic TT			Hypoxic TT		
	Basal	**Post-ex.**	**Recovery**	**Basal**	**Post-ex.**	**Recovery**
			Erythrocytes (pmol/mg Hb)			
Sphingosine	0.498 ± 0.126	0.491 ± 0.084	0.553 ± 0.14	0.349 ± 0.068	0.353 ± 0.073	0.353 ± 0.066
dhSph	0.095 ± 0.037	0.134 ± 0.045*	0.151 ± 0.034*#	0.079 ± 0.027	0.127 ± 0.034*	0.121 ± 0.028*
S1P	6.53 ± 1.31	6.29 ± 1.1	5.93 ± 1.22*	5.26 ± 1.02	5.27 ± 1.15	4.91 ± 0.95
dhS1P	5.57 ± 0.89	8 ± 1.23*	7.91 ± 1.98*	3.66 ± 0.9	5.27 ± 1.38*	5.46 ± 1.36*
Ceramide	67.4 ± 15.5	69.6 ± 11.7	66.1 ± 13.9	52.1 ± 12.9	48.4 ± 11.5	49.9 ± 12.9
dhCer	9.73 ± 6.09	10 ± 5.23	9.51 ± 5.41	7.07 ± 4.43	5.98 ± 2.36	6.42 ± 3.9
Sphingomyelin	1267 ± 178	1141 ± 142	1174 ± 176	1349 ± 162	1311 ± 165	1295 ± 169
			Platelets (pmol/mg protein)			
Sphingosine	51.7 ± 19.9	53.2 ± 20.6	60.6 ± 26.5	46.5 ± 15.2	40.8 ± 13.7	41.7 ± 18.1
dhSph	9.64 ± 4.66	13.2 ± 6.5*	15.28 ± 8*#	9.18 ± 3.88	9.74 ± 3.23	10.77 ± 4.32
S1P	651 ± 206	590 ± 153	640 ± 115	653 ± 170	584 ± 108	600 ± 148
dhS1P	231 ± 99	303 ± 98*	341 ± 87*	250 ± 79	300 ± 67*	334 ± 88*#
Ceramide	655 ± 204	600 ± 205	668 ± 305	672 ± 155	597 ± 160	614 ± 180
dhCer	366 ± 143	337 ± 143	404 ± 168#	407 ± 119	360 ± 89	351 ± 122
Sphingomyelin	21663 ± 5434	19328 ± 3566	21622 ± 3358	20251 ± 3319	18845 ± 2733	19371 ± 3827

The results are means ± SD. * – $p < 0.05$ vs. the basal value, # – $p < 0.05$ vs. the post-exercise value. S1P – sphingosine-1-phosphate, dhCer – dihydroceramide, dhS1P – dihydrosphingosine-1-phosphate, dhSph – dihydrosphingosine, Hb – hemoglobin.

base-1-phosphates concentrations in their major plasma pools, and the rate of their release from RBCs. In addition, the effect of hypoxia on exercise-induced changes in sphingolipid metabolism has not been studied so far.

The total plasma S1P concentration was transiently increased following GE in normoxia, a similar response was observed in our previous report where athletes performed identical exercise protocol on a rowing ergometer [8]. Here we found that this effect results entirely from the increased concentration of the HDL-bound S1P, which is considered to be the plasma S1P pool with the highest biological activity [23, 24]. Interestingly, endurance training was also reported to selectively increase the HDL-bound S1P [9]. In line with our previous study [8], the GE-induced elevation in plasma S1P concentration was not associated with changes in its content, or the rate of its release, in blood cells, which argues against the involvement of either RBCs or platelets in this phenomenon.

On the other hand, the TT in both normoxia and hypoxia induced an increase in the total plasma dhS1P concentration, resulting from elevation in HDL- as well as albumin-bound pools. We found that this effect was a consequence of increased availability of dihydrosphingosine in the plasma, which caused accumulation of dhS1P in RBCs and platelets, and ultimately augmented dhS1P release to the circulation. In general, these results are in line with our previous study where athletes performed a similar submaximal exercise (60 min at 65% of VO_{2max}) on a rowing ergometer [8]. However, in the present report the total plasma S1P concentration was reduced during the recovery period due to a decrease in the amount of the albumin-bound pool. A similar tendency was observed by Bergman et al. [25] in the serum of untrained subjects following 2 hours of recovery from a 90-minute exercise on a cycle ergometer. It should be noted that acute changes in plasma volume induced by exercise did not contribute to alterations in sphingolipid concentrations found in our study, since they were expressed on a protein, not volume basis.

We can only speculate on the origin of dihydrosphingosine released to the circulation during the TT. In our previous report, dihydrosphingosine was not found to be released across a leg either at rest or during exercise and recovery period [8]. However, our experiment on rats identified liver as a prominent source of plasma dihydrosphingosine [26]. It is, therefore, possible that the rate of its hepatic release increases during prolonged submaximal exercise.

As already mentioned in the introduction, Sun et al. [10] found that S1P levels in both plasma and RBCs are elevated following ascent to high altitude. However, in the present study, contrary to our initial hypothesis, hypoxia did not augment the exercise-induced increase in circulating sphingoid base-1-phosphate concentration. The response observed for dhS1P following TT was not affected by concomitant exposure to simulated altitude. Surprisingly, the increase in plasma S1P concentration induced by GE was abolished when the exercise was performed under hypoxic conditions. This effect was associated with a reduction in S1P content in erythrocytes and platelets, that was not observed in normoxia, which indicates that blood cells contributed to this phenomenon. In addition, hypoxia enhanced the reduction in plasma S1P concentration observed during the recovery from TT. First of all, the reduction was no longer limited to the albumin-bound pool, since a similar effect was found also for the HDL-bound S1P. Secondly, in hypoxia the decrease in the former pool developed already at the end of the exercise.

The lack of increase in the concentration of circulating S1P under exposure to simulated altitude could be explained by insufficient strength and duration of the hypoxic stimulus. In our study, the athletes spent up to 90 minutes at a simulated altitude of 2,000 m, whereas Sun et al. [10] exposed the subjects to 5,260 m for at least 12 h. Moreover, they showed that in mice exposure to a simulated altitude of 5,260 m lasting more than 6 h was required to increase the concentration of S1P in plasma and erythrocytes. The fact that in our study hypoxia tended to reduce the concentration of circulating S1P may seem puzzling. It should be noted, however, that D'Alessandro et al. [27] found that the time course of changes in erythrocyte S1P content during exposure to high altitude included a brief period of initial reduction preceding the subsequent increase.

Several studies showed that plasma S1P concentration decreases upon strong activation of inflammatory pathways. Such effect was observed following lipopolysaccharide administration to mice, as well as in patients with acute pancreatitis, sepsis, and COVID-19 infection [28–31]. In our recent paper including results obtained during the same experiment, we found that hypoxia markedly augmented oxidative stress and inflammatory response during both GE and TT [32]. It is, therefore, possible that these factors were responsible for the hypoxia-induced reduction in the post-exercise concentration of circulating S1P.

It is widely accepted that live high-train low (LHTL) model of altitude training is more effective in terms of athletic performance improvements than the live high-train high strategy [33]. In addition, an effective variation of the LHTL model has been developed, where athletes live and perform low-intensity training at moderate altitude and only high-intensity training takes place at low altitude [34]. Our observation that hypoxia abolishes the increase in plasma S1P concentration induced by high-intensity exercise supports the notion that in altitude programs this type of training should be performed at low altitude. Furthermore, considering the important role of extracellular S1P in muscle regeneration [7], our results raise a question as to whether hypoxic training should be implemented during recovery from injuries.

Sun et al. [10] found that elevation in the content of intracellular S1P plays a key role in hypoxia-induced increase in 2,3-bisphosphoglycerate (2,3-BPG) level in RBCs. The mechanism underlying this effect involves S1P-induced anchoring of deoxy-Hb to the plasma membrane resulting in enhanced release of membrane-bound glycolytic enzymes to the cytosol. We have recently reported that

concentration of 2,3-BPG in RBCs sampled during the same experiment was reduced following the TT in hypoxia [35]. The effect developed despite the lack of changes in the content of S1P in erythrocytes. Our findings indicate that S1P is not involved in alterations in RBCs 2,3-BPG content induced by acute exercise in hypoxia.

S1P and dhS1P were shown to activate S1PRs with equal potency [36] and, therefore, it is commonly assumed that they induce similar effects. This is, however, not always the case. First of all, these two sphingoid base-1-phosphates are characterized by different distribution between plasma fractions and show distinct transport protein preferences [37]. Secondly, extracellular S1P was reported to have a much shorter half-life than dhS1P [38]. It was also found that dhS1P induces stronger S1PR-mediated reduction in intracellular cAMP concentration and higher magnitude of calcium mobilization compared to S1P [38, 39]. In some studies S1P and dhS1P were even shown to induce opposite effects [40, 41]. Interestingly, in our study plasma S1P concentration was increased only in response to a maximal exercise, whereas that of dhS1P only by prolonged submaximal effort. This observation suggests that S1P and dhS1P may be involved in different aspects of muscle adaptation to exercise. As already mentioned in the introduction, S1P emerged as an important regulator of muscle function [7]. However, the role of dhS1P in muscle biology has not been studied so far and represents an interesting area for further research.

CONCLUSIONS

In summary, we found that simulated 30 km time trial increased dhS1P concentration in plasma (both HDL- and albumin-bound) and blood cells, as well as the rate of dhS1P release from erythrocytes, regardless of oxygen availability. The plasma concentration of S1P was, however, reduced during the recovery phase, and this trend was augmented by hypoxia. On the other hand, graded exercise until exhaustion in normoxia induced a selective increase of HDL-bound S1P in the plasma. This effect disappeared when the exercise was performed under hypoxic conditions, and it was associated with reduced S1P level in blood cells. We conclude that submaximal exercise elevates total plasma dhS1P concentration via increased availability of dihydrosphingosine resulting in enhanced dhS1P synthesis and release by blood cells. Maximal exercise, on the other hand, induces a selective increase in HDL-bound S1P, which is a consequence of mechanisms not related to blood cells. We also conclude that hypoxia does not affect the exercise-induced changes in metabolism of circulating dhS1P, but reduces post-exercise plasma S1P concentration. Therefore, extracellular S1P does not seem to be involved in adaptation to acute exercise in hypoxia.

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Conflict of interest declaration

The authors declare no conflict of interest.

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Association of the *IL1A* and *IL6* polymorphisms with posttraining changes in body mass, composition, and biochemical parameters in Caucasian women

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ABSTRACT: Polymorphisms located in *IL1A* and *IL6* are promising markers of obesity-related traits; however, studies concerning their potential impact on the effectiveness of lifestyle interventions are lacking. Therefore, the aim was to examine the association between the polymorphic sites located in *IL1A* (rs1800587) and *IL6* (rs1800795, rs1800796, and rs1800797) and the body's response to a 12-week training program. We studied the genotype distribution in a group of 168 Caucasian females in whom body mass and composition parameters, the lipid profile, and glucose levels were measured before and after the exercise period. Our results showed that carriers of the *IL1A* rs1800597 CC genotype exhibited a significant decrease in total body water (TBW) in response to training ($p = 0.045$). Additionally, carriers of the *IL6* rs1800797 GG and GA genotypes demonstrated a posttraining decrease in body mass index (BMI) ($p = 0.039$). Haplotype analysis revealed that only rare haplotypes, namely, GGA, CGG and CCG (rs1800795, rs1800796, and rs1800797, respectively), were linked to changes in phenotype, yet assessing individual haplotype effects was not possible. Studies of the interactions between these genes showed that carrying the TC-GG genotype (rs1800587-rs1800795 and rs1800587-rs1800796) may be associated with greater posttraining decreases in fat mass percentage (%FM) and fat-free mass (FM). Carriers of the CC-CG genotype (rs1800587-rs1800795) had significantly greater changes in triglycerides (TGL) over the training period. Our study showed that the *IL1A* and *IL6* genotypes, either individually, in haplotype, or in gene-gene combination, may modify training-induced changes in body mass, composition, glucose levels, and the lipid profile in healthy women.

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Obesity related traits

INTRODUCTION

Cytokines have been defined as "one group of protein cell regulators variously called lymphokines, monokines, interleukins, interferons and chemokines produced by a wide variety of cells in the body that play an important role in many physiologic responses" [1]. They consist of over 200 secreted factors that create a dynamic highly complex and intricate network of elements controlling the immune response [1, 2]. Circulating levels of cytokines reach a characteristic profile in specific metabolic states, e.g., in obesity, which is considered a chronic, low-grade inflammatory disease [3]. Additionally, it has been demonstrated that physical activity and weight change induce considerable variations in the levels of several cytokines [4]. Physiologically and anatomically, fat and muscle tissues are connected and play an important role in metabolism [5]. Taken together, these proteins and the genes encoding them are very promising subjects of research on posttraining changes in body mass, composition, and biochemical parameters.

Interleukin-1 (IL-1) is a superfamily of pleiotropic cytokines with numerous functions in mostly immunity, inflammation, and hemopoiesis. This family contains 11 members, including interleukin-1 alpha (IL-1α), which is a major proinflammatory cytokine [6]. This interleukin is produced in cells including macrophages, monocytes, neutrophils, and hepatocytes. It has been shown to contribute to obesity and insulin resistance. Elevated blood serum IL-1α levels were associated with increased body mass in humans [3, 7]. The *IL1A* gene, similar to other IL-1 family genes, is located on chromosome 2 (2q14.1) and contains 8 exons. Numerous studies have described associations between single nucleotide polymorphisms (SNPs) of *IL1A* and numerous diseases, such as obesity [8, 9], metabolic syndrome [10], cardiovascular disorders [11], asthma [12], and cancer [13]. One of the functional polymorphisms affecting *IL1A* expression is rs1800587 (-889 C > T), which is localized in the promoter region of the gene [14].

Interleukin-6 (IL-6) is a multitarget, pleiotropic cytokine that is a key player in the control of immune responses, inflammation, hematopoiesis, and host defense mechanisms. It is secreted by white adipose tissue, skeletal muscle, and the liver [15]. Elevated IL-6 levels have been correlated with increased body mass, waist circumference, and free fatty acid levels [16], with a decrease in plasma circulating IL-6 after weight loss [17]. The protein is encoded by the *IL6* gene, which is localized on the short arm of chromosome 7 (7p15.3) and contains 5 exons. The promoter region includes numerous SNPs that affect gene transcription in specific types of cells [18]. For the purposes of this article, three polymorphisms, namely, rs1800795 (-174 G > C), rs1800796 (-572 G > C), and rs1800797 (-597 A > G), with the greatest impact on *IL6* gene expression, were selected.

Although the *IL1A* and *IL6* polymorphisms are promising genetic markers of obesity-related traits, data are lacking concerning the potential impact of these SNPs on the effectiveness of lifestyle interventions such as regular physical activity. Thus, the aim of this study was to establish whether the *IL1A* (rs1800587) and *IL6* (rs1800795, rs1800796, and rs1800797) polymorphisms would affect the post-training changes in selected body mass, composition, and biochemical measurements. To examine the possible association between genotypes, haplotypes, and interactions between these two genes and physical outcomes, we evaluated the allele and genotype distribution in a group of young healthy females participating in 12 weeks of aerobic training.

MATERIALS AND METHODS

Ethics statement

The experiment was approved by The Ethics Committee of the Regional Medical Chamber in Szczecin (no. 09/KB/IV/2011 and 01/KB/VI/2017) and was conducted ethically according to the Strengthening the Reporting of Genetic Association studies statement (STREGA) and World Medical Association Declaration of Helsinki. All participants obtained an information sheet about the aim, procedures, risks and benefits of the experiment, and a written consent form. Pseudonymization was applied as the method of data protection.

Participants

One hundred sixty eight Polish Caucasian females (n = 168; age: 21 ± 1 years; body height: 168 ± 2 cm; body mass: 61 ± 2 kg) were chosen for this study. The following inclusion criteria were considered: low level of physical activity self-reported with the use of the Global Physical Activity Questionnaire (according to the World Health Organization in the Polish adaptation); no metabolic, musculoskeletal or neuromuscular diseases; refrained from using medications and supplements for 6 months prior to the start of the experiment; as well as nonsmokers.

Dietary program

The participants who met the inclusion criteria took part in a dietary program and were expected to keep a balanced diet based on their personal dietary plan which was established during a nutritional meeting involving a recommendation of an adequate diet matched with personal energy needs and nutritional status. The medium daily macronutrient ratio was proposed (expressed as a percentage of total calories): 45–65% from carbohydrates, 20–35% from fat (reducing the intake of saturated fats and increasing the intake of unsaturated fats), and 10–20% from protein. The women were also instructed to maintain a daily cholesterol intake of less than 300 mg with a minimum dietary fiber intake of 25 g. The quality and quantity of food and drink were assessed during weekly consultations.

Training Phase

At first, a week-long familiarization period (3 training units, 30 min each, at ~50% of maximum heart rate – HRmax) preceded a proper training. A 12-week (36 training units) experimental training program was divided as follows:

- stage 1: 1–3 weeks (9 training units), 60 min each, at 50–60% of HRmax, tempo 135–140 beats per minute (BPM);
- stage 2: 4–6 weeks (9 training units), 60 min each, at 60–70% of HRmax, tempo 140–152 BPM;
- stage 3: 7–9 weeks (9 training units), 60 min each, at 65–75% of HRmax, tempo 145–158 BPM;
- stage 4: 10–12 weeks (9 training units), 60 min each, at 65–80% of HRmax, tempo 145–160 BPM.

Each training unit included a warm-up (10 min), the main aerobic exercise (43 min), and cool-down period (7 min). A program combining high and low impact styles was described in detail previously [19].

Body mass and composition measurements

Before and after the realization of the 12-week training program, the chosen body mass and composition parameters were assessed with the bioimpedance method using an electronic scale Tanita TBF 300 M (Arlington Heights, Illinois, United States) as previously described [19]. The following parameters were noted:

- total body mass (BM, kg);
- body mass index (BMI, -);
- basal metabolic rate (BMR, kcal);
- fat mass (FM, kg);
- fat free mass (FFM, kg);
- fat mass percentage (%FM, %);
- total body water (TBW, kg).

Biochemical Analyses

Biochemical analyses were performed before the beginning of the training program and repeated after the 36th training unit. Fasting blood samples were obtained from the elbow vein in the morning.

The analyses were performed at once after the blood collection as previously described in detail [19]. The selected parameters received using the Random Access Automatic Biochemical Analyzer for Clinical Chemistry and Turbidimetry A15 (BioSystems S.A., Barcelona, Spain) were:

- total cholesterol (TC, mg/dL);
- triglycerides (TGL, mg/dL);
- high-density lipoprotein (HDL, mg/dL);
- low-density lipoprotein (LDL, mg/dL);
- blood glucose (BG, mg/dL).

Genetic Analyses

Genomic DNA was extracted from the buccal cells using a GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Steinheim, Germany) according to the manufacturer's recommendation. All samples were genotyped in duplicate, using TaqMan® Pre-Designed SNP Genotyping Assays (Applied Biosystems, Waltham, MA, USA) on a C1000 Touch Thermal Cycler (Bio-Rad, Feldkirchen, Germany) instrument according to the manufacturer's procedures. The assays (C___9546481_30, C___1839697_20, C___9546481_30, and C___1839695_20) included primers and fluorescently labeled (FAM and VIC) probes to discriminate the *IL1A* and *IL6* alleles.

Statistical analyses

Statistical analyses were performed in R (https://cran-r.project.org, accessed on 7 October 2021, version 4.1.0). An HWChisq function from Hardy-Weinberg v. 1.7.4 R package was used to test for Hardy–Weinberg equilibrium. No variants violating HW equilibrium were observed. To check the normality of the distribution of the random variable, the Shapiro-Wilk test was used. The influence of the *IL1A* and *IL6* polymorphisms on training response was performed using two-way ANOVA with repeated measures with one between-subject factor (genotype) and one within-subject factor (time: before training vs after training) and FDR adjustment after. To test the significance of the effect of the applied training on the study variables, the Wilcoxon paired rank-order test was used. Haplotype analysis was conducted with haplo.stats v. 1.8.7 R package and haplo.glm regression function. Haplotypes for which the occurrence rate exceeded 1% were included. Percentage change overtraining was used as the dependent variable, while the *IL6* haplotypes were used as the independent variables. Interactions between genes were analyzed in a general linear model (GLM) using the following penetrance models: dominant model (DOM), recessive model (REC) and homozygote-heterozygote model (or heterozygote-homozygote, HOM-HET/HET-HOM). In addition, each of the four possible genotype combinations for the HOM-HET model was analyzed individually (HOM1-HET, HOM2-HET, HET-HOM1, HET-HOM2), where HOM1 is the homozygote for the risk allele in question and HOM2 is the homozygote for the major allele. The level of statistical significance was set at $p < 0.05$.

RESULTS

Individual SNPs analysis

Genotype and allele frequencies of the interleukin genes polymorphism are shown in Table 1–4. All markers variants were tested for Hardy-Weinberg equilibrium (HWE) and no significant deviations from theoretical frequencies were found (*IL1A* rs1800587 p = 0.852; *IL6* rs1800795 p = 0.438; rs1800796 p = 1.0; rs1800797 p = 0.438).

TABLE 1. Training responses by the *IL1A* rs1800587 genotypes.

Para- meter	TT (n = 85)		TC (n = 70)		CC (n = 13)		Genotype	Training	Genotype × training
	Pre	Post	Pre	Post	Pre	Post			
BM (kg)	60.3 ± 8.0	59.5 ± 7.9	61.4 ± 7.0	60.8 ± 6.9	58.1 ± 8.5	57.4 ± 8.3	0.289	**< 0.0001**	0.864
BMI (-)	21.6 ± 2.4	21.4 ± 2.3	21.8 ± 2.4	21.6 ± 2.4	20.1 ± 2.4	19.9 ± 2.5	0.053	**0.0001**	0.926
%FM (%)	23.9 ± 5.5	22.2 ± 5.8	24.1 ± 5.3	23.1 ± 5.5	22.8 ± 5.9	21.2 ± 5.1	0.583	**< 0.0001**	0.120
FM (kg)	14.7 ± 5.2	13.6 ± 5.3	15.1 ± 4.9	14.4 ± 5.0	13.6 ± 5.3	12.6 ± 4.8	0.482	**< 0.0001**	0.273
FFM (kg)	45.4 ± 3.4	46.0 ± 3.5	46.2 ± 2.9	46.6 ± 2.9	44.3 ± 3.2	44.6 ± 3.3	0.095	**0.005**	0.483
TBW (kg)	33.2 ± 2.7	33.7 ± 2.6	33.9 ± 2.1	34.1 ± 2.1	33.2 ± 3.8	32.8 ± 2.6	0.233	0.433	**0.045**
TC (mg/dL)	169 ± 26	169 ± 29	169 ± 24	167 ± 26	184 ± 19	174 ± 21	0.293	0.065	0.238
TGL (mg/dL)	76 ± 24	80 ± 27	83 ± 40	85 ± 33	90 ± 33	101 ± 72	0.094	0.130	0.696
HDL (mg/dL)	64 ± 13	61 ± 13	66 ± 14	62 ± 15	67 ± 12	60 ± 12	0.785	**0.0001**	0.737
LDL (mg/dL)	89 ± 22	92 ± 26	86 ± 22	88 ± 22	100 ± 23	93 ± 15	0.275	0.795	0.282
BG (mg/dL)	79 ± 10	76 ± 11	78 ± 10	76 ± 9	78 ± 13	72 ± 8	0.705	**0.001**	0.388

Mean ± standard deviation; p values (ANOVA) for main effects (genotype and training) and genotype × training interaction; bold p values – statistically significant differences ($p < 0.05$).

TABLE 2. Training responses by the *IL6* rs1800795 genotypes.

Para- meter	GG (n = 55)		CG (n = 78)		CC (n = 35)		Genotype	Training	Genotype × training
	Pre	Post	Pre	Post	Pre	Post			
BM (kg)	60.3 ± 8.4	59.5 ± 8.2	61.3 ± 8.0	60.5 ± 8.0	59.4 ± 5.0	58.9 ± 5.3	0.505	**< 0.0001**	0.488
BMI (-)	21.6 ± 3.0	21.3 ± 2.9	21.8 ± 2.2	21.6 ± 2.1	21.1 ± 1.9	21.0 ± 2.0	0.390	**< 0.0001**	0.109
%FM (%)	23.7 ± 6.1	22.5 ± 6.4	24.2 ± 5.4	22.7 ± 5.4	23.5 ± 4.5	22.2 ± 5.0	0.847	**< 0.0001**	0.831
FM (kg)	14.7 ± 5.8	13.9 ± 5.8	15.2 ± 5.1	14.1 ± 5.1	14.1 ± 3.6	13.2 ± 4.0	0.599	**< 0.0001**	0.562
FFM (kg)	45.6 ± 3.3	45.9 ± 3.2	46.0 ± 3.5	46.5 ± 3.5	45.0 ± 2.3	45.8 ± 2.8	0.383	**< 0.0001**	0.191
TBW (kg)	33.4 ± 2.4	33.7 ± 2.5	33.7 ± 2.5	34.0 ± 2.5	33.0 ± 2.9	33.5 ± 2.0	0.447	**0.002**	0.669
TC (mg/dL)	168 ± 21	166 ± 21	169 ± 27	168 ± 32	174 ± 25	173 ± 25	0.497	0.375	0.974
TGL (mg/dL)	83.1 ± 39.0	81.8 ± 27.7	77.2 ± 30.2	84.0 ± 42.9	82.5 ± 23.8	85.9 ± 24.2	0.824	0.270	0.376
HDL (mg/dL)	65.9 ± 12.3	61.9 ± 13.1	64.7 ± 13.6	59.7 ± 13.7	64.7 ± 14.7	63.1 ± 13.9	0.679	**0.00009**	0.306
LDL (mg/dL)	85.7 ± 18.8	88.0 ± 17.2	89.2 ± 23.5	91.5 ± 26.5	92.5 ± 23.1	92.4 ± 25.3	0.411	0.364	0.814
BG (mg/dL)	79.4 ± 10.9	77.2 ± 9.9	78.7 ± 9.6	75.8 ± 10.4	75.0 ± 8.0	72.3 ± 9.1	**0.034**	**0.002**	0.933

Mean ± standard deviation; p values (ANOVA) for main effects (genotype and training) and genotype × training interaction; bold p values – statistically significant differences ($p < 0.05$).

TABLE 3. Training responses by the *IL6* rs1800796 genotypes.

Para- meter	GG (n = 143)		CC+CG (n = 25)		Genotype	Training	Genotype × training
	Pre	Post	Prew	Post			
BM (kg)	60.3 ± 7.1	59.5 ± 7.0	62.3 ± 10.1	61.7 ± 9.7	0.207	**0.00009**	0.675
BMI (-)	21.5 ± 2.3	21.3 ± 2.3	22.1 ± 3.0	21.9 ± 2.9	0.211	**0.0004**	0.476
%FM (%)	23.8 ± 5.3	22.5 ± 5.4	24.1 ± 6.6	22.8 ± 6.9	0.794	**< 0.0001**	0.993
FM (kg)	14.7 ± 4.7	13.7 ± 4.8	15.6 ± 6.7	14.8 ± 6.7	0.349	**< 0.0001**	0.718
FFM (kg)	45.5 ± 3.0	45.9 ± 3.1	46.7 ± 4.0	47.1 ± 3.8	0.086	**0.001**	0.842
TBW (kg)	33.3 ± 2.5	33.7 ± 2.3	34.2 ± 2.9	34.6 ± 2.7	0.098	**0.017**	0.783
TC (mg/dL)	171 ± 24	169 ± 26	163 ± 26	165 ± 33	0.246	0.915	0.261
TGL (mg/dL)	79.8 ± 28.2	83.2 ± 34.3	82.6 ± 50	86.4 ± 38.9	0.643	0.317	0.955
HDL (mg/dL)	65.2 ± 13.4	61.4 ± 13.1	64.5 ± 13.2	59.3 ± 15.7	0.589	**0.0002**	0.535
LDL (mg/dL)	90.0 ± 22.3	90.8 ± 23.3	81.7 ± 18.9	88.9 ± 25.6	0.251	0.061	0.134
BG (mg/dL)	78.0+9.8	75.6 ± 10.2	78.8 ± 9.9	75.1 ± 9.3	0.929	**0.006**	0.561

Mean ± standard deviation; p values (ANOVA) for main effects (genotype and training) and genotype × training interaction; bold p values – statistically significant differences ($p < 0.05$).

TABLE 4. Training responses by the *IL6* rs1800797 genotypes.

Para- meter	GG (n = 55)		AG (n = 78)		AA (n = 35)		Genotype	Training	Genotype × training
	Pre	Post	Pre	Post	Pre	Post			
BM (kg)	60.8 ± 8.4	60.1 ± 8.1	61.0 ± 8.1	60.0 ± 8.0	59.5 ± 5.1	59.1 ± 5.4	0.717	**< 0.0001**	0.240
BMI (-)	21.6 ± 3.0	21.4 ± 2.9	21.8 ± 2.2	21.5 ± 2.1	21.0 ± 1.9	21.0 ± 2.0	0.397	**< 0.0001**	**0.039**
%FM (%)	24.0 ± 5.9	22.9 ± 6.1	24.0 ± 5.6	22.4 ± 5.5	23.4 ± 4.4	22.1 ± 5.0	0.813	**< 0.0001**	0.506
FM (kg)	15.0 ± 5.6	14.3 ± 5.6	15.0 ± 5.2	13.9 ± 5.2	14.0 ± 3.5	13.2 ± 4.0	0.586	**< 0.0001**	0.249
FFM (kg)	45.3.3 ±	46.0 ± 3.2	45.9 ± 3.4	46.3 ± 3.4	45.2 ± 2.4	46.0 ± 2.8	0.749	**< 0.0001**	0.191
TBW (kg)	33.5 ± 2.4	33.8 ± 2.5	33.6 ± 2.5	33.9 ± 2.5	33.1 ± 3.0	33.7 ± 2.1	0.780	**0.002**	0.635
TC (mg/dL)	169 ± 22	168 ± 26	170 ± 27	167 ± 30	172 ± 24	172 ± 24	0.774	0.516	0.680
TGL (mg/dL)	83.5 ± 38.9	83.1 ± 27.4	77.4 ± 30.2	83.6 ± 42.9	81.3 ± 24.1	84.8 ± 25.0	0.839	0.254	0.536
HDL (mg/dL)	66.0 ± 12.2	61.5 ± 13.0	64.6 ± 13.6	59.8 ± 13.7	64.8 ± 14.7	63.4 ± 13.9	0.680	**< 0.0001**	0.260
LDL (mg/dL)	86.1 ± 19.3	90.0 ± 19.9	89.9 ± 23.7	90.5 ± 25.6	90.5 ± 22.0	91.5 ± 24.7	0.769	0.253	0.627
BG (mg/dL)	79.2 ± 10.8	76.7 ± 10.1	79.0 ± 9.4	75.8 ± 9.9	74.7 ± 8.3	73.0 ± 10.3	0.061	**0.003**	0.780

Mean ± standard deviation; p values (ANOVA) for main effects (genotype and training) and genotype × training interaction; bold p values – statistically significant differences ($p < 0.05$).

To investigate the effect of training and polymorphisms of the *IL1A* and *IL6* genes a two-way ANOVA with repeated measures was conducted (for the *IL6* rs1800796 one rare homozygote and heterozygotes were pooled) (Tables 1–4). We found two significant (but not after FDR adjustment) genotype × training interactions suggesting a modulating effect of interleukin genes variants on training induced changes in the TBW (*IL1A* rs1800587; Table 1) and BMI (*IL6* rs1800797; Table 4). Carriers of the *IL1A* rs1800597 CC genotype exhibited a significant decrease in TBW in response to applied training in comparison to TC and TT genotypes ($p = 0.045$). The posttraining values of BMI were significantly lower compared with pretraining for the *IL6* rs1800797 GG and GA genotypes ($p = 0.039$).

Haplotype analysis

Haplotypes were reconstructed for the *IL6* gene. Six haplotypes were present in 168 women. The three main haplotypes: GGG – 47.5%, CGA – 43.1%, GCG – 7.6% (rs1800795, rs1800796, rs1800797, respectively) with frequencies greater than 1% accounted for 98.2% of the total haplotypes. Examination of haplotype structure and frequencies across these *loci* suggested a high linkage between the rs1800795 and rs1800797, which was confirmed by a linkage disequilibrium (LD) pattern for the 1000G CEU population. Reconstructed haplotypes were tested for association with body composition- and lipid-related phenotypes. We did not find any significant associations for the main haplotypes. However, rare haplotypes comprising of haplotypes GGA, CGG and CCG were associated with smaller changes of several phenotypes under a recessive model (Table 5), yet estimating of individual haplotype effects was not possible.

Gene-gene interactions

Given a strong linkage disequilibrium between the rs1800795 and rs1800797 variants of the *IL6*, pairwise gene-gene interactions were examined across *IL1A* rs1800587, *IL6* rs1800795, and *IL6* rs1800796 variants. The *IL1A* rs1800587 had a significant interaction effect with *IL6* rs1800795 (Table 6) and with *IL6* rs1800796 (Table 7) on a change in %FM, FM, and TGL (only *IL1A* rs1800587 and *IL6* rs1800795) under the homozygote-heterozygote model. A change in %FM and FM over training period tended to be higher in carriers of the compound *IL1A* heterozygous (TC) and *IL6* homozygous (GG) genotype. Although these interactions were nominally significant none of them remained significant after multiple-test correction. In contrast, an effect of the *IL1A* rs1800587 and *IL6* rs1800795 interaction on a change in TGL remained significant after correction. Women carrying the *IL1A* risk allele homozygous CC genotype and *IL6* rs1800795 CG heterozygous genotype had significantly higher change in TGL over training period (coeff. 77.31, SE = 16.45, FDR $p < 0.0001$, Table 6).

DISCUSSION

Numerous studies have confirmed that systematic physical activity, in addition to diet, plays a key role in the regulation of body mass and body composition and, as a consequence, in the prevention of obesity [20]. Although mutual muscle-to-fat signaling factors have been identified, we are only in the early stages of understanding the complex pathways in which they participate. Thus, knowledge of the structure, function, and genetics of cytokines, such as IL-1α and IL-6, is essential for the development of a sensible intervention strategy for obesity prevention by enabling accurate prediction of

TABLE 5. *IL6* haplotype analysis, a linear model with a relative change as the dependent variable, and baseline values (before training) as independent variables.

		CGA	GCG	Rare genotypes (GGA, CGG, CCG)
BM (kg)	OVER	0.17 (0.17), p = 0.339	0.36 (0.34), p = 0.290	0.48 (0.66), p = 0.472
	DOM	0.07 (0.26), p = 0.798	0.22 (0.35), p = 0.543	0.40 (0.66), p = 0.550
	REC	0.32 (0.29), p = 0.280	2.43 (1.52), p = 0.112	**-5.89 (1.84), p = 0.002**
BMI (-)	OVER	0.09 (0.06), p = 0.114	0.16 (0.11), p = 0.131	0.18 (0.21), p = 0.409
	DOM	0.04 (0.08), p = 0.659	0.11 (0.11), p = 0.328	0.14 (0.21), p = 0.532
	REC	0.17 (0.09), p = 0.059	0.77 (0.45), p = 0.093	**-1.99 (0.34), p < 0.0001**
%FM (%)	OVER	-0.11 (0.25), p = 0.656	0.02 (0.48), p = 0.962	0.70 (0.94), p = 0.456
	DOM	-0.30 (0.37), p = 0.430	-0.12 (0.50), p = 0.813	0.65 (0.94), p = 0.486
	REC	-0.03 (0.42), p = 0.953	2.61 (2.20), p = 0.236	**-7.51 (3.13), p = 0.017**
FM (kg)	OVER	-0.10 (0.18), p = 0.583	0.16 (0.35), p = 0.638	0.60 (0.68), p = 0.379
	DOM	-0.30 (0.27), p = 0.271	0.06 (0.36), p = 0.870	0.55 (0.86), p = 0.421
	REC	-0.01 (0.30), p = 0.961	2.06 (1.54), p = 0.184	**-6.63 (1.64), p < 0.0001**
FFM (kg)	OVER	0.26 (0.14), p = 0.062	0.24 (0.27), p = 0.362	-0.26 (0.53), p = 0.625
	DOM	0.32 (0.21), p = 0.135	0.22 (0.28), p = 0.434	-0.30 (0.53), p = 0.576
	REC	0.41 (0.23), p = 0.075	0.10 (1.19), p = 0.931	**5.49 (1.19), p < 0.0001**
TBW (kg)	OVER	0.14 (0.14), p = 0.346	0.29 (0.28), p = 0.300	0.14 (0.55), p = 0.794
	DOM	0.18 (0.22), p = 0.409	0.32 (0.29), p = 0.273	0.13 (0.55), p = 0.808
	REC	0.13 (0.25), p = 0.610	-0.40 (1.31), p = 0.763	0.17 (12.50), p = 0.989
TC (mg/dL)	OVER	1.20 (2.27), p = 0.597	2.15 (5.24), p = 0.682	12.37 (8.44), p = 0.145
	DOM	0.42 (3.40), p = 0.901	1.65 (5.48,) p = 0.763	1.84 (8.45), p = 0.163
	REC	2.16 (3.89), p = 0.579	1.59 (2.03), p = 0.938	**-7.11 (5.028e-03), p < 0.0001**
TGL (mg/dL)	OVER	2.14 (3.36), p = 0.524	0.02 (6.54), p = 0.997	-6.43 (12.70), p = 0.613
	DOM	4.52 (5.06), p = 0.373	2.00 (6.80), p = 0.769	-5.91 (12.69), p = 0.642
	REC	3.73 (4.54), p = 0.412	-32.92 (27.43), p = 0.232	**171 (17), p < 0.0001**
HDL (mg/dL)	OVER	0.76 (1.11), p = 0.498	-1.54 (2.15), p = 0.475	4.64 (4.22), p = 0.273
	DOM	-0.44 (1.68), p = 0.794	-2.12 (2.26), p = 0.349	4.03 (4.22), p = 0.341
	REC	2.99 (1.92), p = 0.121	-2.67 (1.00), p = 0.790	**-31.09 (5.504e-03), p < 0.0001**
LDL (mg/dL)	OVER	0.12 (2.07), p = 0.955	3.75 (4.06), p = 0.357	8.97 (9.37), p = 0.340
	DOM	-0.07 (3.13), p = 0.983	2.88 (4.40), p = 0.513	8.68 (9.82), p = 0.378
	REC	-0.76 (1.87), p = 0.833	18.59 (18.71), p = 0.322	**-6.54 (4.208e-03), p < 0.0001**
BG (mg/dL)	OVER	-1.16 (1.00), p = 0.247	-0.96 (2.13), p = 0.652	5.10 (3.87), p = 0.189
	DOM	-0.96 (1.49), p = 0.520	-0.57 (2.12), p = 0.796	5.32 (3.73), p = 0.156
	REC	-2.37 (1.72), p = 0.171	-1.48 (9.11), p = 0.871	**-20.73 (2.653e-03), p < 0.0001**

Table cells show model coefficients, coefficient's standard error in parentheses and raw p values—statistically significant when $p < 0.05$. OVER- overdominant model; DOM- dominant model; REC – recessive model.

TABLE 6. Analysis of the *IL1A* rs1800587 × *IL6* rs1800795 interaction.

	DOM	REC	HOM-HET	HOM1-HET CC-CG	HOM2-HET TT-CG	HET-HOM1 TC-CC	HET-HOM2 TC-GG
BM (kg)	0.15 (0.26), p = 0.577	0.59 (0.71), p = 0.407	0.06 (0.24), p = 0.807	-0.42 (0.92), p = 0.644	-0.12 (0.28), p = 0.677	0.40 (0.51), p = 0.433	0.16 (0.33), p = 0.626
BMI (-)	0.02 (0.08), p = 0.768	0.23 (0.23), p = 0.308	0.002 (0.08), p = 0.976	-0.16 (0.29), p = 0.592	-0.06 (0.09), p = 0.470	0.14 (0.16), p = 0.407	0.06 (0.11), p = 0.582
%FM (%)	0.23 (0.37), p = 0.537	-0.48 (1.01), p = 0.632	0.67 (0.34), p = 0.051	0.57 (1.30), p = 0.660	-0.19 (0.40), p = 0.636	1.18 (0.72), p = 0.103	**0.93 (0.46), p = 0.045**
FM (kg)	0.05 (0.27), p = 0.861	-0.10 (0.73), p = 0.891	0.41 (0.25), **p = 0.010**	0.21(0.94), p = 0.825	-0.16 (0.29), p = 0.570	0.66 (0.52), p = 0.213	**0.69 (0.33), p = 0.040**
FFM (kg)	-0.12 (0.21), p = 0.579	0.75 (0.57), p = 0.187	-0.13 (0.19), p = 0.497	-0.92 (0.73), p = 0.210	0.10 (0.22), p = 0.644	-0.56 (0.41), p = 0.168	-0.03 (0.26), p = 0.896
TBW (kg)	-0.25 (0.22), p = 0.246	-1.15 (0.58), p = 0.050	-0.19 (0.20), p = 0.348	-0.89 (0.75), p = 0.238	0.01 (0.23), p = 0.961	-0.49 (0.42), p = 0.244	-0.04 (0.27), p = 0.874
TC (mg/dL)	1.16 (3.38), p = 0.732	-10.46 (9.16), p = 0.255	-3.84 (3.09), p = 0.217	8.26 (11.72), p = 0.482	-2.99 (3.58), p = 0.404	-0.53 (6.61), p = 0.936	-3.79 (4.22), p = 0.370
TGL (mg/dL)	8.08 (5.01), p = 0.109	-8.32 (13.63), p = 0.542	1.26 (4.62), p = 0.786	**77.31 (16.45), p < 0.0001**	-3.71 (5.36), p = 0.491	5.34 (9.87), p = 0.589	-4.78 (6.31), p = 0.450
HDL (mg/dL)	-0.50 (1.68), p = 0.765	-3.11 (4.54), p = 0.493	0.46 (1.55), p = 0.767	-2.55 (5.82), p = 0.661	-1.27 (1.78), p = 0.477	5.24 (3.26), p = 0.109	0.79 (2.10), p = 0.706
LDL (mg/dL)	0.35 (3.12), p = 0.911	-4.16 (8.47), p = 0.624	-4.16 (2.85), p = 0.146	-3.49 (10.84), p = 0.748	-0.95 (3.31), p = 0.774	-5.46 (6.04), p = 0.368	-3.70 (3.91), p = 0.345
BG (mg/dL)	-0.37 (1.50), p = 0.803	-6.06 (4.00), p = 0.132	-0.68 (1.37), p = 0.619	-0.64 (5.17), p = 0.901	-0.82 (1.58), p = 0.607	-1.27 (2.92), p = 0.665	0.47 (1.86), p = 0.803

IL1A (rs1800587) – G risk allele, *IL6* (rs1800795) – C risk allele. Table cells show model coefficients, coefficient's standard error in parentheses and raw p values—statistically significant when $p < 0.05$. DOM – dominant model: at least one risk allele at both *loci*; REC – recessive model: homozygotes for the risk allele at both *loci*; HOM-HET – homozygote-heterozygote model including four possible genotype combinations, where HOM1 is the homozygote for the risk allele and HOM2 is the homozygote for the major allele.

TABLE 7. Analysis of the *IL1A* rs1800587 × *IL6* rs1800796 interaction.

	DOM	REC	HOM-HET	HOM1-HET CC-CG	HOM2-HET TT-CG	HET-HOM1 TC-CC	HET-HOM2 TC-GG
BM (kg)	0.33 (0.49), p = 0.497	Na	0.18 (0.24), p = 0.464	Na	0.09 (0.44), p = 0.829	Na	0.10 (0.25), p = 0.693
BMI (-)	0.14 (0.16), p = 0.364		0.04 (0.08), p = 0.630	Na	0.06 (0.14), p = 0.671	Na	0.002 (0.08), p = 0.979
%FM (%)	0.04 (0.70), p = 0.950	Na	**0.77 (0.34), p = 0.025**	Na	-0.01 (0.62), p = 0.982	Na	**0.93 (0.46), p = 0.045**
FM (kg)	0.05 (0.51), p = 0.924	Na	**0.53 (0.25), p = 0.034**	Na	0.24 (0.45), p = 0.594	Na	0.44 (0.26), p = 0.092
FFM (kg)	0.31 (0.39), p = 0.431	Na	-0.23 (0.20), p = 0.233	Na	-0.04 (0.35), p = 0.914	Na	-0.24 (0.20), p = 0.243
TBW (kg)	0.39 (0.40), p = 0.339	Na	-0.11 (0.20), p = 0.584	Na	0.12 (0.36), p = 0.742	Na	-0.15 (0.21), p = 0.480
TC (mg/dL)	-0.15 (6.31), p = 0.081	Na	1.16 (3.12), p = 0.710	Na	5.04 (5.61), p = 0.370	Na	-0.45 (3.25), p = 0.889
TGL (mg/dL)	0.30 (9.40), p = 0.975	Na	-0.52 (4.69), p = 0.912	Na	2.43 (8.39), p = 0.773	Na	-0.31 (4.86), p = 0.949
HDL (mg/dL)	-3.79 (3.10), p = 0.224	Na	1.49 (1.55), p = 0.339	Na	0.24 (2.79), p = 0.931	Na	1.61 (1.61), p = 0.318
LDL (mg/dL)	2.97 (5.85), p = 0.613	Na	-0.09 (2.89), p = 0.976	Na	3.77 (5.18), p = 0.468	Na	-1.84 (3.00), p = 0.540
BG (mg/dL)	-0.47 (2.78), p = 0.866	Na	0.44 (1.38), p = 0.749	Na	-1.08 (2.47), p = 0.663	Na	0.88 (1.44), p = 0.541

IL1A – G risk allele, IL6 (rs1800795) – C risk allele. Table cells show model coefficients, coefficient's standard error in parentheses and raw p values—statistically significant when $p < 0.05$. DOM – dominant model: at least one risk allele at both *loci*; REC – recessive model: homozygotes for the risk allele at both *loci*; HOM-HET – homozygote-heterozygote model including four possible genotype combinations, where HOM1 is the homozygote for the risk allele and HOM2 is the homozygote for the major allele.

individual training results involving weight loss and improved health [21, 22]. This study aimed to examine the impact of the four polymorphic sites located in the *IL1A* and *IL6* genes on the posttraining changes in the body mass and composition parameters, lipid profile, and glucose levels in Caucasian women taking part in a 12-week training program. To our knowledge, this is the first novel study to evaluate the association between genotypes, haplotypes, gene–gene interactions and physical outcomes. Therefore, our results cannot be directly compared to previous studies.

When tested individually, the statistical analyses showed two significant genotype × training interactions (for TBW and BMI). First, the carriers of the *IL1A* rs1800587 CC genotype exhibited a decrease in TBW in response to applied training. This finding suggests that this genotype may be unfavorable for achieving the desired training-induced TBW changes. This genotype may cause faster water loss in women and lead to disorders in the body's homeostasis. Previously, this polymorphism was associated with the levels of IL-1α expression; specifically, the TT genotype significantly increased the transcriptional activity of *IL1A* with respect to the CC genotype. Thus, an increase of the mRNA and protein levels was observed in the plasma of carriers of the TT genotype [14]. Additionally, several studies have reported that chronic inflammatory diseases, including obesity, rheumatoid arthritis, Alzheimer's disease, and periodontitis, are associated with the T allele [8, 23–25]. Thus, it was expected that the T allele carriers might show a higher BMI. However, Um et al. showed a reverse association between the T allele and a lower BMI value in obese healthy women [8]. Unfortunately, studies are lacking that describe the effect of the *IL1A* rs1800587 polymorphism on the posttraining changes in TBW and other body composition parameters, so further research is needed to confirm our observations.

Second, the carriers of the *IL6* rs1800797 GG and GA genotypes demonstrated a significant decrease in BMI in response to regular exercise. This result implies that these genotypes may be considered favorable factors for achieving the planned training-induced BMI change. Thus, *IL6* rs1800797 AA homozygotes should maintain more restrictive dietary habits and undergo more intense exercise than carriers of the G allele to achieve similar postworkout effects. The number of previous studies is small, and they have shown various results among different populations. Previously, the rs1800797 GG genotype was associated with higher levels of IL-6 expression. Some researchers have revealed the contribution of the *IL6* rs1800797 G allele to the development of type 2 diabetes and metabolic syndrome, suggesting a potential protective effect of the A allele against obesity and inflammation [26, 27]. On the other hand, Boeta-Lopez et al. did not report any significant connections between the rs1800797 G allele and obesity (categorized by BMI and waist circumference) and metabolic traits [28]. Genotyping in another study also showed a lack of significant association of rs1800797 with type 2 diabetes [29]. Our experiment only showed an association between GG and GA genotypes and a higher decrease in BMI in response to training, suggesting that these genotypes are favorable in the context of weight loss. This finding is inconsistent with some mentioned studies, which indicated that the G allele is the risk allele [26, 27]. However, interventional studies that include training programs are lacking, so we are unable to compare the results. More experimental studies are required to confirm the protective or harmful role of the *IL6* rs1800797 genotypes.

In addition, we identified a statistically significant association between the genotype and the glucose level; specifically, the carriers of the *IL6* rs1800795 GG and CG genotypes had an elevated glucose level during the entire study period compared with the CC genotype. Our results suggest that carrying these genotypes may have adverse effects on carbohydrate metabolism, and consequently, this polymorphism should be considered a genetic marker to determine predisposition to the development of hyperglycemia. This finding is in accordance with some previous studies, which found that the *IL6* rs1800795 G allele, generally associated with higher levels of IL-6 expression, is linked to a high risk of type 2 diabetes in many populations [26, 29, 30]. However, opposite results have been obtained in studies conducted on obese and type 2 diabetic participants, as well as some healthy individuals. In particular, the rs1800795 C allele has been associated with a high risk of obesity, cardiovascular disorders, and developing obesity-related metabolic disorders such as insulin resistance [31–33].

These inconsistent results related to individual SNP analysis led us to study the haplotypes of all *IL6* promoter variants and *IL6*-*IL1A* interactions. Only simultaneous analysis of multiple SNPs can provide additional unique information about the relationships between gene variants and observed phenotypic traits, as well as insight into the dependency among genetic markers [34], which was confirmed in this study. When the obtained results were included in the haplotype analysis performed for three polymorphic sites located in the *IL6* gene, no significant associations were revealed between the GGG, CGA, and GCG main haplotypes (rs1800795, rs1800796, and rs1800797, respectively) and physical outcomes. However, rare haplotypes such as GGA, CGG and CCG were linked to changes in several phenotypes, yet assessing individual haplotype effects was not possible. While these results did not better define the influence of the *IL6* promoter region on posttraining changes, they pave the way for future research. Previously, a few studies including haplotype analysis have revealed that the variability in the *IL6* gene is significantly associated with obesity, type 2 diabetes, and metabolic syndrome [33, 35]. Ramirez-Lopez et al. have shown significant associations between haplotype GCG/GCG (rs1800797, rs1800796, and rs1800795, respectively) and hyperglycemia; between GCG/GCG and high high-sensitivity C-reactive protein; between AGC/AGC and obesity; and between GGG/GCG and low HDL levels [33]. In an obesity case-control study comparing haplotypes of the *IL6* promoter variants (rs1800797, rs1800796, and rs1800795, respectively), Hamid et al. found the AGC haplotype to be more frequent in the lean group than among obese participants, whereas the GGG haplotype was more frequent among obese subjects. The

researchers also suggested that the rare haplotype AGC/GCG is associated with type 2 diabetes and that the common haplotype AGC/GGG is associated with metabolic syndrome [35]. The mechanisms by which *IL6* promoter SNPs might cause an increased risk of obesity are still unknown, but it might be because of an effect on insulin resistance, suggesting a complicated role of IL-6 in body composition and glucose metabolism [36].

Previous results have revealed that IL-1α stimulates the expression of *IL6* gene transcripts and peptide production, which is regulated by cyclic adenosine monophosphate (cAMP) [37]. Thus, we performed an analysis of the interactions between the two genes encoding these interleukins, which showed that carrying TC-GG genotypes in both *IL1A* rs1800587 and *IL6* rs1800795 as well as *IL1A* rs1800587 and *IL6* rs1800796 may be associated with greater decreases in %FM and FM in response to applied training. This combination of genotypes may be considered beneficial, and carriers of these genotypes might more easily achieve the expected posttraining effects in terms of body composition changes. Additionally, the combination of the CC-CG genotypes in *IL1A* rs1800587 and *IL6* rs1800795 might be unfavorable for achieving the desired training-induced changes in lipidogram (TGL) parameters. In other study conducted on the Caucasian population, Maculewicz et al. (2022) analyzed the association between 5 polymorphisms of the interleukin 10 gene (*IL10*) and body composition parameters in 131 physically active participants. They revealed an association between BMI and CCGTA haplotype (rs1518111, rs1878672, rs3024496, rs3024498, and rs3024505, respectively) [38]. This finding indicates that there is a need to conduct additional studies involving other interleukin coding genes.

Although studies have confirmed that interleukin levels were determined according to weight change induced by lifestyle interventions [39], assessing individual variation in these marker levels is still very imprecise. Recently, it has been shown that people with the same genotype respond similarly to exercise and diet, indicating that some genetic variants play an important role in the determination of individual differences in response to lifestyle change [21, 22]. However, very few investigators have studied the biological variation of a particular analyte over a period of time, which makes it difficult to compare results. In addition, differences in the chosen methodology, inclusion criteria of participants, and interactions with the ethnic background and other genetic or environmental factors that influence the study population are reasons to explain why the search for genetic markers of the functional response of the human body to physical activities is very complicated and why the obtained results may be contradictory [22].

CONCLUSIONS

The results of our experiment suggest that *IL1A* and *IL6* genotypes, either individually, in haplotype, or in gene-gene combination, may modify training-induced body mass, body composition, glucose level, and lipid profile changes in Caucasian females. The most important findings of our study are as follows: 1) carrying the *IL1A* rs1800597 CC genotype may be unfavorable for achieving the desired training-induced TBW changes; 2) carrying the *IL6* rs1800797 GG and GA genotypes may be favorable for achieving the planned training-induced BMI changes; 3) carrying the *IL6* rs1800795 GG and CG genotypes may have adverse effects on glucose metabolism; 4) only rare haplotypes (GGA, CGG and CCG) were linked to changes in several phenotypes, yet assessing individual haplotype effects was not possible; 5) carrying the TC-GG genotypes in both *IL1A* rs1800587 and *IL6* rs1800795 as well as *IL1A* rs1800587 and *IL6* rs1800796 may be associated with greater decreases in %FM and FM in response to applied training and may be considered beneficial; and 6) the combination of the CC-CG genotypes in *IL1A* rs1800587 and *IL6* rs1800795 may be unfavorable for achieving the desired training-induced changes in TGL levels.

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Conflict of interest declaration

The authors declare no conflict of interest.

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Fasting reduces satiety and increases hunger but does not affect the performance in resistance training

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ABSTRACT: Intermittent fasting (IF) has been suggested to reduce body fat percentage and improve non-communicable chronic diseases. However, little is known about resistance training (RT) and the subjective perception of hunger under fasted conditions. This study aimed to examine the effects of overnight fasting (12 h or 16 h fasting) on the maximum voluntary isometric contraction (MVIC) and countermovement jump (CMJ) performance in resistance-trained young male adults. In RT sessions, the maximum number of repetitions (MNR) and the total volume load (TVL) were evaluated in the back squat and leg press 45°. The volunteers performed all tests and the RT session in 3 different conditions: fed state, 12 and 16 hours of IF. The subjective perception of hunger was applied through an adapted visual analogue scale (adVAS). The results showed that strength and power variables did not change significantly: MVIC ($p = 0.960$), CMJ ($p = 0.986$), MNR back squat ($p = 0.856$), MNR leg press 45° ($p = 0.998$), TVL ($p = 0.954$). However, hunger was significantly greater after the 16-hour fasting ($p = 0.001$) compared to 12 hours of fasting and the fed state. Also, the desire to eat was greater after 16 hours ($p = 0.001$) compared to 12 hours of fasting and the fed state. This study indicates that IF for 12 or 16 hours does not significantly impair strength and power, but the longer the fasting duration, the greater are the hunger and desire to eat.

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INTRODUCTION

Intermittent fasting (IF) is a recurrent eating pattern with energy restriction interspersed with periods of *ad libitum* food intake [1]. Some authors have suggested that IF induces metabolic adaptations characterised by the mobilisation and utilisation of stored fat as an energy source (lipolysis), which have been associated with favourable effects on insulin sensitivity, reducing fat mass and improving some markers of non-communicable chronic diseases [2]. The 16/8 IF protocol (16 consecutive hours of fasting and 8 hours of eating) may be the most effective and sustainable because it is the caloric restriction most closely to the human dietary pattern [2, 3]. The 16/8 IF became popular as the basis for the "Leangains Diet", which associates this nutritional strategy with resistance training (RT) [4]. RT is performed to preserve and mitigate losses of muscle mass caused by caloric restriction [5, 2], impairment of muscle strength and reduced training performance proportionally to the duration of fasting [5]. Such deleterious effects can occur due to the reduction in muscle glycogen stores and glycaemia, with an increase in the rating of perceived effort (RPE) [6, 2]. However, the few studies that associated resistance training and 16/8 IF [3, 7, 8] claim that body composition, especially lean mass and strength performance, is not negatively affected by IF 16/8. However, these studies performed the strength test sessions and the RT in the fed state. In contrast, Naharudin et al. [9] discovered that fasting reduces the maximum number of repetitions (MNR) acutely performed in the back squat during the fasting state (10–13 h overnight) in trained individuals who skipped breakfast. Some studies identified bad mood and reduction in general disposition, motivation and exercised enjoyment in

List of abbreviations

adVAS	Adapted visual analogue scale
CMJ	Countermovement jump
FS	Feed state
IF	Intermittent fasting
IF-12	Fasting 12 hours
IF-16	Fasting 16 hours
MNR	Maximum number of repetitions
MVIC	Maximum voluntary isometric contraction
ND	Normal diet
RPE	Rating of perceived effort
s-RPE	Session rating of perceived effort
RT	Resistance training
TVL	Total volume load
PAR-Q	Physical Activity Readiness Questionnaire
1RM	One repetition maximum test

training in fasting conditions [7, 10, 11]. Diminished levels of blood glucose and increased cortisol levels may be considered the main factors contributing to the negative affective state and the decrease in overall mood and pleasure experienced during physical exercise while fasting [7, 10, 11]. Such factors could limit adherence to IF in the long term. However, in an investigation lasting 4 weeks, Stratton et al. [7] did not identify a significant impact of IF on subjective perceptions of fullness, desire to eat, or hunger after 8 hours of IF before the RT session. Furthermore, no other studies were found that investigated subjective perceptions of hunger and fullness during fasting in RT.

Therefore, the acute effect of fasting on strength performance, that is, on the ability to perform training sessions during the fasting period, has not yet been evaluated, even though a reduction in physical performance is expected to be proportional to the length of fasting time [5]. However, no studies have compared performance, hunger and fullness perceptions in RT at different fasting times, which can also interfere with diet and training plan adherence.

Thus, the objective of the present study was to verify the performance of trained individuals and the subjective perceptions of hunger and satiety, in the fasting state, after 12 and 16 hours. The hypotheses were that acute fasting would negatively influence performance and increase subjective perceptions of hunger and satiety proportionally to the fasting time.

MATERIALS AND METHODS

Participants

The volunteers were 16 men (24.9 ± 3.12 years; 79.2 ± 6.42 kg; 1.76 ± 0.05 m; fat mass percentage of 18.4 ± 4.87%). The total RT experience of the volunteers was 2.9 ± 0.6 years, and the mean relative maximum lifted mass (1RM) in the squat was 2.14 ± 0.47 kg. Furthermore, the volunteers were experienced in performing the back squat and leg press 45° exercises with good technique and concentric failure. Therefore, they are classified as trained [12].

This study included only eutrophic individuals who were not undergoing any nutritional intervention and had not consumed any nutritional ergogenic resources for at least 30 days prior to the study. Additionally, participants were required to have reported no joint or muscle injury within six months of the study and to have answered "no" to all questions on the Physical Activity Readiness Questionnaire (PAR-Q), a questionnaire that assesses the safety and risks of physical activity.

The sample size was determined based on the effect size of fasting on strength performance, as reported by Naharudin et al. [9]. Consequently, we recruited the exact number of participants required for the calculation of the sample size, considering an effect size of 0.98, power of 0.95, and alpha level of 0.05. The search for volunteers was carried out by publicising the study and its inclusion criteria in the institution's regional training centres, social networks and research team. Upon reaching the expected number, the volunteer's search was interrupted. There was no exclusion of participants before, during or after the study. All volunteers signed the Participant Consent Form agreeing to participate in the study. This study was submitted to the Ethics and Research Committee of the Federal University of Minas Gerais (CAAE: 47069421.0.0000.5149). The approval protocol number is 4.389.077.

Procedures

In this study, the acute assessment of single and non-consecutive days of IF 16/8 and IF 12/12, classified as time-restricted feeding, was performed. Thus, the IF protocols consisted of a window of 8 or 12 hours of ad libitum feeding interspersed with a period of 16 or 12 hours without ingesting food, caffeine or stimulants, respectively [1, 7].

The volunteers were trained to register all foods consumed and their portions in the FT app through descriptions and photographs. On the day of the experimental testing and training sessions, the reports were reviewed to ensure accordance with the fasting protocol. All sessions were performed in the morning. The schedules and the last meal respected the volunteers' preferences.

The fasting experimental conditions were:

- Fed state (FS): strength tests and RT session in the morning, one hour after breakfast ingestion, as identified in the FT of experimental session three.
- Fasting 16 hours (IF-16): breakfast omission on the session day and starting the test session after 15 hours of fasting, concluding 16 hours of fasting at the end of the session.
- Fasting 12 hours (IF-12): breakfast omission on the day of the session and starting the test session after 11 hours of fasting, concluding 12 hours of fasting at the end of the session.

Experimental design

This study was a randomised crossover clinical trial. The IF was characterised by 12 and 16 hours of overnight fasting. Initially, in the first experimental session, the study was presented to all participants along with a demonstration on how to use the app for food tracking (FT). At the demonstration, the researchers guided the participants to download the food tracking app onto their smartphones and enter their personal information, such as height, weight and age. They also explained how to log all foods and beverages consumed throughout the day, including portion sizes, at the appropriate times. Also, the body composition was determined along with the Physical Activity Readiness Questionnaire (PAR-Q). Total body mass and composition were assessed using an Inbody 270 bioimpedance scale (Inbody Co., Ltd., Korea) and height using a portable stadiometer. Participants were advised to wear lightweight clothing, remove metal objects, stay well hydrated for 24 hours, urinate at least 30 minutes before the assessment, and abstain from alcohol and caffeine. Additionally, all participants were assessed in the FS but were advised to avoid eating a large meal and engaging in strenuous exercise in the 24 hours preceding the assessment. Furthermore, the volunteers were instructed to maintain their usual eating habits throughout the

study period, which was verified through food recall and photographic records. Therefore, participants had either the same dinner the night before the intermittent fasting protocol or their regular breakfast as usual.

In the second experimental session, the volunteers performed a familiarisation session of the equipment and procedures used in the tests and RT session. Then, in experimental session three, they performed the one-repetition maximum test (1RM) in the back squat and in experimental session four, they performed the same test in the leg press 45°. This test was performed to prescribe the intensity of the RT session [12].

The fifth to the seventh experimental sessions were designed for strength and power tests, maximum voluntary isometric contraction (MVIC) and the countermovement jump (CMJ), respectively, in addition to the RT in one of the three experimental conditions: fed state (FS), 12 hours of fasting (IF-12) and 16 hours of fasting (IF-16).

The subjective sensations of hunger, satiety and glycaemia were collected before and after the strength and power tests and after RT. In addition, the lactate concentration was registered before the tests and after the experimental session. The RPE was collected 60 seconds after each series of exercises, and the session-RPE was collected 30 minutes after the end of the experimental sessions. The volunteers used the FT app Dietbox smartphone (Dietbox, version 7.9.0., Brazilian food composition table) one day before experimental sessions three to seven.

Between experimental sessions one and two, there was a 24-hour pause. Therefore, the intervals between experimental sessions two, three, four and five were at least 48 hours. Between experimental sessions five, six and seven, intervals of at least 96 hours were used to prevent a possible effect of fasting from accumulating and promote complete recovery between experimental conditions [13]. The study design is presented in Figure 1.

Performance in experimental sessions

The physical performance assessment consisted of the MVIC tests for the back squat [14] and the CMJ test [15]. The total load volume (TVL) (total number of repetitions × weight lifted) was used to monitor the performance of RT sessions [16]. The interval between tests was 5 minutes and 10 minutes between the last test and the RT session. In the RT session, five sets were performed with repetitions until concentric failure, at 70% of 1RM in the back squat and leg press 45° exercises. The pause between sets and exercises was 2 minutes. In addition, the first series of each exercise was used as a reference for evaluating and comparing MNR performed between experimental conditions [7].

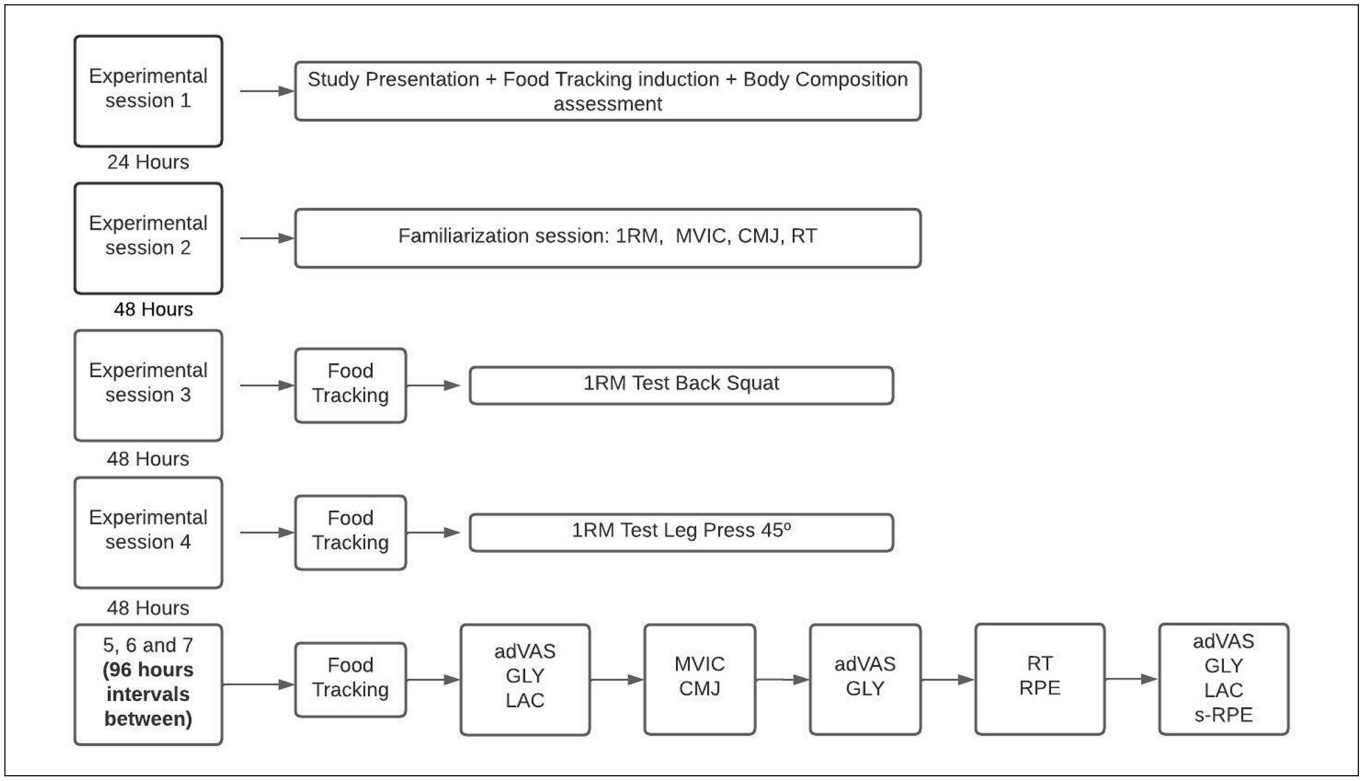


FIG. 1. Study design.
1RM: 1 Repetition Maximum Test; MVIC: Maximum Voluntary Isometric Contraction Test; CMJ: Countermovement Jump Test; FT: Food Tracking; adVAS: adapted Visual Analogue Scale; RT: Resistance Training; GLY: Glycemia; LAC: Lactate concentration; RPE: Rating Perceived Effort; s-RPE: Rating Perceived Effort ater the end of the session.

Strength and power tests

The back squat and leg press 45° techniques in the tests and RT were standardised, similar to the study by Claudino et al. [15]. Adjustments were allowed according to the volunteers' preferences and experiences, recorded and repeated in all experimental tests and training conditions. The experimental sessions to familiarise the participant's strength tests (1RM and MVIC) was the same protocol followed by Drummond et al. [14]. The power was assessed through the CMJ test and its familiarisation [15].

The 1RM test protocol was performed following what was proposed by Drummond et al. [14]. The MVIC test consisted of 3 maximal voluntary isometric actions in back squat lasting 6 seconds with a 3-minute interval between actions. The position was standardised with knee and hip flexion at 90º, determined by the Angle Meter app (version 1.9, iOS). The highest strength value was recorded for comparison [14]. This test was performed on a force platform, model PLA3–1D-7KN/JBA (ZbStaniak; Warsaw, Poland, 1 N precision), connected to a computer interface with the MAX5 program (version 5.1; JBA, ZbStaniak), which presented the force curve as a function of the time [14].

The CMJ protocol followed the procedures suggested by Claudino et al. [15]. Participants completed a 5-minute warm-up consisting of cycling and dynamic stretching. Subsequently, they stood still with their hands on their hips and flexed their knees to approximately 90 degrees. After a brief pause, they executed a maximal vertical jump, aiming to maintain their legs extended and their arms static throughout the jump. The volunteers performed a single set of four consecutive jumps. The CMJ was performed on the same MVIC force platform. The means of peak power in watts (W) and maximum height in centimetres (cm) of the jumps were recorded and compared in each experimental condition [12].

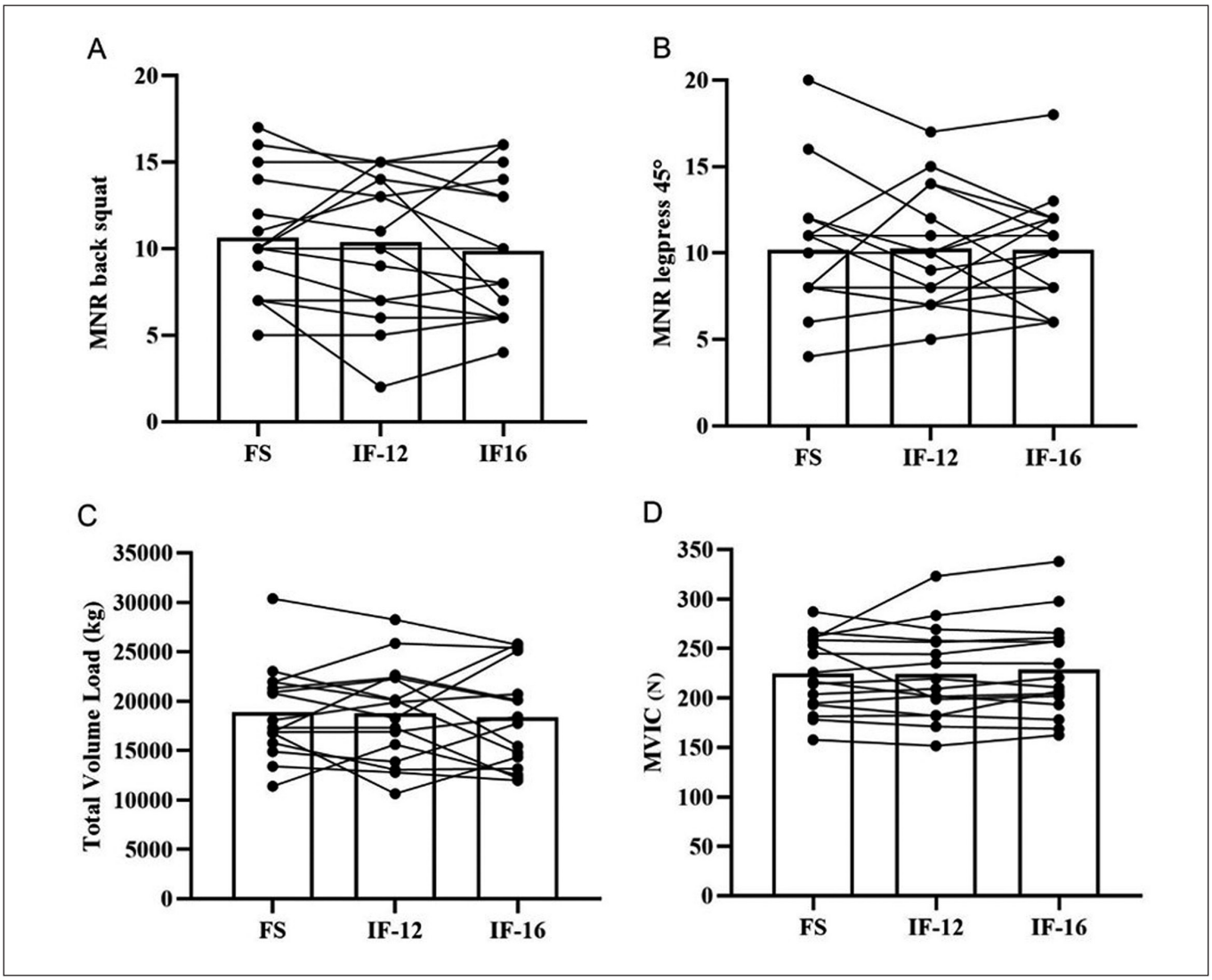


FIG. 2. Individual MNR variations in the back squat (A), leg press 45° (B), training TVL (C) and MVIC test (D) in relation to three different feeding conditions.

Subjective perceptions of hunger and satiety

The present study assessed subjective perceptions of hunger and satiety using an adapted visual analogue scale (adVAS) [17]. Aiming to facilitate the visual analysis of the volunteers, registering and analysing the answers, a scale from 1 to 10 was adapted and standardised following a visual scale with answers anchored in the initial and terminal portions that describe the extreme sensations. For instance: 1 = "no, not at all"; 10 = "yes, a lot"). The participants were instructed to verbally communicate their perceptions so that the quantitative answer could be recorded. Before, during, and after the experimental session, the adVAS variables "hunger," "desire to eat," and "fullness" were recorded.

The subjective perceptions of hunger and satiety were used as an assessment parameter of hunger itself, while the desire to eat and fullness were used as subjective parameters of satiety [7, 17].

Rating of perceived exertion (RPE)

The RPE was collected 60 seconds after the end of each series of exercises. The familiarisation with RPE was performed in the experimental sessions of the 1RM test. In addition, 30 minutes after the end of the training session, the volunteers were asked about their perception of the training session exertion (session-RPE) without the acute session fatigue. On the scale proposed by Foster et al. [18], the volunteers indicated the value corresponding to the perceived exertion in the test session and training. Session-RPE was calculated from the Borg CR-10 scale × training duration (minutes) [18, 19].

Glycaemia and lactate concentration

The glycaemia measurement was used in this study to ensure that the volunteers were not in a hypoglycaemic state before starting the test and training sessions [19]. Moreover, glycaemia and lactate concentration response were investigated to analyse the use of energy substrates during effort [12, 20].

Glycaemia was also determined at the end of the tests and after the resistance training session to monitor this parameter's behaviour between the participants. The glucometer used was the FreeStyle model (Abbott, Brazil). In addition, blood lactate was measured before and after the experimental session (after 3 minutes of rest) [21] with the Accutrend lactometer (Roche Diagnostics, Switzerland). The procedures adopted were the same as those used by Franchini et al. [20].

Statistical analysis

The data were expressed as mean ± standard deviation. Data normality was analysed using the Shapiro-Wilk test, and homogeneity of variances using the Levene test. The ANOVA one-way repeated measures test was used to compare the variance of the physical performance of individuals in the strength, power test sessions, resistance training, RPE, session-RPE and the appetite parameters investigated through the adVAS in the conditions FS, IF-12 and IF-16. When the variables did not present a normal distribution, Friedman's non-parametric equivalent test was used. The ANOVA two-way test for repeated measures was used to analyse the glycaemia and lactate concentration variance. Friedman's non-parametric equivalent test was used to analyse the times when the adVAS was applied during the experimental sessions. A significance level of 0.05 was adopted, and the Bonferroni post hoc test was used when necessary. The epsilon (ε^2) and eta squared (η^2) effect sizes were calculated and classified as trivial(< 0.004), small (0.004–0.25), moderate (0.025–0.64) or strong (> 0.64) [22]. Data analysis was performed using the statistical software Jamovi 1.6.23.0.

RESULTS

The MVIC, training session performance, glycaemia and lactate concentration results showed normal distribution and verified homogeneity. In contrast, session-RPE, back squat and leg press 45° RPE did not show normal distribution. In the CMJ, no statistically significant differences were identified in the mean maximum height (cm) and peak power (W) between the experimental conditions ($p > 0.05$) (Table 1). The strength performance (N) in the MVIC test was

TABLE 1. Performance in tests and resistance training session (mean ± standard deviation)

	FS	IF-12	IF-16	p-value	F	η^2	classification (η^2)
CMJ (cm)	35.2 ± 0.04	35.2 ± 0.04	35.4 ± 0.04	0.986	0.015	0.001	Trivial
CMJ (W)	1880 ± 263.13	1945.03 ± 291.90	1914.63 ± 369.69	0.807	0.215	0.008	Trivial
MVIC (N)	224.84 ± 38.09	224.28 ± 45.86	228.65 ± 48.14	0.960	0.332	0.002	Trivial
TVL	18852.24 ± 4541.88	18749.35 ± 4897.08	18337.33 ± 5124.48	0.954	0.048	0.002	Trivial
MNR Back Squat	10.63 ± 3.44	10.38 ± 4.06	9.88 ± 4.06	0.856	0.156	0.007	Trivial
MNR Leg press 45°	10.19 ± 3.82	10.25 ± 3.38	10.19 ± 3.17	0.998	0.002	0.000	Trivial

CMJ: Countermovement Jump; cm: Centimetres; W: Watts; N: Newton; MVIC: Maximum Voluntary Isometric Contraction; TVL: Total Volume Load; MNR: Maximum Number Repetition; FS: Feed State; IF-12: 12-hour Intermittent Fasting; IF-16: 16-hour Intermittent Fasting; F: ANOVA One-way repeated measures; η^2: Effect Size;

similar in the three experimental conditions ($p = 0.960$; $F = 0.332$) (Table 1). The MNR in both exercises and the TVL of the training sessions were also similar between the three experimental conditions (Table 1). The individual performances in the MVIC test, MNR and TVL are shown in Figure 2.

Hunger was significantly greater in the 12-hour fasting (pre: $p < 0.001$, $t = -7.903$; during: $p < 0.001$, $t = -5.229$; post-session: $p < 0.002$, $t = -4.389$) and 16-hour fasting (pre: $p < 0.001$, $t = -8.950$; during: $p < 0.001$, $t = -6.157$; post-session $p < 0.001$, $t = -5.467$) compared to the fed state. However, there was no statistically significant difference in hunger between the 12-hour and 16-hour fasting at any time (pre: $p = 1.000$, $t = -1.047$; during: $p = 1.000$, $t = -0.928$; post-session: $p = 1.000$, $t = -1.078$) (Table 2).

The desire to eat was significantly greater in the 12-hour fasting (pre: $p < 0.001$, $t = -6.177$; during: $p < 0.001$, $t = -4.766$; post-session: $p < 0.048$, $t = -3.423$) and in the 16-hour fasting (pre: $p < 0.001$, $t = -7.870$; during: $p < 0.001$, $t = -6.025$; post-session: $p < 0.001$, $t = -4.586$) compared to the fed state. However, there was no significant difference in the desire to eat between the 12-hour and the 16-hour fasting (pre: $p = 1.000$, $t = -1.694$; during: $p = 1.000$, $t = -1.256$; post-session: $p = 1.000$, $t = -1.433$). Finally, it was identified that the fullness in the moments before ($p < 0.001$, $t = 7.442$), during ($p < 0.001$, $t = 8.009$) and after a session ($p < 0.001$, $t = 4.260$) was greater in the fed condition compared to the 12-hour fasting and the 16-hour fasting (pre: $p < 0.001$, $t = 8.166$; during: $p < 0.001$, $t = 9.540$; post-session: $p < 0.048$, $t = 5.390$). No significant differences were found in fullness between the 12-hour and the 16-hour fasting (pre: $p = 1.000$, $t = 0.724$; during: $p = 1.000$, $t = 1.531$; post-session: $p = 1.000$, $t = 0.770$) (Table 2).

In the back squat, the mean RPE was similar ($p = 0.951$, $\chi^2 = 0.101$) between the conditions (FS:8.41 ± 1.08; IF-12: 8.34 ± 1.04; IF-16: 8.55 ± 1.10). The effect size was trivial ($\varepsilon^2 = 0.00261$). Likewise, the RPE in leg press 45° was also similar ($p = 0.654$, $\chi^2 = 0.848$) in the experimental conditions (FS: 8.84 ± 0.936; IF-12: 8.86 ± 0.726; IF-16: 9.09 ± 0.906), with a trivial effect size = ($\varepsilon^2 = 0.01804$). The session-RPE values were also similar in the experimental condition (FS: 250 ± 34.9; IF-12: 252 ± 27.4; IF-16: 256 ± 28.5), without statistically significant differences ($p = 0.913$, $\chi^2 = 0.182$) and also with a trivial effect size ($\varepsilon^2 = 0.00387$).

The glycaemia was similar before, during and after the experimental session ($p = 0.236$; $F = 1.47$) in all conditions, with trivial effect sizes ($\eta^2 = 0.012$). As for the interaction between the times of glycaemia measurement and for each different condition, no statistically significant difference was found ($p = 0.056$; $F = 2.40$), and the effect size was considered trivial ($\eta^2 = 0.038$) based on the 95% confidence interval (CI) (Table 3). The initial lactate concentration was similar in the three evaluated experimental conditions ($p = 0.2$; $F = 1.67$). However, the lactate concentration was higher in the post-session compared to the pre-session ($p < 0.001$) in all experimental conditions, with a trivial effect size ($\eta^2 = 0.01$), considering a 95% confidence interval (CI) (Table 3).

DISCUSSION

The present study results showed that the performance in the RT session and strength tests did not change significantly in any of the fasting conditions compared to the fed state. However, the perception of fullness significantly decreased, and hunger increased, similarly in the fasting conditions. Therefore, the hypotheses of the study were partially confirmed.

In studies with trained males submitted to eight and four weeks of intervention respectively, Moro et al. [23] and Stratton et al. [7] compared the results of maximal strength of an RT programme associated with IF 16/8 and a normal diet (ND). The performance improved

TABLE 2. Moments of collection of adVAS parameters concerning feeding conditions, Δ (% fasting12h/fed), Δ (%fasting16h/fed), effect size and classification (ε^2)

adVAS	FS	IF-12	Δ (%)	IF-16	Δ (%)	ε^2	Classification (ε^2)
(Hunger – Pre)	1.44 ± 0.73	6.63 ± 2.03*	460.42	7.31 ± 2.39*	507.64	0.68	Strong
(Hunger – During)	2.38 ± 1.50	6.25 ± 2.35*	262.61	6.94 ± 2.32*	291.60	0.52	Moderate
(Hunger – Post)	3.19 ± 2.29	6.75 ± 2.52*	211.60	7.63 ± 2.06*	239.18	0.41	Moderate
(Desire to eat – Pre)	3.50 ± 1.97	7.38 ± 1.86*	210.86	8.44 ± 1.46*	241.14	0.58	Moderate
(Desire to eat – During)	4.00 ± 2.28	7.31 ± 1.89*	182.75	8.19 ± 1.68*	204.75	0.45	Moderate
(Desire to eat – Post)	4.00 ± 2.13	6.69 ± 2.52*	167.25	7.81 ± 1.97*	195.25	0.35	Moderate
(Fullness – Pre)	7.31 ± 1.82	2.81 ± 1.80*	38.44	2.38 ± 1.50*	32.56	0.59	Moderate
(Fullness – During)	7.31 ± 1.45	3.06 ± 1.81*	41.86	2.25 ± 1.18*	30.78	0.64	Strong
(Fullness – Post)	6.56 ± 2.16	3.19 ± 2.10*	48.63	2.63 ± 1.93*	40.09	0.41	Moderate

*Statistically significant differences of parameters adVAS in the fed condition compared to 12-hour fasting (IF-12**)** and 16-hour fasting (IF-16) conditions; FS: Feed State; Δ: Difference; ε^2: Effect Size

TABLE 3. Glycemia and lactate concentration results.

	FS	CI 95%	IF-12	CI 95%	IF-16	CI 95%
Glycemia (Pre)	98.1 ± 9.75	93.4–102.9	89.9 ± 9.62	85.1–94.6	88.9 ± 9.05	84.1–93.6
Glycemia (During)	95.6 ± 8.87	91.3–99.8	86.2 ± 7.48	82.0–90.4	90.1 ± 8.76	85.9–94.4
Glycemia (Post)	90.1 ± 11.8	84.9–95.2	88.4 ± 7.10	83.2–93.5	90.8 ± 11.2	85.6–96.0
Lactate (Pre)	1.94 ± 0.98	1.45–2.44	2.17 ± 1.24	1.68–2.67	1.56 ± 0.613	1.06–2.05
Lactate (Post)	11.3 ± 2.60	9.43–13.2	12.2 ± 4.20	10.34–14.1	10.2 ± 4.06	8.36–12.1

FS: Feed State; CI: Confidence Interval; IF-12: 12-hour Intermittent Fasting; IF-16: 16-hour Intermittent Fasting.

at the end of the intervention, but no significant differences were found between the conditions. Moro et al. [24], who followed the same volunteers as Moro et al. [23] for 12 months, reached the same conclusion. These results indicate that IF 16/8 did not influence strength training, similarly to the present study. Tinsley et al. [25] evaluated and compared the performance of MVIC of women submitted to RT plus IF 16/8 and ND for eight weeks. A significant and similar increase was identified after the intervention in both conditions. In the present study, maximal MVIC strength was acutely assessed on single days in a 12-hour or 16-hour fast and showed no significant differences between the fasting conditions and the fed state. However, in the present study, the chronic effect of RT was not investigated. Only the RT session performance was evaluated and compared.

The present study also assessed jump height (cm) and peak power (W) of the countermovement jump (CMJ) and did not identify significant differences in either of these parameters between the different feeding conditions. These findings are consistent with previous studies conducted by Tinsley et al. [25, 26] and Stratton et al. [7]. The improvement in strength performance in RT associated with IF was principally due to neuromuscular adaptations to the RT protocol, and possibly the IF did not negatively interfere with this adaptation process [3, 7, 25, 26].

However, the studies that investigated RT associated with IF only performed and assessed the strength tests in the fed state when the volunteers did not perform fasting. Thus, to the author's knowledge, the acute effect of IF on strength performance has not been investigated. This partially limits the discussion of the results. New studies are needed to investigate the acute and chronic effects on strength when strength tests and RT are performed during fasting.

The training performance parameters TVL and the MNR in back squat were also not influenced by the acute fasting of 12 or 16 hours in the present study. Contrary to these findings, Naharudin et al. [9] found an increase of approximately 15% in the MNR in a back squat in the fed state compared to overnight fasting (10 h–13 h fasting). However, in a subsequent study, where the same volunteers were submitted to the same fasting protocol, Naharudin et al. [10] found that MNR in back squats was higher in placebo conditions than control conditions, with no significant repetitions between carbohydrate and viscous placebo ingestions. Therefore, fasting would not have interfered with performance in the RT session, corroborating the present study's findings.

In all experimental conditions, the maintenance of glycaemic levels was observed in the present study during and after the RT session. Thus, a potential daily oscillation range of liver glycogen during fasting and feeding periods had no deleterious impact on RT performance or glycaemic levels [10]. Furthermore, the RT session alone may not deplete substantial muscle glycogen when individuals recover between training sessions after a night's sleep [10]. The present study results indicate that muscle glycogen stores were sufficient for RT session demands, which would explain the similar performance between the experimental conditions.

The lactate concentrations, characteristic of the proposed RT protocol, were similar in all conditions. Thus, fasting did not influence production and utilisation of this metabolite. Therefore, since the MNR and TVL did not undergo significant changes, the hypothesis that the primary energy source used in the RT session came from previous muscle glycogen stores is reinforced.

The results showed that the perception of hunger and the desire to eat were significantly higher in the fasting conditions. On the other hand, the feeling of fullness was lower in the fasting condition compared to the fed state. However, the altered fasting appetite perceptions were not sufficient to negatively impact strength and power performance.

The RPE and session-RPE remained high without significant differences in all experimental conditions, even with increased hunger and reduced satiety. These results corroborate the maintenance of performance in the evaluated conditions. No other studies have investigated the effect of IF on RPE in RT, whether acute or chronic, but it is still worth pointing out that the volunteers were verbally motivated to perform at maximum during the RT sessions and power and strength tests, which could justify the maintenance of RPE at high levels in all conditions, even if the participants were uncomfortable with the feeling of hunger. However, this might obfuscate the volunteer's actual performance under routine training. Nevertheless,

it is recommended that in subsequent studies, the volunteers receive no motivation during the training session in order to more accurately represent their reality during training and, consequently, their performance.

Slater et al. [11] found that fasting before evening exercise was associated with reduced motivation and exercise enjoyment. Furthermore, Naharudin et al. [10] and Stratton et al. [7] identified a reduction in general disposition and poor mood in fasting conditions. These factors can also influence adherence to diet and training planning, especially in RT. Thus, new investigations on the effects of IF on hunger, mood, fullness, and motivation should be conducted.

Among other limitations that have been previously mentioned, it is important to consider some factors that may reduce the applicability and reliability of the study when interpreting the results. One of these limitations is the use of the FT app to control and monitor food intake. Despite the tool's limitations, the researcher took measures to ensure that the participants adhered correctly to the stipulated fasting protocol. This included sending individual and group reminders via mobile phone the day before data collection and reinforcing the guidelines on the day of the sessions. Controlling the repetition time was another possible improvement. This would be done to minimise external interference with the regular movement pattern of the volunteer.

CONCLUSIONS

Our findings suggest that IF may not influence the performance of maximum strength, power, and the performance of an RT session, regardless of the fasting period. Consequently, resistance-training males, their coaches, nutritionists and other associated professionals can choose fasting for 12 or 16 hours as a nutritional strategy without negatively influencing strength performance.

However, the reduction in the subjective perceptions of satiety and the increase in hunger can make it challenging to adhere to training and diet planning, reducing their effectiveness.

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Conflict of interest declaration

The authors declare no conflict of interest.

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Comparison of the external load of professional goalkeepers in different weekly training sessions

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ABSTRACT: The aim of this study was to compare the external load of professional goalkeepers (GKs) in different training sessions of the microcycle. Three professional GKs (age: 28.1 ± 6.9 years; stature: 190.1 ± 1.9 cm; body mass: 84.8 ± 1.1 kg) were monitored by GPS devices during different training sessions according to the days since/until the match day (MD) at MD+1, MD-4, MD-3, MD-2 and MD-1. Different external load measures were calculated: total distance, distance covered at > 14 km·h^{-1}, acceleration load, player load, number of dives, dive load, number of explosive efforts of displacement, number of low intensity (< 0.3 m), medium intensity (0.3–0.4 m), high intensity (> 0.4 m) and medium-high intensity jumps (> 0.3 m). The results showed that there is a decrease in the external load as the GKs' training sessions approach the match, with the lowest value of external load observed at MD-1, and the highest external loads at MD+1 and MD-4. This analysis of the external load demands of professional soccer GKs provides new information that will be useful to inform professionals when planning and implementing training and/or recovery strategies for soccer GKs during the microcycle.

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INTRODUCTION

During a soccer match, one of the goalkeeper's (GK) primary roles is to protect their team's goal, performing multiple individual actions that directly influence the outcome of a match [1]. It is a very different role compared to the rest of the players due to the possibility of touching the ball with the hands, and the specific positioning on the field, with consequences of errors very directly related to the match outcome [2, 3]. Other important functions of GKs include delivery of the ball during the initiation of an attack and taking control of the field of play [1]. The motor actions performed by these players are generally explosive and of short duration, and include diving, catching, jumping, throwing and explosive accelerations/decelerations [4].

Several studies have quantified the activity profile of GKs during soccer matches [2, 5, 6]. GKs cover a distance during a match that is close to 50% of that covered by outfield players [5], comprising only a few short sprints, typically of very short distance [4, 5, 7]. Considering that a GK plays in a limited space, it is more difficult for him/her to reach a large number of high-velocity actions [8, 9]. However, GKs' high-intensity actions are highly decisive in a match, especially the explosive actions performed in short distances between 0 and 5 m, which are the most prevalent during competition [5]. In the Spanish League, the distances covered between the 2011–2012 and 2016–2017 seasons did not significantly change among GKs, but players in the teams at the bottom of the ranking covered longer distances at high-speed running than their peers in the top ranked teams [6]. This may be because matches between unbalanced opponents produce more open game contexts than those between balanced opponents [10]. Other studies suggest that, in the second half of matches, the number of actions performed by GKs and the number of goal opportunities/goals increase due to physical fatigue of the outfield players [4, 5]. Therefore, more dives and explosive efforts are observed among GKs during the final 15 min of the match [4].

It is extremely important to know the match demands of each player so that training programmes can be adjusted appropriately [5, 7]. In this way, it is possible to design specific exercises that take into account the physical demands placed on a GK. However, the most commonly reported external load variable in GKs is the distance covered at different ranges of speed [5, 11]. For this reason, the loads imposed by high-intensity movements with minimal changes in position (saves, clearances, slides, etc.) are usually undervalued [12]. When

it comes to the GK's training, it is important to improve players' ability in explosive movements by combining short high-intensity exercises with sufficient rest periods [13], because the most frequent competitive actions are the save, control and clearing [14].

Nonetheless, there is very little information regarding the GK's training load during a typical training week. Malone et al. [7] described the load imposed on a single GK as a function of the number of days until/since the match day (MD), finding that the highest values of external and internal load occur at MD-3 for most variables, while the lowest values are found close to the match (MD-1 and MD+1). Recently, Moreno-Pérez et al. [12] observed higher external load values in most of the variables investigated at MD-4, with a decrease in activity as training sessions approached the match.

However, there is little information on relevant and specific variables for GK training such as the number of explosive actions performed, the number of jumps of different intensities and dives. Therefore, the aim of this study was to compare the external load of professional GKs from an elite European club in different training sessions within the typical microcycle, according to the number of days until/since the MD.

MATERIALS AND METHODS

Subjects

Three professional soccer GKs (age: 28.1 ± 6.9 years; stature: 190.1 ± 1.9 cm; body mass: 84.8 ± 1.1 kg) belonging to the same Spanish First Division team participated in the study. The training sessions were carried out on a natural grass field at 11:00 a.m. All GKs were familiar with the training protocols prior to the investigation. The monitored sessions occurred between September and January of the relevant season. Only sessions belonging to weeks with a single match were included in the analyses. Finally, as an inclusion criterion, only sessions in which GKs completed all the planned training time were included. The statistical power was computed with the G*Power stand-alone power analysis program (version 3.1.9.6 for Windows, Institut für Experimentelle Psychologie, Düsseldorf, Germany). For the total sample size of 114 records, an effect size of 0.50 and an α error of probability of 0.05, the power (1–β err prob) was 0.99. Data arose as a condition of the players' employment whereby they were assessed daily; thus no authorization was required from an institutional ethics committee [15]. Nevertheless, this study complied with the Declaration of Helsinki and was approved by the Ethics Committee of the University of the Basque Country (UPV/EHU, code: M10-2021-153).

Measures

The external training load was collected using GK-specific 10 Hz GPS devices (Vector, Catapult innovations Ltd., Australia). Players wore the GPS devices from the beginning to the end of each training session. The GPS device was fitted to the upper back (i.e., between the shoulder blades) of each player in a specifically designed neoprene harness to minimise movement artefacts. After each training session, the data were extracted to a computer and analysed using the manufacturer's software (OpenField, version 2.4.0 Build #55403; Catapult innovations Ltd., Australia).

The external load variables analysed were the following: total distance covered (m), distance covered at > 14 $km \cdot h^{-1}$ (m), player load (PL, AU), acceleration load (Aload, AU), number of dives (n), total dive load (AU), number of high intensity (> 0.4 m), medium intensity (0.3–0.4 m), low intensity (< 0.3 m) and medium-high intensity (> 0.3 m) jumps, the number of total jumps (n) and the number of explosive displacement efforts (n; combined number of high-intensity accelerations (> 3 $m \cdot s^{-2}$), decelerations (< -3 $m \cdot s^{-2}$) and change of directions to the left and right).

PL is a measure expressed in arbitrary units obtained through the sum of the accelerations across all axes of the tri-axial accelerometer embedded in the GPS units. Previous research on this indicator has reported high intra- and inter-device reliability [16], and it has been shown to be a valid metric to measure training load in soccer players [17]. The Aload is a variable calculated making all accelerations and decelerations positive, providing an indication of the total acceleration requirements of the athlete, irrespective of velocity [18]. A previous study [19] showed an inter-unit reliability of 2–3% regarding Aload, and this is lower (i.e., higher reliability) than what is typically seen between devices using more commonly used acceleration-based assessments (number of accelerations/decelerations and distance covered in different intensity thresholds). The jumping intensity thresholds were based on previous studies [4]. The dive load quantifies the "work" performed by a GK during a dive, and it is calculated as the sum of three sub-metrics: pre-dive load – the sum of all movements of the GK during the second before the impact; dive impact – the force with which the GK hits the floor; and post-dive load – the sum of all movements of the GK during the second after impact. Hence, dive load = pre-dive load + dive impact + post-dive load.

It was previously reported that the ability of the GPS (and the inertial measurement unit) devices to detect GK-specific action counts was very high, with a nearly perfect correlation (r = 0.903) with the number of counts via video analysis, the accuracy to detect dives being better than that to detect jumps [20]. Although jump height presents high test-retest reliability (intra-class correlation coefficient = 0.86–0.88), it displays mean bias of -2.74 cm when compared to the criterion 3D motion analysis system [21].

The number of satellites used to infer GPS signal quality, horizontal dilution of precision and the average of the GNSS quality were 11.7 ± 0.8 satellites, 0.8 ± 0.1 and 66.8 ± 9.7%, respectively.

Procedures

The study was conducted during the 2019–2020 competitive season and a total of 114 individual GPS files from training sessions were analysed. All training sessions performed on the field were monitored using GPS devices. They were activated according to the

manufacturer's guidelines ~40 min before commencing each training session and players wore the same device throughout the study in order to avoid inter-unit variability.

The microcycle was adjusted to the players' competitive schedule, the state of physical recovery and the conditioning requirements. This study analysed training weeks where players had 6 days between successive matches, and the training week comprised 5 training sessions that clearly focused on the upcoming match [22]. Training load data were analysed with respect to the number of days before or after a match (MD minus or plus [MD- or MD+] approach) [22, 23]. The training sessions of a typical microcycle described below encompass specific contents for GKs, combined with exercises with outfield players:

MD+1: GKs combined training with players who had completed less than 60 minutes in the previous match. In this training session, work was carried out within a technical circuit followed by a positional game and a small-sided game with GKs (area: 30–60 m^2 per player), with exercises where outfield players developed maximal speed and high-speed running and finishing.

MD-4: GKs warmed up with the goalkeeper coach and after that, the training aimed to develop the strength and power capabilities of the players. This session consisted of gym training followed by positional games and a small-sided game with GKs (area: 25–50 m^2 per player). The former comprised intensive exercise with a low number of players per team, small pitch dimensions per player and shorter repetitions.

MD-3: After warming up with the goalkeeper coach, GKs performed an extensive training session, with more players per team, larger pitch dimensions per player and longer repetitions. The structure consisted of specific GK exercises with positional games (area: 70–100 m^2), finishing the session with an 11 vs. 11 game (72 × 65 m).

MD-2: This session aimed to tactically prepare the players for the next match. This session finishes with finishing drills.

MD-1: GKs warmed up with the goalkeeper coach and, after that, they participated with the rest of the outfield players in drills replicating the tactical competition scenarios and concluded with set pieces.

Statistical Analysis

The descriptive statistics were calculated and reported as mean ± standard deviation of the mean (SD) for each training session relative to MD, for each variable. The differences between the independent variable MD (− or +) training session in all measured variables (dependent variable) were examined using repeated measures analysis of variance (ANOVA). Post hoc analyses were performed using Bonferroni's honestly significant difference test. Differences between training sessions were assessed via standardized mean differences (Cohen's d with 90% confidence limits). The interpretation thresholds

TABLE 1. External load metrics (mean ± SD) according to the training session time since/until match day.

Training days	Total distance (m)	Distance > 14 km/h (m)	Player load (AU)	Acceleration load (AU)	Dive Load (AU)	Low intensity jumps (< 0.3 m; n)	Medium intensity jumps (0.3–0.4 m; n)	High intensity jumps (> 0,4 m; n)	Medium-high intensity Jumps (> 0.3 m; n)	Explosive efforts of displacement (n)
MD+1	3221.4 ± 628.9	44.7 ± 38.2	369.1 ± 78.8[b]	1695.2 ± 333.0[b]	438.6 ± 148.0[a,b]	19.7 ± 9.9[a,c]	11.0 ± 10.1[d]	4.1 ± 4.6	15.1 ± 12.1	53.8 ± 17.5[a,b,c]
MD-4	3611.3 ± 1141.7[b]	30.0 ± 21.8	377.2 ± 120.4[b]	1846.4 ± 631.5[b,c]	453.1 ± 215.4[a,b]	14.3 ± 8.9	4.3 ± 3.6	4.5 ± 5.1	8.8 ± 7.4	49.2 ± 15.4[a,b,c]
MD-3	3296.4 ± 484.9[b]	49.9 ± 43.5	318.9 ± 44.4	1415.6 ± 206.5	245.3 ± 132.0	10.0 ± 6.3	6.5 ± 6.4	5.0 ± 4.6	11.4 ± 9.6	28.5 ± 14.2
MD-2	2958.2 ± 738.9	37.7 ± 33.6	322.3 ± 71.6	1376.1 ± 335.4	316.0 ± 101.6	11.6 ± 6.1	6.7 ± 4.8	4.0 ± 4.6	10.7 ± 7.1	35.3 ± 12.4
MD-1	2654.3 ± 635.8	36.2 ± 32.7	294.8 ± 69.7	1185.5 ± 306.9	256.7 ± 125.1	12.9 ± 9.4	6.6 ± 5.7	3.7 ± 3.0	10.3 ± 7.5	31.2 ± 14.0
ES; p:	ES: 0.3–1.2; p < 0.001	ES: 0.0–0.4; p = 0.557	ES: 0.3–1.2; p = 0.002	ES: 0.5–1.6; p < 0.001	ES: 0.1–1.3; p < 0.001	ES: 0.1–1.2; p = 0.008	ES: 0.01–0.9; p = 0.045	ES: 0.01–0.4; p = 0.858	ES: 0.1–0.6; p = 0.286	ES: 0.2–1.6; p < 0.001

Note: MD is match day; MD+1: is the training session 1 day after the previous match; MD-4 is the training session 4 days before the next match; MD-3 is the training session 3 days before the next match; MD-2 is the training session 2 days before the next match; MD-1: is the training session 1 day before the next match; [a] > MD-3; [b] > MD-1; [c] > MD-2; [d] > MD-4.

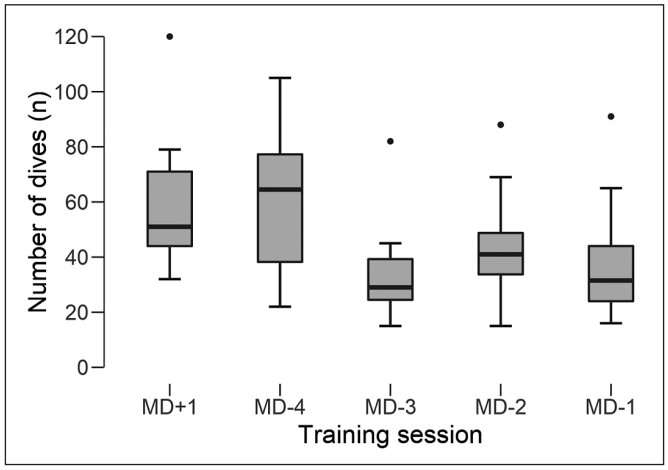


FIG. 1. Number of dives according to the training session time since/until match day.
Note: MD is match day; MD+1: is the training session 1 day after the previous match; MD-4 is the training session 4 days before the next match; MD-3 is the training session 3 days before the next match; MD-2 is the training session 2 days before the next match; MD-1: is the training session 1 day before the next match; ●: higher number of dives than MD+1, MD-3, MD-2 and MD-1 ($p < 0.001$).

for standardized effect size (ES) were as follows [24]: < 0.2 (trivial), 0.2–0.6 (small), 0.6–1.2 (moderate), 1.2–2.0 (large), and > 2.0 (very large). JASP version 0.7.5 was used to conduct the analysis and statistical significance was set at $p < 0.05$.

RESULTS

Table 1 presents the absolute external load variables obtained by GKs in training sessions according to the days before/after the MD. Regarding global indicators, MD-4 displayed higher values than MD-3, MD-2 and MD-1 in Aload, TD and PL (effect size, ES ranged from 0.3 to 1.6). MD+1 showed the highest load in the number of explosive efforts of displacement, and number of low, medium and medium-high intensity jumps (ES: 0.2–1.2), while MD-3 resulted in the highest number of high intensity jumps (> 0.4 m) with respect to the rest of the training sessions (ES: 0.01–0.4). Furthermore, a greater distance covered at > 14 $km \cdot h^{-1}$ at MD-3 was observed (ES: 0.2–0.4), while sessions MD+1 and MD-4 had a higher dive load (ES: 0.5–1.3).

Figure 1 shows the absolute number of dives that GKs performed across the training sessions. At MD+1 and MD-4, a higher number of dives was observed compared to MD-3, MD-2 and MD-1 (ES: 0.4–1.3).

DISCUSSION

The purpose of this study was to investigate the weekly external load dynamics of professional soccer GKs. The main findings of the study were that there was a decrease in the level of external load as the GK approached the match, with the lowest value of external load at MD-1 and the highest level of external load at MD+1 and MD-4.

There is little information in the literature regarding the weekly load dynamics of GKs in professional soccer. However, there seems to be a tendency to reduce the load as training sessions approach the game, similarly to what is observed in outfield players [22]. In our study, the highest load levels were found at MD-4, in agreement with the observations of Moreno-Pérez et al. [12]. However, Malone et al. [7] found that the highest values of training metrics were observed at the MD-3 session. Different training methodologies among clubs may explain the differences in results. Teams that plan training according to tactical periodization will place situations in small pitches at MD-4 [25, 26]. These training situations with a low number of players per team and small pitch dimensions increase the frequency of shooting/finishing [27]; therefore, they can increase the external load imposed on GKs during the session. However, this type of activity is characterized by very little aerial play, which could lead to a reduced average number of jumps [4], this being the main reason for MD-4 attaining the lowest load of the week in the number of medium and medium-high intensity jumps.

The tapering strategy has proven to be effective in increasing match performance [28]. This decline in training load as the match gets closer seems to be a strategy adopted by soccer teams frequently, with the intention of promoting recovery processes before the match [22, 29]. In the case of GKs, previous studies have shown that the lowest external load of the microcycle is at MD-2 [12] or at MD-1 [7]. In our study, there were no significant differences between the two sessions in any of the load variables analysed, but the MD-1 session tended to have a lower external load than the MD-2.

On the other hand, according to the scientific literature, the MD+1 session focuses on the active recovery of GKs [7, 12]. Nevertheless, in our case it seems that the MD+1 session had a similar external load as the MD-4 session, having higher values in some variables and lower in others, with a significant difference in the number of medium intensity jumps (0.3–0.4 m). In our study, three GKs from the same team participated, so only one of the three had played the previous match. In this case, the other two GKs do not need an active recovery session and hence they perform a compensatory training session, this being a possible reason for them displaying an external load similar to MD-4. In addition, a small number of players usually participate in this session [30], which could explain this similarity of loads between MD+1 and MD-4.

In combination with other studies, there is a pattern of total distance covered throughout the microcycle. In our study, the lowest total distance values were found at MD-1, approximately 2600 m, and the highest values at MD-4, approximately 3600 m, being similar to the values reported by Malone et al. [7] and Moreno-Pérez et al. [12]. It is also clear that GKs cover a greater total distance in the match than in training sessions [5, 7, 11].

Given that specific performance variables are missing for GKs and that there is a lack of information that characterizes the type of

training performed, it is difficult to make direct comparisons of the other variables between the different studies. Nevertheless, White et al. [4] found that GKs made approximately 51 dives in training sessions between matches. In our study, there were sessions that accumulated between about 30 and 60 dives, which means that the results are similar in both studies. According to White et al. [4], GKs make about 10 dives during a match, with the number of dives being significantly higher throughout the training week. On the other hand, very similar values of PL can be seen in the MD-4 session of the Malone et al. [7] study and in our study (approximately 350–400 AU). It is known that a GK has an external load of about 550 AU on the PL variable during a match [7], so it can be inferred that in our study GKs showed a lower PL in the MD-4 session than in the matches. Nonetheless, to confirm these inferences, it would be interesting to make an analysis of the match demands in our GKs. It would also be interesting to know the circumstances of the matches, as GKs of teams in the lower zone of the ranking are generally subjected to a higher external load. Future studies concurrently analysing GPS-derived measures and video analysis can help clarify this topic.

The present study has several limitations that should be taken into account. Firstly, only three GKs belonging to a single professional soccer team were monitored. Therefore, the external load distribution in each type of session will be directly affected by the specific load management carried out by the team analysed. In this sense, information about external load management in other professional soccer teams is required. Secondly, completing the external load values with information on internal load or technical-tactical aspects would be relevant in order to know the demands linked to the different types of sessions analysed. Finally, completing information on the external and internal load demanded of goalkeepers with the contextualization of the features of the tasks performed would provide a more practical proposal that goalkeeping coaches could use in the design of training tasks.

CONCLUSIONS

This analysis of the external load demands of professional soccer GKs provides new information that will be useful to inform professionals when planning the preparation and implementation of training and/or recovery strategies for soccer GKs during the course of the competitive microcycle. This study shows that the goalkeepers' load management presents an external load reduction as they get closer to the official match, with the lowest value of external load at MD-1. The first two post-match sessions accumulate the highest external load levels.

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Conflict of interest

The authors did not report any potential conflicts of interest.

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Physical match demands across different playing positions during transitional play and high-pressure activities in elite soccer

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ABSTRACT: This study explored physical match demands across different playing positions during transitional play, to inform the need for position-specific training interventions. Data was collected using 10 Hz GPS units from 10 competitive matches including 23 elite soccer players of the 1st Polish Division (Ekstraklasa) in season 2020–21. A total of 4249 positional observations were made; center backs (n = 884), full backs (n = 972), central defensive midfielders (n = 236), central attacking midfielders (n = 270), central midfielders (n = 578), wingers (n = 778), and attackers (n = 531). Match data reflected distances covered per minute ($m \cdot min^{-1}$): total distance (TD), high-speed running distance (HSRD, > 19.8 $km \cdot h^{-1}$), sprint distance (SD, > 25.2 $km \cdot h^{-1}$), and the frequency of high-intensity accelerations and decelerations (A+D, > 3 $m \cdot s^{-2}$; $n \cdot min^{-1}$). Total absolute sprint distance (SD, > 25.2 $km \cdot h^{-1}$) and total relative sprint distance (Rel B5) were also quantified. A univariate analysis of variance revealed position-specific differences. Significant effects of position were found for all analysed metrics during transitional play (large ESs; $p < .001$). Central attacking midfielders displayed higher TD ($m \cdot min^{-1}$), fullbacks covered highest SD ($m \cdot min^{-1}$) and wingers achieved the highest A+D ($n \cdot min^{-1}$) ($p \leq 0.05$). Centre backs displayed the lowest physical outputs when compared to all other positions, except in A+D ($n \cdot min^{-1}$) during defensive transitions ($p \leq 0.05$). Attackers displayed the highest physical metrics during high pressure activities ($p \leq 0.05$). Coaches should carefully consider positional transitional demands to better inform training design. With specific attention paid to drills that replicate game play.

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INTRODUCTION

Soccer match play is characterized by short linear high-velocity actions and multidirectional accelerations and decelerations, mixed with lower intensity recovery breaks of longer duration [1]. With the most decisive and crucial moments involving explosive actions (high-speed running and sprinting) combined with varied technical skills [1, 2, 3]. Thus, emphasizing the need to carefully consider the inclusion of physical, technical, and tactical demands within modern training design, optimally preparing players for high-intensity passages experienced within game play [4]. Modern microtechnology (i.e., Global Positioning System) has become commonplace in professional soccer allowing quantification of team and individual external loads. GPS has been shown to be a valid and reliable method of quantifying team and positional demands, albeit commonly utilized to display maximum and average outputs over the duration of a session or game [5]. This approach fails to quantify the isolated physical demands experienced by players during transitional play, where short bouts of high-intensity actions are performed [6].

Although average session data provides valuable information about the volume of the activity, it does not represent fluctuations in physical, technical, and tactical intensity. Thus, potentially underestimating the most demanding periods within a modern game and importantly providing no accurate differentiation of these high-intensity passages in relation to individual positional demands [7]. Literature details that distances covered at varied intensities would differ between positional groups [1]. Many studies have reported

that midfield players cover the most total distance, whilst wide players (fullbacks, wingers) and forwards complete more high intensity running [8–9]. Research surrounding peak intensity passages, with specific reference to technical and tactical activities, is limited in elite soccer [10, 11, 12]. Understanding positional differences during crucial high-intensity transitional periods, would provide definitive insight of how to best prepare the players for game demands. Allowing practitioners to contextualize the physical outputs required in specific drills, ensuring the physical demand complements the tactical requirements placed on players by the coaches [13, 14, 15].

Offensive (defense-to-attack) and defensive (attack-to-defense) transitions represent key moments of play in soccer, that directly influence match outcome [10, 16]. During these critical phases of match-play goal scoring opportunities are created, with teams taking risks to capatilise quickly on the opposition conceding possession [16]. The main objective of offensive transitional activities (counter-attack and fast attack) is to achieve numerical superiority in the opponent's half by performing high-speed actions. Contrastingly, the defensive transition is characterized by re-shaping an imbalanced defense to effectively slow down the opposition attack [17]. It is noteworthy that elite soccer teams attack at a very high tempo that lasts below 20 seconds [14, 18]. Counterattacks have been shown to be the most productive playing style for scoring goals [19] often influenced by a number of contextual factors [20]. High-pressure activities were identified as an effective strategy to score goals and create more goal scoring opportunities [17]. These actions have been associated with high fitness levels and high physical demands [21]. TA's have been well described in the literature and directly connected with technical and tactical activities occurring concurrently with other physically stressful moments [17]. Moreover, TA's have a deep context within since it encompasses moments when the ball is in and out of possession (offense and defense, respectively) as well as describing periods of high tactical component such as offensive activities (counter-attack and fast attack) and defensive actions (attack-to-defense transition and high pressure).

It is hypothesized that physical outputs would differ across playing positions during transitions. Moreover, it is suggested that defensive players (center backs, fullbacks, and central defensive midfielders) would be exposed to higher physical stress during defensive phases, with offensive players (wingers, central attacking midfielders, and attackers) within attacking moments of game play. Wide players (fullbacks and wingers) would accumulate greater high-velocity distances during transitional activities. Therefore, the aims of the present study were, 1) investigate physical match demands across different playing positions during transitional play in elite soccer; and 2) identify positional differences in different relative and absolute physical metrics (total distance, high-speed distance, sprint distance, and accelerations/decelerations) during specific short-duration actions such as offensive transitions (counter-attacks), defensive transitions, fast attacks and high-pressure activities.

MATERIALS AND METHODS

Participants

Twenty-three male elite soccer players participated in the present study. Players belonged to a leading team of the 1st Polish Division (Ekstraklasa) in season 2020–21 and they were classified according to playing position, resulting in the following number per position: center backs (n = 4), full backs (n = 5), central defensive midfielders (n = 2), central attacking midfielders (n = 2), central midfielders (n = 2), wingers (n = 5), and attackers (n = 3). Only players who completed at least 60 minutes were included in this investigation, due to pacing strategies utilized by substitutes potentially generating higher outputs than starting players [7, 13]. Players received all information about the project protocol and provided informed consent for the use of match data, in accordance with the Helsinki Declaration. To ensure player confidentiality, all data was anonymised prior to analysis. Ethical approval was provided by the University of Central Lancashire (HEALTH 0104).

Procedures

Data from a total of ten official games including one UEFA CL qualifier and nine Polish domestic league (Ekstraklasa) games (6 wins, 1 draw, and 3 losses) were collected and analysed in the 2020–2021 season. The CL qualifier was included due the opposing team competing at a comparable level, in terms of budget and league position, within another eastern European country. A low number of analysed matches were observed due to a manager change, could have skewed the results due to tactical differences. A total of 4249 individual observations were extracted including 1164 offensive transitions, 1269 defensive transitions, 1120 fast attacks, and 696 high pressure activities. The following number of observations per position were recorded: centre backs (n = 884), full backs (n = 972), central defensive midfielders (n = 236), central attacking midfielders (n = 270), central midfielders (n = 578), wingers (n = 778), and attackers (n = 531). The activity profile of players was captured by MEMS (10 Hz; Vector S7, Catapult Sports, Melbourne, Australia). The GPS model used in this study was worn in each game between the scapulae and contained within the playing jersey inside a mini pocket and thus not affecting mobility of the upper limbs. The players wore these devices in training and games and thus, were familiar with the entire procedure. To get an optimal connection to the satellites, the devices were turned on 15 mins before the start of the game. In accordance with previous guidelines for acceptable GPS coverage, each data was screened for satellite coverage and horizontal dilution of precision (HDOP) using an inclusion criterion of > 6 satellites and ≤ 1.0 respectively [22]. To avoid the inter-unit variations, each player used the same device in matches. The accuracy of this technology has been previously presented in detail [5, 23].

All variables selected for this study have been previously utilised within research [4, 13, 14, 24]. Displaying ICC values between 0.77 (95% CI: 0.62–0.89) (very large) to 1.0 (95% CI: 0.99–1.0) (nearly perfect) for the measurement of distances at different velocity

bands, indicating excellent intra-unit reliability [5, 23]. For mechanical load, acceptable reliability was found for accelerations (CV = 1.4 ± 1.5% to 4.2 ± 1.5%), and acceptable to poor reliability for decelerations (CV = 2.5 ± 1.5% to 10.9 ± 1.5%) [5, 23]. These variables reflected absolute distances covered per minute ($m \cdot min^{-1}$) in the following categories: total distance (TD), high-speed running distance (HSRD, > 19.8 $km \cdot h^{-1}$), sprint distance (SD, > 25.2 $km \cdot h^{-1}$), as well as the number of high-intensity accelerations and decelerations (A+D, > 3 $m \cdot s^{-2}$; $n \cdot min^{-1}$). Also, the variables reflected total absolute sprint distance (SD, > 25.2 $km \cdot h^{-1}$) and total relative sprint distance (Rel B5), which was proposed previously by other authors to represent the functional limits of endurance and sprint locomotor capacities [25]. Relative sprint distance (Rel B5) was set above maximum aerobic speed (MAS) and plus 30% for anaerobic speed reserve (ASR) [25]. An incremental running treadmill test was conducted by the club physiologist to measure VO_{2max} and MAS. The test was performed in the gym environment with a normal ambient temperature and took place on a mechanical treadmill (Technogym, Italy). It began with an initial speed of 10 km/h^{-1} and each stage was increases by 1.5 km/h^{-1}. Five stages were set. Each stage lasted 4 minutes and it was separated by 1 minute passive break. The inclination was set at 1.5%. Polar heart rate monitors (Polar, Norway) and Polar M400 are used to record HR data. Expired gases were analysed breath-by-breath using an online automated gas analysis system (MetaLyzer® 3b-R2; Cortex Biophysik Gmbh, Leipzig, Germany) and accompanying software (MetaSoft® 3). Maximum oxygen uptake (VO_{2max}) was defined as the highest 15-s average oxygen uptake. Velocity (km/h^{-1}) during the maximum oxygen uptake (VO_{2max}) was recorded and set as the maximum aerobic speed (MAS).

TA's were identified and manually created by the club's analysis team in the Catapult Vision video analysis system (Catapult Sports Ltd, Melbourne, Australia) after each match was completed. Phases of play were classified into the following categories: offensive transition (counterattack) (OT), defensive transition (opposition's counterattack) (DT), fast attack (FA), and high pressure (HP), which were previously defined and analysed by others [14, 17]. Offensive transitions and fast attacks ended when the following occurred: a) loss of ball possession (ball of out play and/or cleared by opposition), b) shot on goal, c) decreased tempo of attack due to an effective defense by opposition. Defensive transitions ended if the following events took place: a) possession re-gained, b) ball out of play, c) shot on goal, d) decreased tempo of attack due to an effective defense by opposition. High-pressure activities ended when: a) possession re-gained, b) ball kicked forward by team in possession, c) ball out of play.

The observational methodology REOFUT theoretical framework was used to identify these actions by one member of the club's analysis team [26] and checked by an independent analyst working for the club's software supplier. The analysis procedure using the REOFUT instrument involved in the current body of work was part of the club's analysis protocols implemented daily by the analysis team. Previous literature has demonstrated good to high intra- and inter-observer reliability of this analysis method [17, 27]. Inter-observer and intra-observer analysis indicated adequate levels of reliability for the phases analysed in the study based on Cohen's Kappa calculations (OT: 0.819, 0.964; DT: 0.815, 0.818; FA: 0.959, 0.962; HP: 0.775, 0.896, for inter- and intra-observer reliability, respectively). High levels of objectivity are detailed for OT, DT and FA, and reduced objectivity for HP [28]. Both analysts had 8 years plus experience of working as part of an analysis team within elite football. Prior to the analysis of the ten games reliability and objectivity checks between the two analysts were completed. Both analysts were asked to utilize the REOFUT methodology to analyse two 11v11 training matches which were separated by 7 days, with each game consisting of two 30-minute halves. Data from the Catapult vision software was then downloaded and integrated into the manufacturer's software package (*Openfield*, version 3.2.0) and finally exported into Microsoft Excel (Microsoft Corporation, USA) to make additional calculations for each transitional play. The transition mean average for selected metrics was calculated as the sum of all TA's and divided by their number. For each of the observed transition periods within the two 11v11 games an agreement of > 85% was noted between the two analysts.

Statistical analysis

A descriptive analysis was used, and the results are shown as mean ± standard deviation (SD). Between-matches coefficient of variation (CV) values were computed for transitions for absolute and relative sprint distance (SD and Rel B5, respectively). Statistical analyses were performed using IBM Statistical Package for the Social Sciences (SPSS, Version 27.0, IBM Corporations, New York, USA) with the statistical significance accepted at the 0.05 level. A univariate analysis of variance (ANOVA) was used to quantify main effects for games, transitions, and positions. Interaction effects were also calculated, and any significant main effects associated with games, transitions, and positions were examined using post hoc pairwise comparisons. The assumptions linked to the statistical model were examined to ensure model adequacy. To establish residual normality for each dependent variable, q-q plots were created using stacked standardised residuals. Scatterplots of the stacked unstandardized and standardised residuals were also used to examine the error of variance associated with the residuals. Mauchly's test of sphericity was also done for all dependent variables, with a Greenhouse Geisser correction applied if the test was significant. *A priori* power calculations were conducted using familiarisation trials and pilot data completed by participants matching the criteria described above. Across all isokinetic and stabilomtery measures considered within the thesis, a sample size ≥ 14 players was required to evaluate the interactions associated with all independent variables (for statistical power > 0.8; $p < 0.05$). Partial eta squared (η^2) were computed to estimate effect sizes for all significant main effects and interactions. Partial eta squared was classified as small (0.01–0.059), moderate (0.06–0.137), and large (> 0.138) as suggested previously [29].

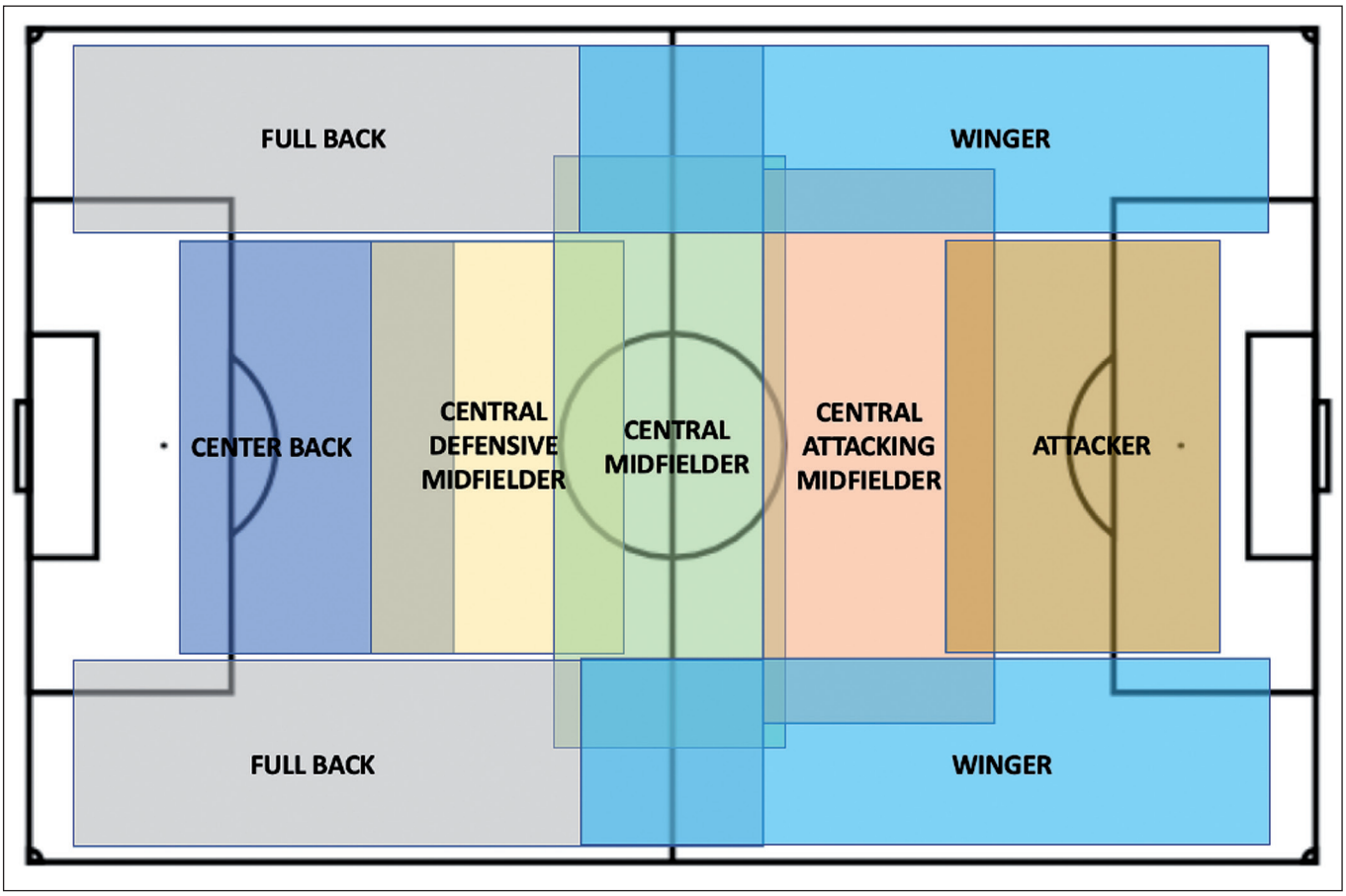


FIG. 1. General and specialised tactical roles based on game analyses. Adapted from Aalbert and Van Haaren [30].

TABLE 1. Mean ± SD for TD ($m \cdot min^{-1}$), high-speed running distance (HSRD) ($m \cdot min^{-1}$), sprint distance ($m \cdot min^{-1}$), accelerations and decelerations (A+D) ($n \cdot min^{-1}$) for each playing position CB (Center Back), FB (Full Back), CDM (Central Defensive Midfielder), CM (Central Midfielder), CAM (Central Attacking Midfielder), W (Winger), and A (Attacker) during transitional activities across 10 official matches.

	All (n = 4249)	CB (n = 884)	FB (n = 972)	CDM (n = 236)	CM (n = 578)	CAM (n = 270)	W (n = 778)	A (n = 531)
Total distance [m · min^{-1}]	206.2 ± 19.0	166.8 ± 63.7^{b,c,d,e,f,g}	204.2 ± 81.4^{a,e}	214.9 ± 59.2^{a,e}	212.3 ± 66.6^{a,e}	234.4 ± 58.6^{a,b,c,d,f,g}	211.7 ± 72.6^{a,e}	203.6 ± 73.7^{a,e}
HSRD [m · min^{-1}]	79.8 ± 11.6	21.7 ± 57.1^{b,c,d,e,f,g}	61.2 ± 94.2^{a,c,d}	44.5 ± 70.4^{a,b,e,f}	45.6 ± 81.9^{a,b,e,f}	67.4 ± 87.2^{a,c,d}	63.4 ± 87.7^{a,c,d}	53.0 ± 77.3^{a}
Sprint distance [m · min^{-1}]	13.2 ± 7.0	5.0 ± 26.7^{b,d,f}	24.2 ± 62.2^{a,c,d,e,g}	5.5 ± 26.2^{b,f}	12.9 ± 42.8^{a,b}	13.5 ± 38.8^{b}	20.0 ± 54.1^{a,c,g}	10.9 ± 37.2^{b,f}
A+D [m · min^{-1}]	1.1 ± 0.3	0.7 ± 2.3^{b,d,f,g}	1.1 ± 2.9^{a,f}	0.9 ± 2.7^{f}	1.1 ± 3.0^{a,f}	1.1 ± 2.6^{f}	1.7 ± 3.5^{a,b,c,d,e}	1.3 ± 2.9^{a}

Note: Significant differences ^{a} CB, ^{b} FB, ^{c} CDM, ^{d} CM, ^{e} CAM, ^{f} W, and ^{g} A ($p < 0.05$).

RESULTS

Figure 1 depicts how players were assigned to their general and specialized playing position in accordance with their tactical roles and responsibilities [30].

Table 1 highlights the mean for selected physical variables for each playing position during transitions that occurred across all 10 analysed matches.

Statistically significant interactions between game, transition and position were found for TD ($m \cdot min^{-1}$) and HSRD ($m \cdot min^{-1}$) (TD: $F_{(131,4010)} = 1.506$, $p < .001$, partial $\eta^2 = .047$; HSRD: $F_{(131,4010)}$

TABLE 2. Mean ± SD and match-to-match variability as CV (%) for absolute sprint distance and relative sprint distance for each playing position CB (Center Back), FB (Full Back), CDM (Central Defensive Midfielder), CM (Central Midfielder), CAM (Central Attacking Midfielder), W (Winger), and A (Attacker) during transitional activities across 10 official matches.

	All (n = 4249)	CB (n = 884)	FB (n = 972)	CDM (n = 236)	CM (n = 578)	CAM (n = 270)	W (n = 778)	A (n = 531)	CV (%)
Absolute sprint distance [m·min^{-1}]	2.2 ± 1.2	0.8 ± 4.1^{b,d,e,f}	4.0 ± 10.6^{a,c,d,e,g}	1.0 ± 4.7^{b,f}	2.1 ± 7.1^{a,b}	2.4 ± 6.8^{a,b}	3.3 ± 8.6^{a,c,f}	1.7 ± 5.6^{b,f}	53.8
Relative sprint distance (Rel B5) [m·min^{-1}]	6.1 ± 2.0	2.6 ± 8.1^{b,c,d,e,f,g}	7.2 ± 13.7^{a,d}	5.3 ± 9.8^{a,f}	5.1 ± 11.5^{a,b,e,f}	8.2 ± 13.3^{a,c,d,g}	8.2 ± 13.1^{a,c,d,g}	5.8 ± 10.2^{a,f}	32.7

Note: Significant differences ^{a} CB, ^{b} FB, ^{c} CDM, ^{d} CM, ^{e} CAM, ^{f} W, and ^{g} A ($p < 0.05$).

= 1.223, p = .045, partial η^2 = .038). A significant interaction between transition and position were identified for SD (m·min^{-1}), A+D (n·min^{-1}), Absolute SD and Relative SD (Rel B5) (SD: $F_{(18,4010)}$ = 6.385, p < .001, partial η^2 = .028; A+D: $F_{(18,4010)}$ = 9.227, p < .001, partial η^2 = .040; Absolute SD: $F_{(18,4010)}$ = 6.211, p < .001, partial η^2 = .027; Relative SD (Rel B5) $F_{(18,4010)}$ = 16.337, p < .001, partial η^2 = .068). Removal of the CL game for analysis indicated negligible change to the observed interactions for game, transition and position (TD: $F_{(131,4010)}$ = 1.514, p < .001, partial η^2 = .044 and HSRD: $F_{(131,4010)}$ = 1.293, p = .02, partial η^2 = .038) and for transition and position (Rel B5) (SD: $F_{(18,4010)}$ = 6.672, p < .001, partial η^2 = .031; A+D: $F_{(18,4010)}$ = 9.081, p < .001, partial η^2 = .041; Absolute SD: $F_{(18,4010)}$ = 6.826, p < .001, partial η^2 = .031; Relative SD (Rel B5) $F_{(18,4010)}$ = 16.907, p < .001, partial η^2 = .074). There was a statistically significant main effect of transitions on duration ($F_{(3,456)}$ = 9.997, p < .0005, partial η^2 = .062). Offensive transitions (OT) duration was significantly higher than all other transitions ($p \leq 0.05$).

Post hoc tests for all ten games revealed that central attacking midfielders (CAM) had higher TD (m·min^{-1}) compared to other positional groups ($p \leq 0.001$). Wingers (W), fullbacks (FB) and central attacking midfielders (CAM) ran higher HSRD (m·min^{-1}) and relative sprint distance (Rel B5) from other positions ($p \leq 0.05$). Fullbacks (FB) covered highest SD (m·min^{-1}) and Absolute SD ($p \leq 0.05$). Wingers (W) had the highest number of A+D (n·min^{-1}) compared to other groups ($p \leq 0.05$). In contrast, center backs (CB) showed

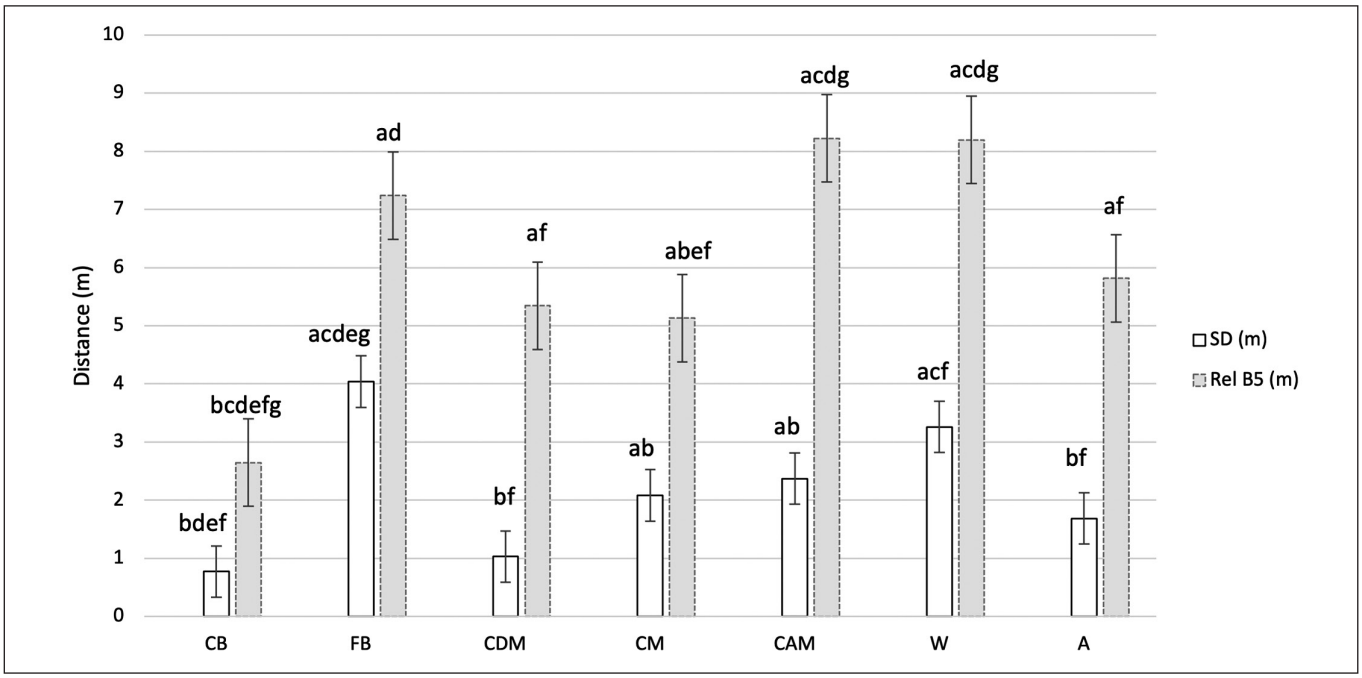


FIG. 2. Comparisons between all playing positions CB (Center Back), FB (Full Back), CDM (Central Defensive Midfielder), CM (Central Midfielder), CAM (Central Attacking Midfielder), W (Winger), and A (Attacker) in mean absolute sprint distance – SD (m) (> 25.2 km·h-1) and mean relative sprint distance - Rel B5 (m) (MAS + 30% ASR) during transitional activities (offensive transitions; defensive transitions; fast attacks; high pressures) across 10 official matches. **Significant differences a CB, b FB, c CDM, d CM, e CAM, f W, and g A ($p < 0.05$).

lower TD ($m \cdot min^{-1}$), HSRD ($m \cdot min^{-1}$), A+D ($n \cdot min^{-1}$), and Relative SD (Rel B5) compared to other positions ($p \leq 0.05$). Also, they had lower Absolute SD than fullbacks (FB), wingers (W), central midfielders (CM), and central attacking midfielders (CAM) ($p \leq 0.05$).

No statistical significance was found between central midfielders (CDM and CM) and wingers (W) for TD ($m \cdot min^{-1}$) ($p > 0.05$). Wingers (W), fullbacks (FB), central attacking midfielders (CAM), and attackers (A) detailed no significant difference positionally for HSRD ($m \cdot min^{-1}$) and relative SD (Rel B5) ($p > 0.05$), however for these metrics these positions were higher than all other positions ($p \leq 0.05$). No difference between wingers (W) and fullbacks (FB) for SD ($m \cdot min^{-1}$) and Absolute SD ($p > 0.05$). Center backs (CB) and central defensive midfielders (CDM) had lowest SD ($m \cdot min^{-1}$) ($p \leq 0.05$), A+D ($n \cdot min^{-1}$) and Absolute SD, a no statistically significant difference between them ($p > 0.05$). No statistically significant difference was found for Fullbacks (FB), wingers (W), and central attacking midfielders (CAM) for Relative SD (Rel B5) ($p > 0.05$).

Table 2 displays the mean absolute and relative sprint distance for each playing position during all analysed transitions accompanied by match-to-match variability for these physical metrics.

Figure 2 displays differences in all playing positions for mean absolute and relative sprint distance across all transitions.

Figure 3 depicts differences between all playing positions in mean distances covered per minute ($m \cdot min^{-1}$) for TD, HSRD, SD and mean number of accelerations/decelerations – A+D ($n \cdot min^{-1}$) across all transitional activities.

Analysis of positional effect identified that center backs (CB) had the lowest TD ($m \cdot min^{-1}$), HSRD ($m \cdot min^{-1}$), and A+D ($n \cdot min^{-1}$), a statistically significant difference from all other positions ($p \leq 0.05$) during offensive phases such as offensive transitions (OT) and fast attacks (FA). In contrast, they achieved highest A+D ($n \cdot min^{-1}$) during defensive transitions (DT), a statistically significant difference with offensive players such as attackers (A), wingers (W), and central attacking midfielders (CAM) ($p \leq 0.05$).

Detailed positional analysis also revealed that SD ($m \cdot min^{-1}$) was highest for fullbacks (FB) ($p \leq 0.05$) during defensive transitions (DT) and fast attacks (FA). Similarly, central midfielders (CM) had high SD ($m \cdot min^{-1}$), a statistically significant difference with attackers (A)

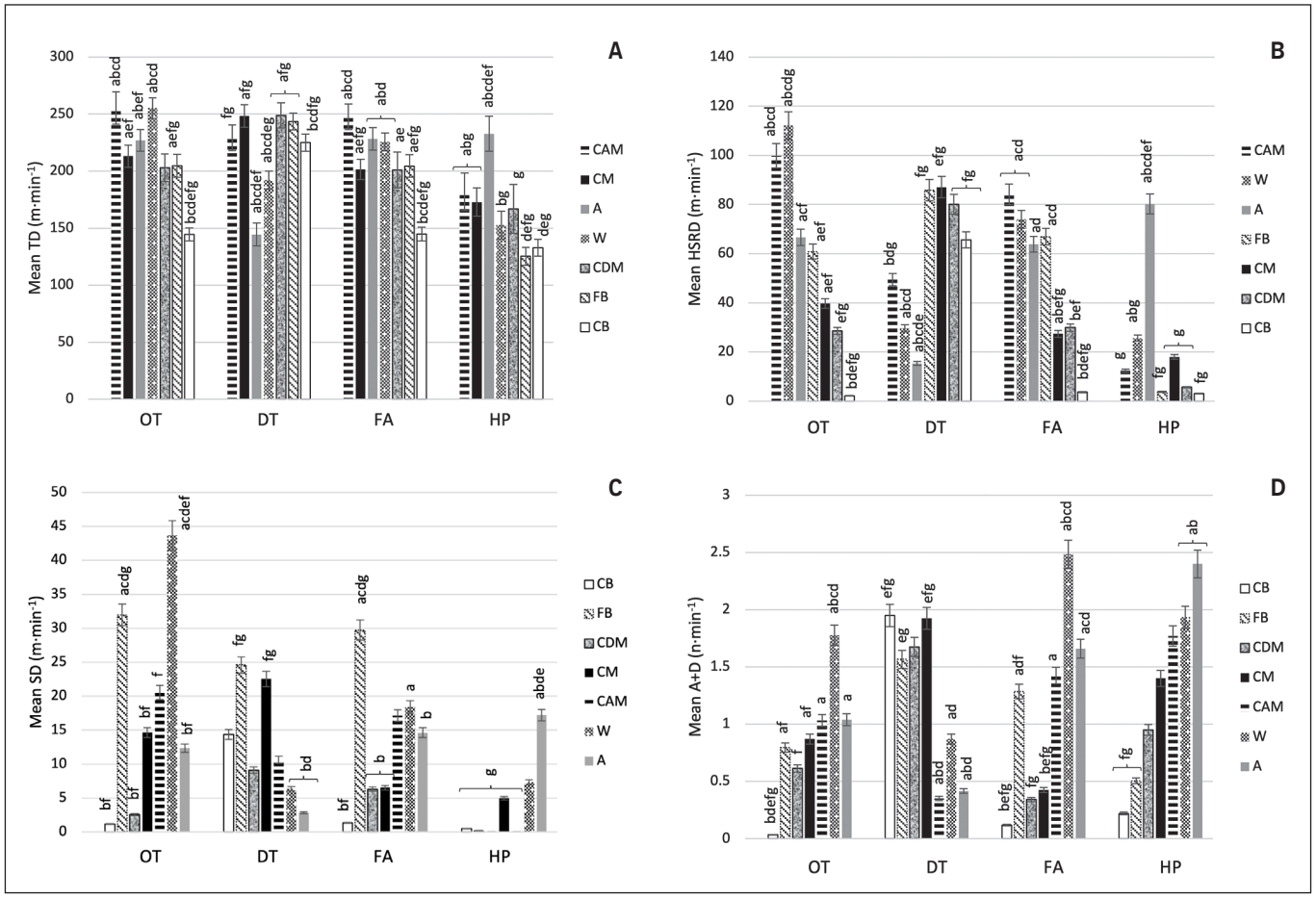


FIG. 3. Comparisons between playing positions CB (Center Back), FB (Full Back), CDM (Central Defensive Midfielder), CM (Central Midfielder), CAM (Central Attacking Midfielder), W (Winger), and A (Attacker) in a) mean TD ($m \cdot min^{-1}$) b) mean HSRD (> 19.8 $km \cdot h^{-1}$; $m \cdot min^{-1}$), c) mean SD (> 25.2 $km \cdot h^{-1}$; $m \cdot min^{-1}$), and d) mean A+D (> 3 $m \cdot s^{-2}$; $n \cdot min^{-1}$) during different transitions: OT = Offensive transition; DT = Defensive transition; FA = Fast attack; HP = High pressure.
**Significant differences [a] CB, [b] FB, [c] CDM, [d] CM, [e] CAM, [f] W, and [g] A ($p < 0.05$).

and wingers (W) ($p \leq 0.05$) during defensive transitions (DT). Central attacking midfielders (CAM) revealed significantly higher TD ($m \cdot min^{-1}$), HSRD ($m \cdot min^{-1}$) from other groups ($p \leq 0.05$) during offensive transitions (OT) and fast attacks (FA).

Wingers (W) showed statistically higher TD ($m \cdot min^{-1}$), HSRD ($m \cdot min^{-1}$), SD ($m \cdot min^{-1}$), and A+D ($n \cdot min^{-1}$) ($p \leq 0.05$) during offensive transitions (OT). Also, this positional group revealed highest output in A+D ($n \cdot min^{-1}$) during fast attacks (FA), a statistically significant difference with other positions ($p \leq 0.05$). Attackers (A) had significantly lower TD ($m \cdot min^{-1}$) and HSRD ($m \cdot min^{-1}$) compared to all other positions ($p \leq 0.05$) during defensive transitions (DT). However, this positional group had highest TD ($m \cdot min^{-1}$), HSRD ($m \cdot min^{-1}$), and SD ($m \cdot min^{-1}$) from other positions ($p < .001$) and were statistically higher in A+D ($n \cdot min^{-1}$) from all defenders (CB, FB) ($p \leq 0.05$) during high pressure (HP). No other significant positional differences were identified for any other physical metrics ($p > 0.05$).

DISCUSSION

The aims of the present study were to 1) investigate physical match demands across different playing positions during transitional play in elite soccer; and 2) identify positional differences in different relative and absolute physical metrics during offensive transitions (counter-attacks), defensive transitions, fast attacks and high-pressure activities. The main findings of our study indicated that physical performance during transitions varied between different playing positions. The activity profile of elite soccer players within 90-min match play has been found to be highly dependent on the tactical role and playing position [1, 31–32]. Previous research has identified midfielders covering the longest average distance followed by forwards and defenders [8]. Fullbacks, midfielders, and offensive midfielders were found to cover a greater high-speed distance, whilst central defenders and attackers did more high acceleration movements [33].

In the current body of work, center backs (CB) experienced the lowest absolute and relative physical demands. This finding is consistent with previous research investigating whole and peak match demands in elite soccer [15, 34]. This positional group was found to be less active in offensive transitions (ball possession) but achieved highest number of accelerations and decelerations – A+D ($n \cdot min^{-1}$) during defensive transitions (DT) (out of possession). It is noteworthy to acknowledge that accelerations and decelerations are more physically demanding than speed-based activities [35–36] and have been linked to match result in modern soccer [37]. Therefore, center backs should be regularly exposed to adequate accel/decel stimuli in training to be optimally prepared for high mechanical demands during collective defensive actions in matches. It was also revealed that this positional group's locomotor metrics were raised when a team was out of ball possession [24]. In the current study, center backs (CB) along with central defensive midfielders (CDM) had the lowest SD ($m \cdot min^{-1}$), absolute SD, and A+D ($n \cdot min^{-1}$). Interestingly, these two positional groups showed very similar patterns for high-intensity metrics such as HSRD ($m \cdot min^{-1}$), SD ($m \cdot min^{-1}$), and A+D ($n \cdot min^{-1}$) during all transitions, suggesting similarities within parts of their training content. Similar training modes could be applied to best prepare them for high physical demands during transitional play, especially when the ball possession is lost and a rapid transition from offense to defense required.

In contrast, fullbacks (FB) covered the highest sprint distance in all transitions and near-maximum velocity actions. Predominantly displaying this during defensive transitions (DT) and fast attacks (FA), which is consistent with previous work [15, 24, 38]. It has been shown that maximum speed activities over longer distances were not adequately used in soccer training [39]. This should be considered when planning training for fullbacks. They often link defensive and offensive responsibilities, resulting in them accumulating high speeds over longer distances [13]. Prompting the need for them to be provided with regular high-velocity exposures during a weekly microcycle. It is important to remember high-velocity distances were found to be directly related to the training status [40] and recognized as one of the key indicators of the physical performance in professional soccer [41]. Fullbacks should be conditioned to cope with higher sprint distance exposure to best prepare them for competition. Allowing them to deliver high performance, meet their positional requirements and importantly keep them free from injury. Due to the fatiguing nature of these repeated high-velocity actions coaches should consider employing appropriate recovery strategies post training and game play. This is due to their link to increased injury risk [42–43]. Future research should consider analysing the effectiveness of recovery strategies post increased exposure to these high-velocity demands.

Additionally, central attacking midfielders (CAM) exhibited higher TD ($m \cdot min^{-1}$) and HSRD ($m \cdot min^{-1}$) than other positions. Interestingly, wingers (W) demonstrated the highest number of accelerations and decelerations per min – A+D ($n \cdot min^{-1}$) during offensive actions while in possession of the ball such as counterattacks and fast attacks. These findings are consistent with previous studies [15, 44]. These rapid changes of speed are crucial in elite soccer and the ability to accelerate, decelerate and change direction quickly has been linked to success in field-sport teams [3, 35] and soccer match result [37]. Interestingly, during counterattacks wingers (W) showed the highest output in other variables per minute ($m \cdot min^{-1}$) such as TD, HSRD and SD, which indicated the importance of this positional group during rapid changes from defensive to offensive transition. Previous literature detailed the significance of high-velocity metrics for creating opportunities and scoring goals, which has very strong implications on performance [17]. Counter-attacks demonstrated the need to generate high-velocity outputs to exploit opponents, open space and create goal scoring opportunities [45–46].

It has been shown that offensive midfielders covered the greatest locomotor distance per minute – TD ($m \cdot min^{-1}$) [38], while wide midfielders (wingers) accumulated the highest A+D ($n \cdot min^{-1}$) in 4-4-3 formation [24]. Findings consistent with the present study. Attackers (A) experienced lower physical output in defensive transitions (DT)

but reached higher metrics during high pressure activities (HP), again consistent with conclusions drawn within literature [24, 44]. Activities that require players to re-gain possession, squeeze space and block penetrating forward passes, have been shown to generate a greater frequency of high-intensity accelerations and decelerations [14, 47]. These actions are very reactive to the opposition movement to apply immediate pressure on the ball. It has been shown that nearly half of all ball winning turnovers across different European leagues, were a result of high pressing in the offensive (opposition) half. Resulting in more goals scored [45, 48]. Thus, demonstrating how crucial it is to use attackers as the first line of defense, aiming to win the ball back and apply high pressure when out of possession.

Finally, mean transition performance for each playing position demonstrated higher match-to-match variability for absolute sprint distance (SD) than for relative sprint distance (Rel B5) (53.8% vs 32.7%, respectively). Indicating the high unpredictability of contemporary football matches and the complexity of modern training design for practitioners [49–50]. Global zones widely adopted in soccer enable workload comparisons to be made between athletes, but don't reflect players' individual physical characteristics [3, 34, 50–51]. Relative speed zones could be more appropriate in elite soccer players analysis to assess true workload, detect fatigue and/or adaptation within a game [52] and therefore, should be explored more in future research.

Prescribing training based on whole/average match data [7], utilizing one physical variable (i.e. distance per min), while neglecting other contextual factors like position, limits specificity. Consequently, not fully reflecting the high physical stress players are exposed to in short and specific high-intensity passages within a soccer match [13, 15, 39, 53]. It is therefore critical for coaches to contextualize soccer-specific short-lasting blocks, that expose players to maximum physical outputs. Describing the tactical phase of play and positional role to best prepare athletes for competition, decrease the injury risk and improve performance [22]. Previous research has shown that utilising principles of overload of short-lasting maximum intensity activities within a training block, are beneficial to soccer players in impacting their performance and causing various physiological adaptations [2, 43, 54]. According to Bortnik et al. [14], if transitions were well described in relation to positional high-intensity and high-velocity metrics, they could offer new multivariate insights into the physical demands of the modern game [14]. Thus, allowing managers and fitness practitioners to plan a clearer contextualized plan for training prescription for the team and individual position [15]. To the authors knowledge, this is the first study to describe physical demands and positional differences in detail during the key phases of a soccer game (transitions).

The findings of this study should be used to inform training intensity to ensure that sessions reflect different physical requirements among varied positional groups. Utilising this approach within varying tactical drills (offensive and defensive) when in or out of possession. For instance, during the midweek overload block (MD-4 and/or MD-3), coaches could use specific drills which encompass transitional activities (defense-to-offense transitions, offense-to-defense transitions, and fast attacks) as well as transitional games (large-, medium-, and small-sided) to over-stimulate locomotor and mechanical demands, increasing the high-velocity stress placed on players [15, 39, 44]. Alternatively, practitioners could utilize different SSG's to apply high pressure without the ball (defensive) and mimic high-intensity periods with the ball (offensive). By changing the number of players, size of the pitch and introducing a goalkeeper, the locomotor demands could be over- or under-stimulated relative to the WCS periods [38]. Large-sided games and small-sided games have been found to be the most appropriate mode of soccer-specific training (midweek) to overstimulate different physical variables in relation to the 5- and 10-min peak match demands (125% sprint demands, and 150% high-intensity accelerations/decelerations, respectively) [38]. However, SSG's could potentially under-stimulate locomotive variables such as distance, high speed, and sprinting [38]. Thus, emphasizing the importance of utilizing transitional activities to replicate the position-specific maximum physical demands experienced by the team or individuals during competition. This approach should be replicated within end-stage rehabilitation sessions to increase performance and reduce the risk of future injury. Importantly, these physical demands would change in relation to individual physical capacity, willingness to complete, coaching style, level of opposition, and other situational and contextual factors [49, 55–56].

Studies investigating the impact of contextual variables (match location, match half, and match outcome) on WCS within a soccer match are limited [24, 44, 57]. Contextual factors most likely determine the physical stress players are exposed to during the most demanding passages in modern match play, particularly for the high-velocity activities. This should be further explored [49]. The present body of work did not analyse effective playing time. Previous research has recently shown that physical demand increases with greater playing time (< 65 min) regardless of the playing position [58]. Our study analysed ten games (small sample) of only one club. Thus, future research should investigate a larger sample across a higher number of teams in a season. Also, consideration should be given to analysis of the impact of substitutions on physical metrics during TA's, comparing training to game play and determine the impact of additional contextual factors such as formation, venue, score, to gain deeper knowledge about the true physical demands of the modern football game. The present study compared absolute to relative sprint distance across different positions during TA's, but future research should also consider analysing peak intensity periods using acceleration thresholds to better relate to the players individual physical capacities during these actions.

CONCLUSIONS

Understanding differences in physical absolute and relative outputs across different playing positions during peak intensity periods is important to practitioners. The present investigation shows that the key physical metrics are largely increased compared to the 90-min

averages when contextualized into transitions. Also, differences exist in physical outputs across positions during these highest physical demands players are exposed to in match play. This study provides guidance for coaches and practitioners on how to prescribe short and specific high intensity activities in training that integrates physical and technical-tactical aspects (offensive and defensive) to mimic maximum physical demands of competition in relation to different positional groups. This knowledge has direct implications for training prescription in relation to high-intensity and high-velocity activities within a weekly microcycle to best prepare each positional group for peak intensity passages in football match.

Key points summary

- Transitional activities largely increased physical demands across all positions compared to the 90-min averages.
- Coaches could use strategies that mimic these physical demands and replicate the positional specificity by designing appropriate drills in training, selecting offensive/defensive activities in/out of the ball possession, manipulating pitch size and the number of players to best prepare athletes for their position-specific role in the modern game and reduce their risk of injury (underpreparedness).
- Center backs experienced the lowest physical metrics during all TA's but achieved the highest number of high-intensity accelerations and decelerations in defensive transitions.
- Fullbacks covered the highest sprint distance during all transitions, which was most evident in defensive transitions and fast attacks.
- Central attacking midfielders ran higher total distance and high-speed running distance from other positions during offensive transitions (counter-attacks) and fast attacks.
- Wingers had the highest number of high-intensity accelerations and decelerations, especially during offensive activities (counter-attacks) and fast attacks.
- Attackers experienced lower physical demands during defensive activities, while reaching higher physical metrics during high pressure activities.

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Conflict of interest

The authors declare no conflict of interest.

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Effects of changing the head coach on soccer team's performance: A systematic review

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ABSTRACT: The aim of this review was to identify and synthesise the most significant literature addressing the effects of changing the HC on soccer team's performance, identifying the most frequently researched outcomes and characterizing their methodologies. A systematic review of PubMed, Scopus, Web of Science and SPORTDiscus databases was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA, 2020) guidelines. The following keywords and synonyms were entered in various combinations in the title, abstract or keywords: "football*" OR soccer AND "coach*" OR "manager*" AND "replacement" OR "turnover" OR "substitution*" OR "change". Solely original articles written in English that contained data about the effects of the change in the HC on performance in professional male soccer were included. A total of 94 titles were identified, of which 24 met the eligibility criteria. The quality of the studies was considered excellent. The most common topic of analysis was the effects of the HC on match outcomes (i.e., win, draw, loss, points won, goals average). Some studies suggest that the HCs dismissal has small but positive impacts on a team's short-term performance, while other findings suggest that little-to-no impact is observed following HC departure. The dismissal of the HC does not guarantee increase success of an underperforming team. Some studies suggest that hiring an appropriate coach could positively affect match performance in the short-term. Due to limited variations in study designs, further research is needed before robust conclusions can be drawn.

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INTRODUCTION

Soccer is a sport with enormous global popularity, mobilizing large masses and with great economic impact. This context tends to create increased pressure on professional clubs, which seek to optimize performance and obtain better results. In the presence of failure, the common tendency is to replace the HC. This replacement usually occurs when a team has a run of negative results over a consecutive period of games, trying to break the negative sequence [1–3].

Contemporary data indicates that the HC turnover rate in professional soccer is high [4–6]. For instance, out of 120 HCs in the top Brazilian soccer league between 2012 and 2017, 87 coaches were dismissed during a season, making the median appointment of a HC around 16 games [5]. Withing the "Big Five" European soccer leagues, only during the 2017/18 season, there were 55 coaching changes [6]. The coach's dismissal usually occurs in response to reductions in a team's performance, typically across a period of two months or twenty games [7]. Bryson et al. [8] report that dismissals in mid-season peak when some leagues have a winter break, suggesting that there are within-season periods where dismissals are more likely to occur.

Even though sacking the HC from elite soccer clubs while the season is in progress could cause substantial disturbances in player and team stability, organizational growth, and financial safety [9], there appears to be three reasons as to why these dismissals occur. These factors relate to intentions to 1) improve performance due to recent match results and unsatisfactory position in the league, 2) increase

the motivation of the players in pre- and post-game contexts, and (3) key stakeholder, sponsors and fans dissatisfaction [10–12].

The HC is the central individual accountable for the teams' results, irrespective of the sport [13–14], with HCs influencing the team's performance, and players' mental wellbeing and physical loads [15]. Some scientific evidence revealed that coach encouragement can be a helpful tool for manipulating the internal and external training load [16–17]. The style of leadership of the HC can also be related with the occurrence of severe injuries [18]. Therefore, match results can be influenced by many variables, with the HC playing a key role in the planning and executing the tactical, technical, physical, and psychological aspects of a soccer team, being the major responsible for the training and competing process.

A review of the literature reveals that the effect of changing coaches has focused mainly on the effects on results, namely victories, defeats, draws, goals scored/conceded and position variation in the league table [3, 9, 19–20]. The scientific evidence is scarce regarding studies that analyse the effects of changing HCs on the locomotor responses of players [21], with some indicating that the change did not cause significant physical changes [22–23]. To the best of our knowledge, only the study of Guerrero-Calderon et al. [24], has verified how coach turnover affects physical responses in players and teams' performances, both in competition and training, presenting some reductions in both contexts with the arrival of a new coach. Additionally, Radzimiński et al. [25], compared match outcome and physical match performance between sacked coaches and new coaches with reference to a control group (teams that did not replace the coach during 3 consecutive seasons). The results suggested that the change may result in short-term improvement in team results and physical performance, however the effect tends to disappear after 5 games following the arrival of the new coach.

Thus, there seem to be insufficiencies in the knowledge related to the performance variables (and its variability) of a professional soccer player when the HC is changed, leading to an increase in recent years in the number of publications on performance and training variables in players caused by the change of coaches [3, 8, 20, 24–25]. This knowledge can provide additional information about a better adaptation to the coach turnover process (i.e., to the "methodological whiplash" and not just "psychological"), in addition to the usefulness in monitoring loads, understanding mood states and well-being of the players, when replacing coaches [24].

Analysing the available literature in relation to the effects of the HC dismissal on teams' performance in variables related to teams' performance (e.g., number of wins, losses, draws, points won, variation in league table and number of goals scored/allowed) and players locomotor responses (e.g., total distance covered, distance per minute, high speed running, max speed, accelerations, decelerations and player workload), might support with stakeholder decision-making processes. Understanding the effects of HC replacement might result in longer-term success for soccer clubs through the support of data-driven decision-making. Therefore, the aim of this article was to systematically review and organize the literature on the effects of changing the HC on professional male soccer teams' performance. This research was also undertaken with a view to identifying frequently researched effects and outcomes, characterising the methodologies, and systematising the evolution of the related research to

TABLE 1. Eligibility criteria.

	Inclusion Criteria	Exclusion Criteria
Population	Professional male soccer players irrespective of the competitive level.	Sports other than soccer (e.g., futsal, beach football, American football, Australian football), female and youth players, amateur senior teams.
Exposure	Exposure to the change of the head-coach independent of duration.	Not controlled for exposure to the change of the head-coach
Comparator	Not mandatory. If possible: Compared with teams that were in same context as the teams that dismissed the HC, but the sacking did not occur.	
Outcome	Locomotor performance (e.g., total distance covered, distance per minute, high speed running, maximum running speed, accelerations, decelerations, and total workload), sports results (e.g., win, lose, draw).	Other measures not related to athletic and match performance, training load, and wellness measures (e.g., injury occurrence).
Other	Only original studies (restricted to articles written in English), published no later than 2010. Only articles that circumscribe dismissals during the course of the season.	Articles not written in English, abstracts, proceedings, book chapters, letters, and other non-original studies.

provide information for clubs' authorities relating to the consequences of replacing the HC.

MATERIALS AND METHODS

Search Strategy: Databases and Inclusion Criteria

This systematic review was reported in accordance with the PRISMA guidelines [26]. Electronic databases (PubMed, Scopus, Web of Science and SPORTDiscus) were searched for relevant publications on 10th June 2023. Searches included the following keywords "football*" OR "soccer" AND "coach*" OR "manager*" AND "replacement" OR "turnover" OR "substitution*" OR "change". Reference managing software (EndNote X9, Thomson Reuters©, New York, NY, USA) was used for managing records.

The inclusion and exclusion criteria can be found in Table 1.

The reference lists of retrieved studies were also reviewed to identify potentially eligible studies not captured by electronic searches. The retrieved records (namely titles and abstracts) were independently screened by two authors (HS and FMC). The same authors also independently screened the full texts. Disagreements between the two authors were deliberated in a collaborative reanalysis. In the case of no unanimity being reached, a third author (HBS) made the final decision. Where doubts occurred in the selection process, all co-authors shared opinions until consensus was achieved.

The data extraction procedure was primarily executed by the lead author (HS) and was verified by two co-authors (FMC and ERG) to corroborate the accuracy and specifics of the data. An adapted Microsoft® Excel datasheet was generated and used to include the data and the main information. In the absence of key data, the primary author (HS) directly contacted the corresponding author by email and/or ResearchGate to acquire the information.

Quality of the Studies and Extraction of Data

As recommended in Faber et al. [27], the methodological assessment process was accessed using the Law et al. [28] scale for quantitative studies (counting 16 items) and was performed by two authors (HS and FMC). The studies were assessed to determine whether they included the following 16 items: the objective (item 1), relevance of background literature (item 2), appropriateness of the study design (item 3), sample included (items 4 and 5), informed consent procedure (item 6), outcome measures (item 7), validity of measures (item 8), significance of results (item 10), analysis (item 11), clinical importance (item 12), description of drop-outs (item 13), conclusion (item 14), practical implications (item 15) and limitations (item 16). Item 9 (details of the intervention procedure) were not applicable because none of the studies included interventions.

For each item, the quality was rated as 1 (meets criteria), 0 (does not meet the criteria fully), or NA (not applicable). A final score expressed as a percentage for each study (Faber et al. 2016). The final score corresponded to the sum of every score in each article, divided by the total number of scored items for that specific research

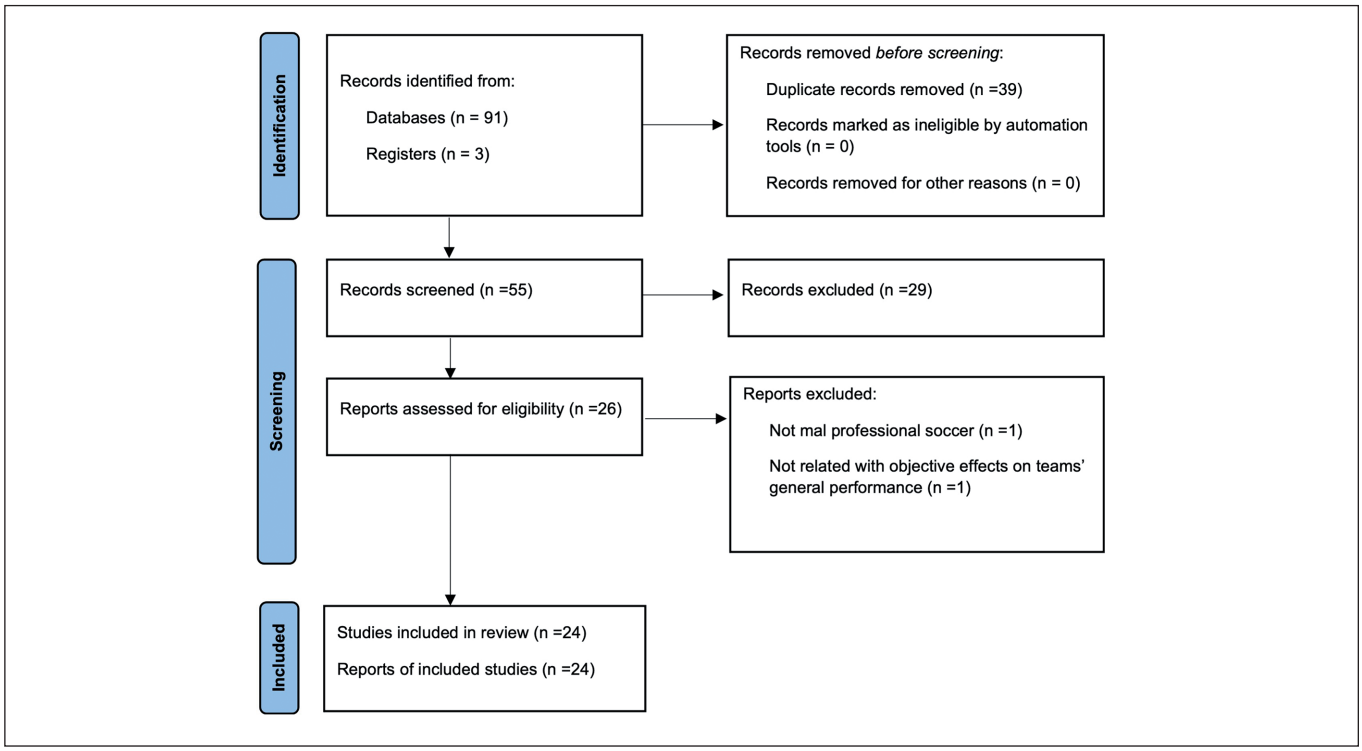


FIG. 1. PRISMA flow diagram.

design. We adopted the classifications of Faber et al. (2016) and Te Wierike et al. [29] and classified the articles as (1) low methodological quality – with a score ≤ 50%; (2) good methodological quality – score between 51 and 75%; and (3) excellent methodological quality – with a score > 75%. The quality analyses were undertaken by the lead author (HS). Disagreements were subsequently solved via diligent debates between two of the authors (HS, FMC). For organizational purposes, the studies were classified into categories according to the major research topics emerged from the content analysis.

RESULTS

Search, Selection, and Inclusion of Publications

The initial searches retrieved 91 titles in the databases (PubMed – 22 articles; Scopus – 24 articles; Web of Science – 27 articles, and SPORTDiscus – 18 articles) and three more articles from Registers. Any duplicates (39 references) were eliminated either automatically or manually. The remaining 55 articles were then assessed for relevance based on their titles and abstracts, resulting in 29 studies being removed from the database. The full text of the remaining 26 articles were examined; 2 were excluded because they did not meet the inclusion criteria. At the end of the screening process, 24 articles were selected for detailed reading and analysis (Figure 1). One study was excluded (n = 1) because of their shortage of relevance with objective effects on teams' general performance (n = 1). The other study (n = 1) was excluded because it contained data not relevant to male professional soccer. The articles considered in this review were published before 2010, with approximately 60 % published in the last 6 years (i.e., 2016 to 2022).

Quality of the Studies

Concerning the quality of studies included, the most significant results were that: (1) the mean score for the studies included in this review was 84.8%; (2) All studies scored above 50%; (3) three studies achieved 100%; (4) only two studies scored between 51 and 75%; and (5) twenty-one studies reached an overall rating above 75%.

Tabela qualidade dos estudos – material suplementar.

General Description of the Studies

Twenty-four studies were included in the qualitative analyses. All analysed studies were carried out in adult male professional soccer. The majority of studies (*n* = 22) were conducted in teams competing in the English Premier League, Spanish La Liga, German 1. Bundesliga, Italian Serie A, French Ligue 1, Portuguese Super Liga,

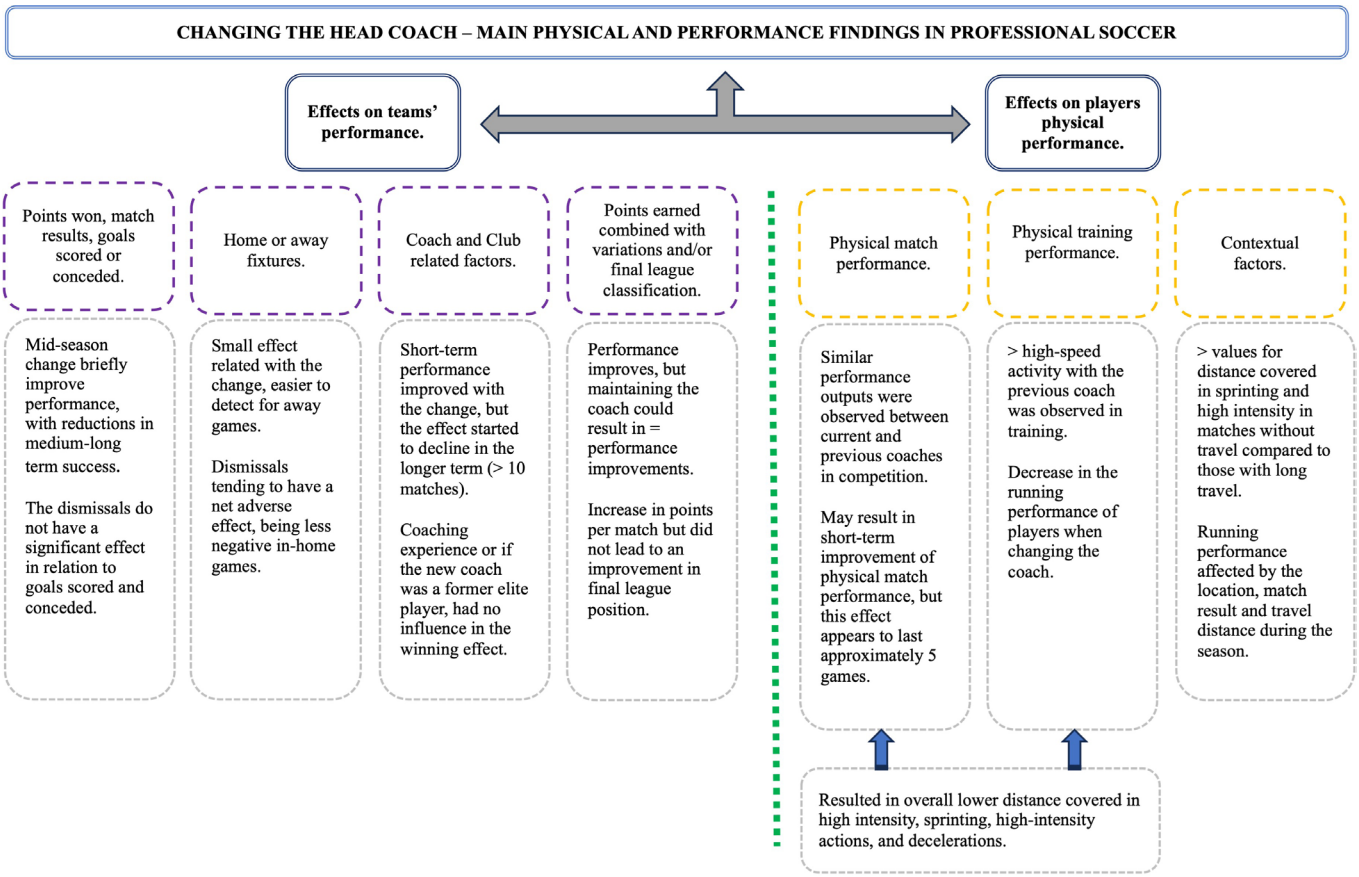


FIG. 2. Main physical and performance findings in professional soccer.

TABLE 2. Studies that analyze the impact of HC replacement using the team's sports performance results and physical performance.

Study	Aim	Sample	Performance Measurement	Study Design	Findings	Quality Score (%)
Radziminski et al. [25]	To examine the physical match performance and match outcome before and after coach turnover compared with a control group containing coaches working continuously for 3 consecutive seasons.	325 performances of teams led by dismissed HCs, 313 of teams led by new HCs, and 580 of teams led by unchanged HCs in first Polish League.	Average number of collected points, total distance, total distance per minute, high-speed running, sprinting and number of high-intensity runs.	Split into periods when the team was led by a coach who was going to be dismissed (DC) and into a period when the team was led by a new coach (NC). For each HC, a group of the last 15 matches was analysed. The first 15 games of NC were taken into consideration.	The study reported that mid-season HC change may result in short-term improvement in team results and physical match performance. However, this effect disappears after a period of approximately 5 games.	92.8%
Bryson et al. [8]	To compare the performance of teams after they have sacked their HC with spells where the HC remains in the job.	Games from the top two divisions of four major European soccer leagues (France, Germany, Italy, and Spain) over the period 2001/02 to 2014/15. 1,327 HC dismissals.	Number of points won by teams.	Included variables such as opposition form, measured by the opponent's league positions, and home advantage, measured by the proportion of home games over the follow up period. Also incorporate a measure of surprise which is the difference between actual and expected performance.	Teams who fire their HC experience small but statistically significant improvements in team performance, although this positive impact is confined to circumstances in which a team holds onto the new HC having sacked the previous coach.	85.7%
Guerrero-Calderon et al. [24]	To analyse the locomotion and metabolic responses of professional players during the four weeks before and after the change of the HC.	Training and match data across 3 professional soccer teams belonging to the top three competitive standards of Spanish soccer have been analysed (n = 1189 events).	Training and match load data.	Training and match load data were analysed separately by HC to compare data between them. To ascertain the effect of the HCC, the individual physical and metabolic responses of players were analysed during 4 weeks before and 4 weeks after the coach dismissal during the competitive period.	Players modify their behaviour in relation to physical performance after the incorporation of a new HC due to the dismissal of the previous at mid-season. In training, players showed greater high-intensity activity with the previous HC rather than with the new one. A similar performance between HCs was found in the competition	92.8%
Gómez et al. [6]	To compare team's performance when coached by new and old HCs. To investigate the impact of a HC change on team's performance according to coach and club related factors.	411 mid-season coaching changes from the 2010–11 season to the 20017–18 season in the Spanish La Liga (n = 85), French Ligue 1 (n = 51), English Premier League (n = 79), German Bundesliga (n = 82) and Italian Serie A (n = 109).	Number of points won by teams.	Linear regression analyses were estimated to determine the winning profile of a new coach. The dependent variable was the points awarded over 1, 2, 3, 4, 5, 10, 15 or 20 matches following the HCC.	Team's short-term performance was improved significantly with a change to a new coach with this impact declining in the longer term (> 10 matches). The winning effect due to the new coach was independent of coach-related factors such as coaching experience or the new coach being a former elite player.	100%
Scelles and Llorca [20]	To examine the effectiveness of dismissing the HC.	In-season leader dismissals and team performance data from games played in the French men's soccer Ligue 1 over the 1994–2016 period (n = 4918 observations, out of the 7990 games played).	Throughout goal difference, position at the table and competitor position.	Used a dummy variable based on the evolution of the team position in the table over the last three games leading to the leader change. Calculated the cumulative difference in the team positions over the same three matchdays between two seasons, one with an HCC after the third matchday and another without an HCC.	Performance improves after a HC dismissal. At the same time, performance improves after a HC dismissal that does not happen (control group), i.e., the HC is not dismissed in a situation when the HC has been dismissed at another time.	100%
Zart and Güllich [3]	To investigate effects of in-season HC changes on the subsequent team performance.	149 HCCs' and 3,960 games between 2010–19 in men's English, German, and Spanish premier soccer leagues.	Points achieved relative to expected points for each game.	Pre- and post-HC change short, medium, and long-term performance (points achieved relative to expected points for each game, taking account of opponents' current strength and whether a game was played at home or away).	Performance showed an instant, strong improvement in the first post-HC change game. The data suggests positive short, medium, and long-term HC change effects at the highest professional soccer level.	100%
Arrondel, Duhautois and Zimmer [12]	To analyse the impact of within-season HC change on club performance.	4 seasons with 18 teams and 16 seasons with 20 teams (392 teams-seasons observations and 7304 games) from French Ligue 1.	Number of points won by teams.	Used an empirical method that takes observable differences between clubs into account (through exact matching) and corrects for unobserved characteristics (through difference-in-differences).	The overall effects of a HC change on team performance are insignificant, except in the short term where they are positive and statistically significant. Between home and away games, the effect is only positive and significant for home games.	78.5%

TABLE 2. Continue.

Study	Aim	Sample	Performance Measurement	Study Design	Findings	Quality Score (%)
Rocaboy and Pavlik [2]	To identify why professional soccer clubs, replace their HC and investigate the effect of HC dismissal on team performance.	English Premier League and the Ligue 1 fixtures for the 2015/16 and 2016/17 seasons. 31 HCC, 18 in the French Ligue 1 and 13 in the English Premier League.	Final league positions of the teams (considering the total wage costs by team) and points won (number of wins, draws and losses).	Used a Monte Carlo simulation (method to produce probability distributions for the final league positions of the teams – this one based on the total wage costs) and compared the final league position with the expected final league position.	HC dismissal results on a drop in the average expected performance compared with the performance expectations at the beginning of the season. Dismissing a coach may enhance performance only if the team under-performed before the dismissal.	85.7%
Scelles and Llorca [40]	To analyse the impact of an HC change on team performance in men's soccer.	3,600 games (94 team-seasons) in French Ligue 1 over the 2000-2016 period.	Points won (considering whether the game was won or not) and the goal difference.	Explanatory variables were used, such as: - home Advantage; the position of the team prior to the Game; the position of the opponent prior to the game; the position of the opponent at the end of the previous season.	An HC change may have a positive impact on team performance, supporting the idea of considering this option when looking for some ways to improve performance.	92.8%
Argentieri, Canova and Manera [41]	To identify the effects of replacing a HC at mid-season due to poor team performance.	38 Serie A games per team and season (over ten seasons – 2007/2008 to 2016/2017).	Player marks given by specialized newspaper and points made by a team in a season.	From the marks, it was calculated an average that served as an indicator for overall team performance in the matches. With the total points obtained by teams at the end of each season it was showed the outcome obtained by each team.	Very low positive impact of the HC change in the short term but a significant negative impact in the long term.	78.5%
Wilson, Plumley and Flint [19]	To examine the effect of HC change in the English soccer industry and explain the association between HC change and organizational performance.	2,816 matches, 525 instances of managerial change in all four main English Football Leagues (EFLs).	Total number of points obtained by a team during an entire season against the total number of matches played by any given team.	The average points per match of those clubs that have experienced managerial change during the last 16 years were analysed. Used league position as an additional proxy to measure sporting success.	The results show significant differences in all four EFLs when considering teams who make a HC change and those who do not. A HC change is more beneficial for clubs in the bottom half of the league.	85.7%
Besters, Van Ours and Van Tuijl [37]	To analyse the performance effects of in-season HC change.	84 in-season managerial changes in English Premier League soccer between 2000/2001 and 2014/2015 seasons, 5700 matches in total.	Number of points won by teams.	Focused on two variables: a treatment group, where the HC had been replaced and measuring the effect of this HCC on the performance; a control group, where the 'hypothetical' change has taken place, to measure the counterfactual effect on the performance.	Some HC changes are successful, while others are counterproductive. On average, performance does not improve following a HCC.	78.5%
Desai, Lockett and Paton [35]	To examine the effects of HC succession on post succession organizational performance.	19 newly appointed leaders in the English Premier League (1996–2010) and 4,452 match observations.	Number of points won by teams.	Different models were analysed, such as: leader to leader entry (HC was directly hired from another organization where that person was a HC); HC domestic experience; HC foreign experience. For control variables, the number of games a leader was in position since the appointment; total number of seasons a leader has held a leader position prior to appointment were some of the used.	HC that moved directly from a HC position at another organization performed better. HCs with domestic top experience are associated with lower post succession performance, while HCs with foreign top coaching experience are associated with higher post succession performance.	85.7%
Muehlheusser, Schneemann and Sliwka [36]	To analyse the impact of HC on performance: the role of team heterogeneity (teams' composition and player abilities)	Dismissals of HC in German Bundesliga 1 between seasons 1994-2010 and 4263 match observations.	Average number of points accumulated in the season up to the match under consideration (HCC).	All procedures were made after identifying teams with identical short-term results. Considered a team's long-term performance history, measuring the average league position in the last three seasons and compared with the season performance at the moment of the HCC.	Significant positive impact but only for homogeneous teams. HCs dismissals showed that individual performance increases to a stronger extent in homogeneous teams.	78.5%

TABLE 2. Continue.

Study	Aim	Sample	Performance Measurement	Study Design	Findings	Quality Score (%)
Van Ours and Van Tuijl [11]	To analyse the causes and consequences of in-season changes of the HC of association soccer teams.	Number of changes of the HC from the highest level of Dutch professional soccer during 14 successive seasons (2000/01-2013/14).	The difference between the actual number of points and the expected number of points, based on the odds of the bookmakers, number of points in recent matches.		Post-dismissal team performance is superior to pre-dismissal team performance. Team performance after "coach dismissals that did not happen" is also better than prior to dismissals that did not happen. There are no positive performance effects for a club dismissing the HC.	78.5%
Flint, Plumley and Wilson [34]	To examine whether HC change was beneficial in acquiring more points per match and whether HC change improved final league position.	A total of 38 league matches per 20 teams in 10 consecutive years/seasons (7600 games) in the English Premier League.	League points and league matches as the main proxy for the analysis and considered the average points per match obtained by each HC. League position before and after the change was considered.	For all analyses, the points per match and league position before HCC was compared with the rest of the season.	HC change led to an increase in points per match but did not necessarily lead to an improvement in final league position.	92.8%
Flores, Forrest and Tena [33]	To analyse the dismissals of soccer HC and their consequences.	420 within-season managerial changes in the top division of Argentine soccer between seasons 1986/87–2005/06 (7000 games).	Points per game.	Team performance was measured at the time of the HCC, in the form of points per game achieved in the preceding four matches and points per game achieved in the season to date, also considering the context of the fixture (away or home game).	There was a difference in impact from HC change according to whether home or away results were examined. The negative effects of change were easier to detect for away games. Dismissals tend to have a net adverse effect on the team, but the effect is perhaps moderated in home games.	78.5%
Maximiano [39]	To investigate whether replacing a HC improves teams' performance.	Portuguese Super Liga seasons between 1999-2005, 3,672 match observations.	Match-level team performance data and a propensity score matching triple difference estimator (average points and average goals scored and conceded in the five matches before/after).	Used a control group with the matches of teams that did not fire the HC but that share similar observable characteristics and an identical pre-firing performance history with those whose HC was fired.	On average, teams perform better with the new HC: more wins, goals and concede fewer. These effects disappear when compared the improvement in performance with the teams that had similar performance and not fired the HC.	78.5%
González-Gómez, Picazo-Tadeo and García-Rubio [31]	To evaluate the impact of a mid-season, change of HC on the sporting performance of professional soccer teams	56 HCC in Spanish soccer teams that played in La Liga between seasons 2001/2002 to 2008/2009.	Throughout points per match (obtained at the end of the season and the extra matches played in other competitions).	Data envelopment analysis techniques were used to compare the performance of a group of teams that have changed their HC mid-season to that group of teams that have stuck with the same HC for the entire season.	A mid-season change of HC improves sporting performance, but it does not allow to perform as well as teams that have not changed HC halfway through the season.	71.4%
Heuer et al. [23]	To study if the HC change during the mid-season has an impact on the subsequent team performance and fitness levels.	14,018 games, starting in the season 1963/64 of the Bundesliga to the 2008/09 season.	Number of goals scored or conceded, and the points won (per match).	Used a statistical framework for accessing the team fitness based on the number of goals scored or conceded, also using the points won (per match) to evaluate teams' overall performance.	Dismissing the HC within the season had basically no effect on the subsequent performance of a team. The HC can bring changes in the physical levels of the team, but these are diluted over time and return to values considered comparable or similar to the previous coach.	78.5%
Lago-Peñas [32]	To investigate the relationship between team performance and HC change over time.	HCCs in male soccer teams that played in the highest national division and the second division in Spain during the seasons from 1997–1998 to 2006–2007.	Percentage of points gained by teams in the matches 1, 2, 3, 5, 10, 15 or 20 prior and following to date of the HCC.	A comparison between the mean team performance levels with the old and a new HC was done over 1, 2, 3, 5, 10, 15 and 20 matches before and after the date of resignation.	No impact of HCC in the long term. The favourable short-term impact of an HCC is followed by continued gradual worsening of results. The HCC effect is non-existent when the comparison is done over 10, 15 or 20 matches before and after termination.	78.5%

TABLE 2. Continue.

Study	Aim	Sample	Performance Measurement	Study Design	Findings	Quality Score (%)
Paola and Scoppa [38]	To study the effects of HC change on teams' performance.	12 seasons of the major Italian soccer league "Serie A" (starting from 1997–1998 to 2008–2009), a total of 4,042 matches.	Points won (per match) and number of goals scored or conceded.	Estimated a number of regressions trying to consider the "Ashenfelter dip" by controlling for team fixed effects, team quality indicators, and lagged match results. Used a matching estimator to estimate the average treatment effect of the HCC, comparing teams that have changed with those hadn't, with similar scenarios.	Changing the HC did not affect team performance, neither when considering as dependent variable the number of points per match nor when looking at the number of goals scored or conceded.	78.5%
Balduck, Buelens and Philippaerts [7]	To examine the short-term effects of midseason HC turnover on soccer team performance.	Game outcomes of Belgian male soccer teams from the first, second and third national divisions from the 1998/99 season to the 2002/03 season.	Points won (four-game point average divided by the seasonal average of points per game).	Define a short term as a span of 4 games prior to and next an HCC. A comparison was made by creating a control (HC not dismissal) and a turnover (HCC in teams with the same performance pattern) groups.	Results showed that changing the HC to improve short-term performance is not the most appropriate way to deal with a bad sequence of results. Teams with the same performance pattern as the turnover group significantly improved after a performance dip without changing HC.	71.4%
Hughes et al. [30]	To test for the short-term and long-term effects of HC change in comparison to the tenures of incumbent top HC.	Results of each competitive game played by every team (more than 5000 soccer matches) between English Premier League seasons of 1994 until 2004.	Points won/Match results (win/draw/loss at home/away games).	Tested for the short-term and long-term effects of HC changes in comparison to the tenures of incumbent top HC. Divided the event window (HCC) into three periods. Ten matches after the change reflect the short term and 30 matches afterwards capture the long term.	Long incumbent tenures are associated with performance far above the average. When looking at change events, change in the short term leads to a brief reprieve in poor performance only for performance to deteriorate in the long term.	92.8%

Dutch First Division and Belgium First Division). The remaining two studies, reported data from the Polish and Argentinian first divisions.

Results are presented in Table 2. Match results or points won were included in thirteen studies [3, 7, 8, 11, 12, 30, 31, 32, 33, 34, 35, 36, 37]. Four other studies combined different sub-topics, such as the points won with the number of goals scored and conceded [23, 38, 39, 40]. One other study used the points won to compare team's performance when coached by the new and the old HC, also scrutinizing the effect of the change with some coach related factors, such as coaching experience or if the new coach being a former elite player could influence the performance [6].

An additional four studies considered the variation in the league table and the final league position, associating this sub-topic with the number of points won, the opponents' strength or table position, the marks given by specialized newspapers to classify players' individual performances, the context of the game (away or home game), and the total wage costs of the teams analysed [2, 19, 20, 34]. Three studies included the physiological component [23–25], with one of these studies analysing the physiological effects for both training and competition [24].

Figure 2 makes a quick synthesis of the main variables under study and consequent key findings that the HCC produces in professional soccer.

DISCUSSION

The aim of this article was to systematically review and organize the literature on the effects of changing the HC on team performance in professional male soccer. Two overarching outcomes were addressed, including, whether dismissal of the HC improves performance after multiple losses, and how performance is influenced with teams that remain with the same HC, despite multiple consecutive losses. The findings can be adopted to determine HC recruitment and retention strategies to inform decision-making processes on whether HCs should be dismissed or remain following periods of unfavorable results.

Effects on teams' performance

Discerning the effects of HC dismissal on performance between studies presented a key challenge given the variation in the performance outcomes. Outcomes varied between points obtained, goals scored and conceded, and league table position, which is often dependent on external factors (i.e., the success of other teams). In relation to studies that focused on the effects of the HC changes on points won or match results [8, 30, 31, 37, 39], a mid-season change of manager appears to briefly improve performance, but with reductions in medium-long term success. In this instance, teams that had not changed the HC performed better in the medium-to-long term that teams that changed the HC mid-season, possibly backing that

long-term investment in the same HC is associated with longer term success versus mid-season management changes.

In the study of Gómez et al., [6], they compared team's performance when coached by new and old HC, but also investigate the impact of a HC change on team's performance according to coach and club related factors. The results showed that team's short-term performance improved significantly with a change to a new HC, but the effect started to decline in the longer term (> 10 matches). Coaching experience or if the new HC was a former elite player, were some aspects analysed, and the winning effect was independent of this coach-related factors.

There were some studies that explored the impact of changing HC according to whether home or away results are examined [3, 12, 33]. These studies reported that there was a small effect related with the change of HCs, which was easier to detect for away games, with dismissals tending to have a net adverse effect on the team, with those effects being more moderated or less negative in-home games.

Considering the studies that focused on the points earned combined with variations and/or final league classification, some of the findings should be interpreted with caution. Scelles and Llorca [20], concluded that team performance improves after a HCs dismissal, however, maintaining the HC on the job could result in similar performance improvements. This evidence seems to support the idea of retaining the HC at the job, with gains related to sports performance and fewer costs associated with the non-dismissal of coaches and coaching stuff, in a mix of financial savings and promoting stability, time and space to develop the work of HC.

Flint, Plumley and Wilson [34] indicate that HC change led to an increase in points per match but did not necessarily lead to an improvement in final league position. Therefore, using league position as a measure of "performance" or "success" in this instance might not be entirely appropriate given that improvements in total points accumulation were observed. Rocaboy and Pavlik [2] showed that HC dismissal results in a drop in the average expected performance compared with the performance expectations at the beginning of the season. This suggests that the dismissal enhances performance solely if the team under-performed before the dismissal.

Two studies reported that the dismissal of a HC does not have a statistically significant effect on the subsequent performance of a team in relation to goals scored and conceded [23, 38], despite some research supporting that such changes seem to have a positive impact on team performance, supporting the idea of considering this option when looking for ways to improve performance [40]. Some studies also found that although performance improves after dismissal, the positive effects dissipate when compared with teams whereby the HC is not dismissed [20, 39]. This notion suggests that often premature dismissal can have detrimental implications for team performance and providing additional time for the HC might result in greater achievements, instead of resetting the process with another HC with the season ongoing.

Two studies used different and interestingly debatable variables for analyses. The first study assessed the difference between the actual number of points attained and the expected number of points based on the odds of the bookmaker [11]. The results demonstrated that post-dismissal team performance was superior to pre-dismissal team performance. Argentieri, Canova and Manera [41] analysed a combination of points won, average score of teams during a season and players ratings given by specialized newspapers. The results showed a small but positive impact of the HC change on short term success, but a significant negative impact longer term. However, the rigour the methodologies employed would appear questionable, given the subjectivity and biases associated with the financial and reputational conflicts of interest involved with bookmaker and journalist interpretations.

Effects on players physical performance

It is important to understand that match outcome (wins, draws, defeats) could be influenced by the physical conditioning processes at individual soccer clubs. Konefal et al. [42] indicated the importance of parameters related to physical performance as highly predictive for the game result in professional soccer. Studies that analyse the modification of players' behaviors in relation to physical performance after the replacement of the HC are scarce and seem to have debatable results.

A study reported that following the mid-season appointment of a new HC, similar performance outputs were observed between current and previous coaches in competition; however, greater high-speed activity with the previous HC was observed in training [24]. These findings are congruent with another study that showed a decrease in the running performance of players when changing the HC in training [21]. There are some variables that need to be considered to try to comprehend these outcomes. The methodological pattern of training can be different, with the two coaches presenting the players with different types of tasks or stimulus in training. For example, one coach applied exercises with large playing areas and, consequently, the players covering greater high-speed distances, and the new coach using drills based with reduced spaces, with more accelerations and decelerations. The new coaches' initial sessions may have been designed to change tactics and playing styles to familiarize the players with new systems and ways of playing. Such sessions that incorporate more of a tactical-based impetus might not be intended to develop the physical component, and as such, might explain the current findings.

Other explanations might relate to the moment of the season when the new coach arrives, with emphasis on the management of training volumes and intensities. The mid-season transfer window can also alter HC practices with possible new signings being cautiously transitioned into new ways of playing. Chmura et al. [43] concluded that players' physical performance tends to decrease after two-thirds of the season. Therefore, load and fatigue management are prioritized at the end of the season but with limited time to

implement the new coaches' principals, tactical behaviors, style of leadership and type of training. Also, a higher number of games in a small period can compromise the preparation of a team, and subsequently leave little time to train specific locomotor capabilities. Contextual factors, such as, the opponent quality, number of players injured and players coming back from injuries, as well as whether the game is a home or away game can also impact the preparation and physical performance of a team. Therefore, it is important that the findings of each of the studies are scrutinized before accurate inferences can be drawn.

Radzimiński et al. [25] found that mid-season replacement of the HC may result in short-term improvement of results and physical match performance, but that this effect appears to last for approximately 5 games. A separate investigation used [23], the goal difference as a proxy for the team's fitness levels (i.e., the greater the goal different the greater the fitness). The findings indicate that the change of coach can bring changes that improve the team's physical performance, but that these are diluted over time and return to values considered comparable to the previous coach. A possible explanation might relate to the commonsense theory that by dismissing the HC and bringing a new one, a psychological shock effect occurs. This shock effect might increase the players motivation instantly [32], but might not persist longer-term.

The gradual decline in physical match performance can be explained by the stabilization of the level of motivation related with the arrival of the new coach. When the psychological effect dissipates, the manager's capability to lead the players is a critical influence in team performance [12]. Other variables such as specific management of volume and intensity of training, management of the injured players, or schedule overloads can be related to this phenomenon. This reiterates that the HC plays a key role in the motivation of the players and that innovative ways to increase the players desire should be sought by the HC.

Augusto et al. [21], stated that changing the HC during the season resulted in overall lower distance covered in high intensity, sprinting, high-intensity actions, and decelerations. Interestingly, the same study indicates that higher values for distance covered in sprinting and high intensity were found in matches without travel compared to those with long-travel, and overall, running performance was affected by the location, match outcome and distance traveled during the season. These variables related with specificities of the schedule and with the volatile environment of a soccer game, are important considerations to be deliberated, when reflecting about the mid-season arrival of a new coach.

Study Limitations

Some limitations should be considered according to the articles that were included in the present review. A limitation is that it only includes studies in English given the databases considered, thereby potentially overlooking other relevant publications written in other languages. Another aspect that was not considered, despite all articles included only reported in season changes of leadership, was the time during the season that the dismissal of the HCs happened. Aspects such as the congestion of games in the schedule at the time of the change of coaches, might be important since performance has shown to be influenced by fixture congested periods [44]. There were only three studies that assessed the physiological components in relation to HC dismissal. Only one of these studies utilized GPS tracking devices that allows you to collect detailed information, allowing the management of training processes and preparation for competition. Quantifying physical and physiological performance parameters using quantifiable metrics to assess the relationship with coach dismissal and performance might present future avenues of research.

Practical Implications and future research

It has been a common practice in professional soccer to fire the HC at mid-season after a period of bad results, to try to reverse the negative streak. Understanding how this process takes place, and how it affects the behaviour and physical performance of the players, this type of information can support changes in tactical-technical and motivational strategies to be adopted during training and competition, optimizing load planning and avoid large variations that could negatively affect the players fitness by coaches and their technical staff. Also, this knowledge about the players physical behaviour could provide various information for clubs' boards, helping to decide whether the dismissal can provide a positive stimulus, or contrariwise be a stress factor.

Some gaps in the available literature were found. First, there is currently a gap and a need for more longitudinal studies related to the analysis of the physiological component after the change of the coach. Structured studies are needed, able to circumscribe and control the largest number of variables that can affect the physical performance of players, to compare the influence and effects that a coach replacement can cause. In this specific situation of physical performance, there seems to be insufficient, or no studies, that could: 1) characterize the dynamics of training and competition in the locomotor performance indicators inherent to the process of replacing the HC; 2) analyse the dependence relationships of training and competition intensity considering the different coach profiles, 3) compare the players' locomotor intensity indicators between a coach replacement process and a continuity process, and 4) verify if the locomotive variables related with the external load and the physical performance of professional football players in training and competition, can work as predictors for the retention or dismissal of professional soccer head coaches.

Another research gap identified during the process of searching for articles for this review, was the need for research on the psychological and environmental aspects impacting the performance of the players subject to coach change. Factors such as the effects caused on players who were playing (or not playing) with the previous coach, associating a comparative analysis with the physical performance of

these same players, would be an interesting evaluation analysis in the context transition generated by the change of coach.

Even in the variable sports results, future research could consider involving outcomes related to the objective performance of the team, namely metrics such as number of shots, crosses or passes, percentage of successful and unsuccessful passes, number of ball recoveries, among others, to achieve an objective comparation related with raw game productivity between two or more different coaches with the same team.

CONCLUSIONS

The findings of this systematic review suggests that dismissal of the HC does not always increase success of an underperforming team. However, some studies suggest that selecting and hiring an appropriate coach could positively affect match performance in professional soccer, particularly in the short-term provided that the coach has sufficient time for longitudinal strategies and philosophies to be implemented. Given the timely requirement for success in soccer, HC dismissals are frequent; but with the notion that persisting with a HC (albeit the correct one) longer-term could enhance a team's performance, perhaps the model in which some board members at soccer clubs operate might need to be adjusted. These findings should be interpreted carefully since there are an abundance of external actors that can influence success when HCs are dismissed and hired, which may not be directly related to the HCs ability to improve performance. Further research should adopt more robust mixed methods study designs that statistically quantity associations with HC dismissal and variables of success, and qualitative analyses of stakeholder perceptions of success within soccer organisations.

Conflict of interest

The authors declared no conflict of interest.

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Testing variations between starters and substitute players in terms of total distance, high-speed running, and sprinting distance: a descriptive study on professional male soccer players

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ABSTRACT: The purpose of this study was three-fold: (i) to compare total distance, high-speed running (HSR) distance, and sprint distance covered per 5-minute epoch by players acting as both starters and substitutes; (ii) to compare the locomotor demands between the moments the players entered the match (45–60, 60–75 and 75–90 minutes); and (iii) to compare the locomotor demands of the players between the variations of the within- and between-playing positions. Twenty-one male professional soccer players competing in the Professional Premier League of one of the European countries were observed over sixteen official matches. The players were monitored during all matches using a Global Navigation Satellite System. The measures collected were total distance (TD; m), distance in HSR, sprint distance, HSR, and sprint counts. Considering the comparisons between the splits over the second half of match play, a significant difference between the starters and the substitutes was observed only for sprint distance in the 90–95 minute split ($Z = -2.023$; $p = 0.043$). Moreover, no substantial differences were found between the moment the substitute player entered the match regarding total distance ($H = 2.650$; $p = 0.266$), HSR distance ($H = 1.738$; $p = 0.419$), and sprint distance ($H = 0.048$; $p = 0.976$). However, the comparison of between-playing positions revealed considerable differences in total distance ($H = 29.246$; $p < 0.001$), and HSR distance ($H = 12.153$; $p = 0.002$) covered by the players acting as starters. In contrast, for substitute players, such differences were reported in HSR distance ($H = 27.892$; $p < 0.001$) and sprint distance ($H = 15.879$; $p < 0.001$). In conclusion, this study suggests that acting as a starter or a substitute does not significantly affect the intensity of effort except during the last periods of match play. However, the contextual factor of performing in a specific playing position plays a significant role both for starters and substitutes.

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INTRODUCTION

The match running demands of professional men's soccer assume a preponderant role in the training process, requiring consistent and objective monitoring on a week-by-week basis [1]. Soccer players are exposed to high loads during a match, and there can be a discrepancy betweenstarters and non-starters [2]. Therefore, appropriate match load monitoring can help coaches better adjust training programmes to players and to improve recovery mechanisms for their players [3].

Load monitoring is commonly categorized into two different and interconnected dimensions [4]: (i) external load (physical demands imposed on players); and (ii) internal load (psychobiological response to a given external load imposed). The load can be quantified through

the use of objective measures, such as heart rate monitors, and/or through subjective measures, including the rate of perceived exertion (RPE). The external load, however, can be assessed using only objective measures, namely Global Navigation Satellite System (GNSS), accelerometers, and inertial measurement units (IMUs) [5]. Modern GNSS have accelerometers, and IMUs are integrated into one device. Given that, coaches can monitor a variety of locomotor measures during both training and matches, which allows them to adjust their training programmes to theirplayers' needs [6].

The most common measures of external load quantified by GNSS include [6]: (i) distances covered at different speed thresholds; (ii) actions associated with changes in speed, such as accelerations and decelerations and/or changes in direction; and (iii) actions measured by the IMUs, such as impacts [6]. The distances covered at different speed thresholds can present significant variations depending on tactical behaviors during a match, a match half, and a field position of each player [7]. On the other hand, accelerometry-based measures can be more dependent on the dynamics of a match and are associated with higher injury incidence [8].

In the last few years there has been increasing evidence regarding match running demands of both starters and non-starters. Nevertheless, most of the available studies on external load quantification in soccer need to consider starters and non-starters [2, 9]. It is known that, compared to non-starters, starter players usually present greater weekly loads for the overall distance-based measures. [10]. However, when considering a soccer match, a study conducted on 1066 professional soccer players showed that the non-starters covered greater high-intensity running distances than the starters [2].

Moreover, the analysis of the match running demands by 15 or 5 minutes has shown higher activity than the match average values [9]. Also, considering that the match running demands are position-dependent, it is paramount to examine the within- and between-players differences according to each player's participation during matches as starters and non-starters. For instance, previous studies reported that increasing the time of a match duration passage decreased the intensity of the overall distance-based measures in all positions. In contrast, the differences among positions increased [11]. On the other hand, decreasing the time of a match duration passage to 5 minutes, no significant differences among positions are observed for high-speed running (HSR) and sprinting distances. This suggests that decreasing the time of the activity observation homogenizes the match running demands imposed on players [11].

To advance our understanding in this field, further research is required to investigate different distance-based measures covered per 5 minutes and to examine within-player and between-player differences based on playing position and type of match participation (starters vs. substitutes). It would be valuable to conduct a study that specifically focuses on understanding the intensities and locomotor demands of starters compared to substitutes, with the aim of determining whether fresh status could potentially enhance locomotor responses. Such research could provide valuable insights for coaches, assisting them in making informed decisions regarding the physical readiness and performance of players. By examining the impact of fresh status on locomotor responses, coaches may gain a better understanding of how to optimize player substitutions and manage player workload effectively.

For the above reasons, the present study aimed (i) to compare total distance, high-speed running (HSR) distance, and sprint distance covered per 5-minute epoch by players acting as both starters and substitutes; (ii) to compare the locomotor demands between the moments the players entered the match (45–60, 60–75 and 75–90 minutes); and (iii) to compare the locomotor demands of the players between the variations of the within- and between-playing positions.

MATERIALS AND METHODS

Experimental approach

We followed a longitudinal study design including sixteen professional male soccer team matches. During the study period, all theplayers were observed. However, for a repeated-measure design, to test starters vs. substitute players, regarding the locomotor demands, we only considered the players who acted as both starters and substitutes. The data collection was performed by a 5-minute epoch, representing the locomotor demands for each 5-minute split during the match. Considering that the substitute players entered only the second half of match play, the comparative analysis of locomotor demands between the starters and the substitutes focused only on this period of time of the matches.

Participants

A single team was selected by convenience. A group of 21 outfield professional football male players (231 observations) from the first team of one of the Polish Ekstraklasa clubs (age: 24.9 ± 3.2 years, body height: 179.6 ± 5.5 cm, body weight: 76.1 ± 5 kg) participated in the research. Sixteen official matches were observed during the autumn season 2022/2023. The study followed the ethical standards for the study involving humans, as described in the Declaration of Helsinki and was approved for Medical Ethic Commity in Gdańsk (Decision number 62/2022). To ensure confidentiality, all data were anonymized before the analysis. The participants were preliminary informed about the study design and signed informed consent after being familiarized with the protocol.

Match load monitoring

Over all the matches, the players used a GNSS unit (10 Hz, Vector S7, Catapult Innovations, Melbourne, Australia; 81 mm × 43 mm × 16 mm). In order to reduce inter-unit variability, the players always used the same device. The GNSS units were placed between the player's shoulder blades and were activated according to a manufacturer's guidelines before kick-off. To avoid potential unit differences, the players wore the same GNSS unit for each match [12]. The data recorded by the units were downloaded after each match for further analysis using Catapult OpenField Cloud Analytics (OpenField 3.9.0

Catapult Sports, Melbourne, Australia). The following variables were selected for the analysis during this study: field time, defined as the time spent on the field (FT; min), total distance (TD; m), distance in high-speed running, defined as running speed between 19.81–25.2 km/h (HSR; m) [13], sprint, defined as velocity greater than 25.2 km/h (SPR; m) [14], High Speed Running Count (HSRC) and Sprint Count (SC). The velocity thresholds chosen are those defined by both tracking system providers. All data from the Tracab system were provided by ChyronHego as a match report. The data from both systems (i.e., Catapult and Tracab) were extracted by 5-min epochs, i.e. splitting the official match time into 5 minute periods.

Statistical procedures

The descriptive statistics were presented as mean, standard deviation, and 95% confidence intervals since the sample revealed non-homogeneity (Levene's $p < 0.005$), and non-parametric tests were executed. As the same players, acting as both starters and substitutes, were compared, the Wilcoxon signed-rank test was used for comparing the locomotor demands. To make a comparison between playing positions, the Kruskal-Wallis test was applied, followed by the Mann-Whitney U test to conduct the pairwise comparisons. The r value was used as effect size, as suggested by previous studies [15]. The statistical procedures were executed in the SPSS software (version 28.0.0.0, IBM, Chicago, USA) for a $p < 0.05$.

RESULTS

Figures 1, 2, and 3 describe the average and the 95% confidence intervals of total distance, HSR, and sprint distance covered in 5-min epochs for the players acting as both starters and substitutes. Over the second half of the matches, total distance covered by the starters during the average 5-min epochs was 523.2 ± 61.8 meters, while for substitutes it amounted to 522.9 ± 87.4 meters. Regarding HSR distance, the starters and the substitutes presented an average of 25.1 ± 27.8 and 27.8 ± 14.6 meters, respectively. Finally, the starters covered an average of 4.7 ± 7.2 meters for sprint distance, while the substitutes had an average of 5.8 ± 7.8 meters.

The Wilcoxon test comparisons between the starters and the substitutes regarding the demands performed in 5-min epochs over the second half of the match revealed no significant differences for total distance ($Z = -1.225$; $p = 0.221$), HSR distance ($Z = -1.914$; $p = 0.056$) and sprint distance ($Z = -1.266$; $p = 0.206$). Considering the comparisons between the splits over the second half, the only significant difference observed between the starters and the substitutes was for sprint distance in the 90–95 minute split ($Z = -2.023$; $p = 0.043$).

Figure 4 presents the descriptive statistics (average and 95% confidence interval) of the average locomotor demands considering the period (45–60 min; 60–75 min; 75–90 min) in which the substitute entered the match. No significant differences were found between the moment the substitute player entered the match for total distance ($H = 2.650$; $p = 0.266$), HSR distance ($H = 1.738$; $p = 0.419$) and sprint distance ($H = 0.048$; $p = 0.976$).

Table 1 presents the descriptive statistics (mean ± standard deviation) comparing the locomotor demands in 5-min epochs between- and within-playing positions. The comparisons of between-playing positions revealed significant differences for total distance ($H = 29.246$; $p < 0.001$) and HSR distance ($H = 12.153$; $p = 0.002$) covered by the players acting as starters. Considering the substitutes, considerable differences between playing positions were found for HSR distance ($H = 27.892$; $p < 0.001$) and sprint

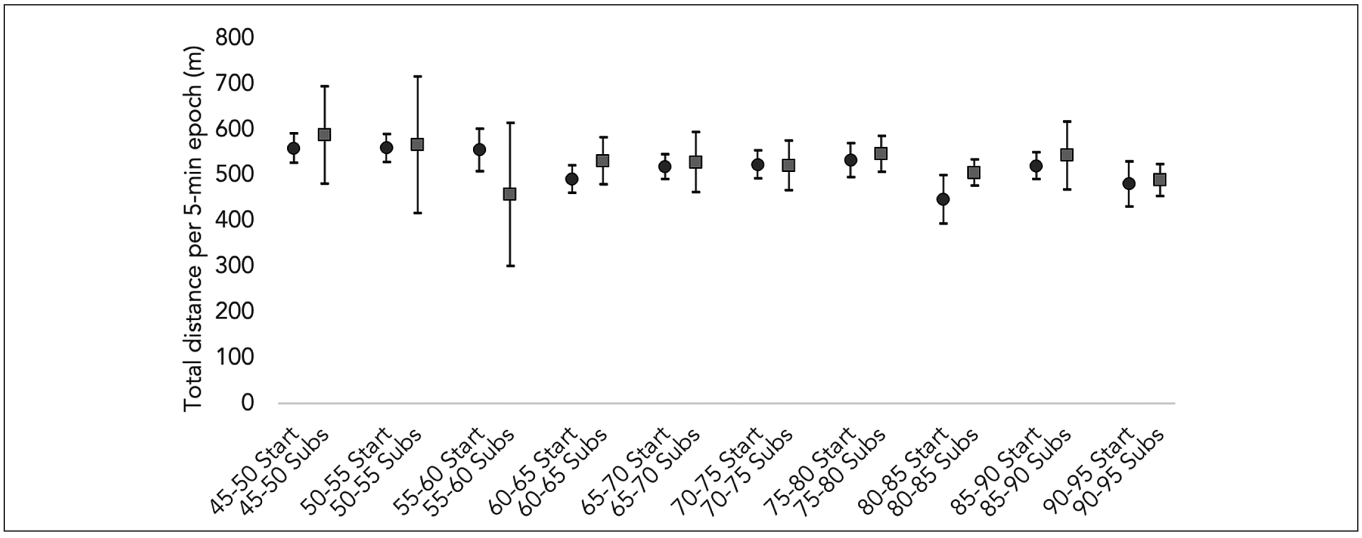


FIG. 1. Descriptive statistics (average and 95% confidence interval) for total distance covered by starters and substitutes in 5-min epochs. Start: starters; Subs: substitutes.

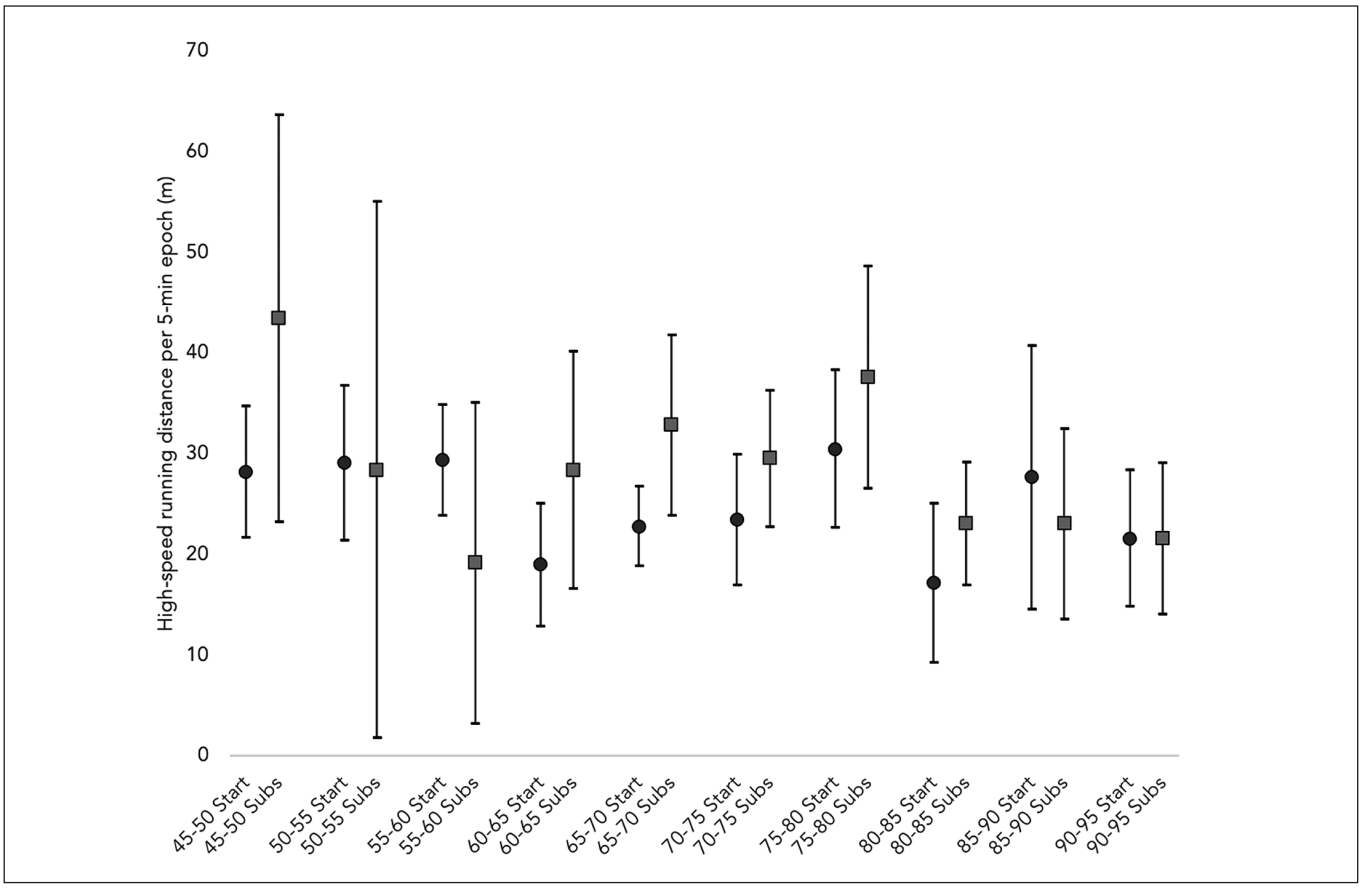


FIG. 2. Descriptive statistics (average and 95% confidence interval) for high-speed running distance covered by starters and substitutes in 5-min epochs. Start: starters; Subs: substitutes.

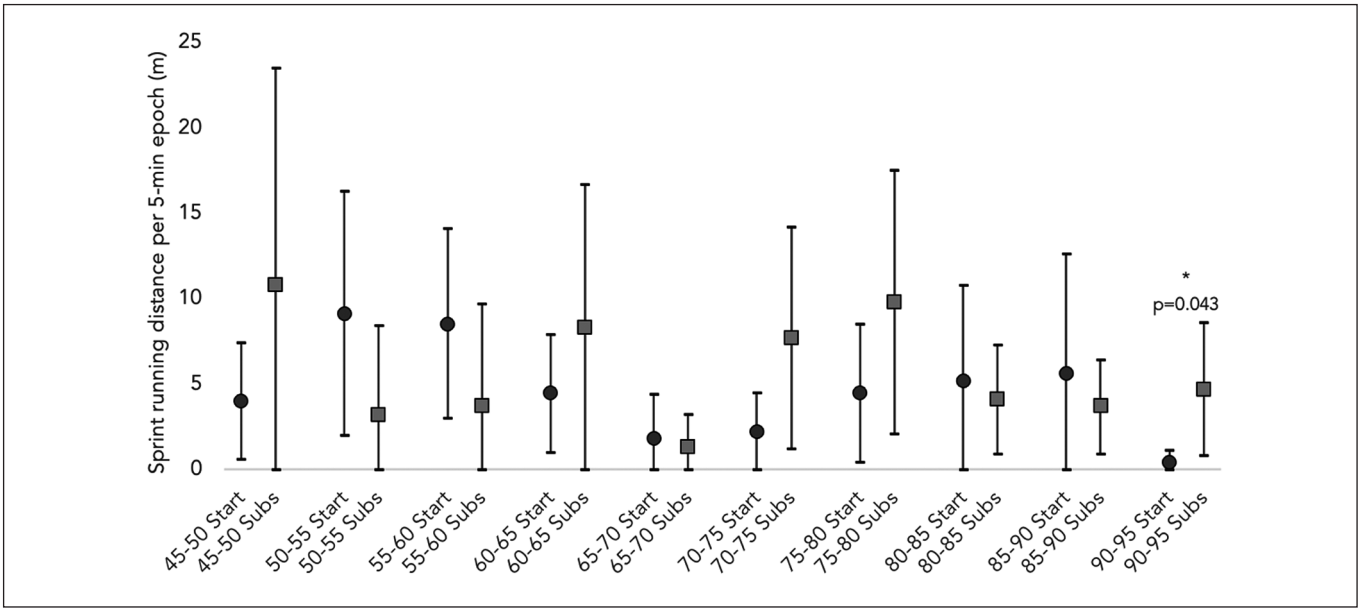


FIG. 3. Descriptive statistics (average and 95% confidence interval) for sprint running distance covered by starters and substitutes in 5-min epochs. Start: starters; Subs: substitutes.

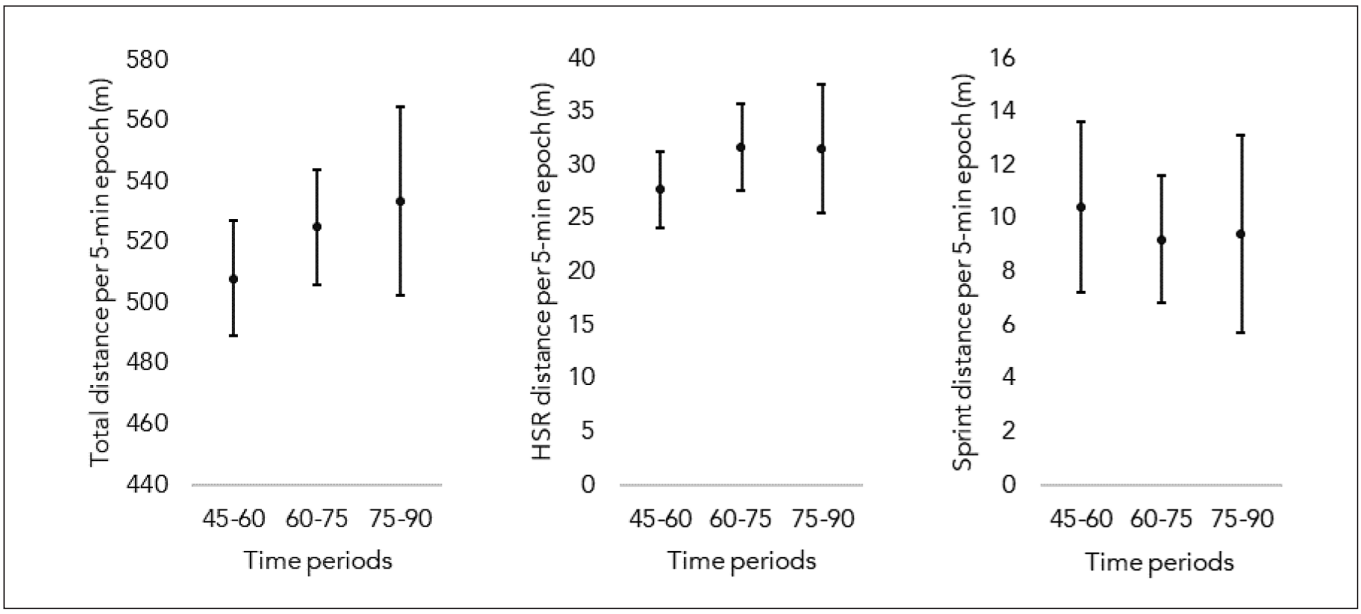


FIG. 4. Descriptive statistics (average and 95% confidence interval) of the average locomotor demands considering the period (45–60 min; 60–75 min; 75–90 min) in which the substitute entered the match.

distance ($H = 15.879$; $p < 0.001$). The starters playing in the position of midfielders covered significantly greater distances than external defenders (+15.9%; $p < 0.001$; $r = –0.446$) and forwards (+11.6%; $p < 0.001$; $r = –0.278$). Similarly, the distance covered by substitutes who played in the position ofmidfielders wassignificantly longer than the distance for external defenders (+26.1%; $p < 0.001$; $r = –0.416$) and forwards (+17.7%; $p < 0.001$; $r = –0.499$). Regarding the starters as forwards sprint distance covered was much longer than the distance covered by external defenders (+369.2%; $p < 0.001$; $r = –0.446$) and midfielders (+238.9%; $p = 0.004$; $r = –0.211$). Similarly, the substitutes playing in the position of forwards covered a significantly greater sprint distance than midfielders (+160.7%; $p < 0.001$; $r = –0.402$).

The within-playing positions analysis revealed no significant differences between the starters and the substitutes in any measures collected ($p > 0.05$).

DISCUSSION

This article compared total distance, high-speed running (HSR) distance, and sprint distance covered per 5-minute epoch by the players acting both as starters and substitutes. Concerning this aim, some differences were reported only in the last time period (90–95 m), during which the substitutes out performed the starters in sprinting. The present study also aimed to compare the locomotor demands between the moments of entering the match (45–60, 60–75 and 75–90 minutes). The comparison showed that, the moment in which the substitution took place did not influence the dependent variables. The current study also analized, the variations of the within- and between-playing positions regarding locomotor demands of the starters and the substitutes. The within-position differences were observed only for HSR among the midfielders, with higher values for the players acting as substitutes than for the starters. Finally, the between-position differences were also observed. The starter midfielders out performed external defenders and forwards in total distance. Similarly, the results were higher for the starters acting as forwards than as defenders. In addition, substitute forwards out performed substitute midfielders in sprinting.

Differences between starters and non-starters were extensively addressed in the soccer-related literature. For example, a previous study revelead that substitute players performed more high-intensity runs than starters [16]. Another study found higher workload demands of official matches for starters than for substitutes, although the non-starters out performed the starters when the variables were relativized by the actual playing time [17]. Interestingly, these acute match-related demands also impact on weekly workload, as was previously shown in the literature [18]. which reinforces the relevance of considering specific training programs for players from different groups (starters and substitutes). In the current study, the differences between starters and non-starters emerged only in the last period of the match. Specifically, the previous study did not examine when substitutes out performed starters, mostly indicating whether this had occurred. When a substitution takes place early in the second half of match play, fatigue-related detrimental effects are not supposed to commit starters, which can keep their regular

TABLE 1. Descriptive statistics (mean ± standard deviation) comparing locomotor demands in 5-min epochs between- and within-playing positions.

	External defenders	Midfielders	Forwards	Kruskal-Wallis test (Between playing positions)	Mann Whitney U p-value and r (effect size) between positions (pairwise comparison)
Total distance at 5-min epoch (m)					
Starters	485.8 ± 24.1	563.1 ± 82.5	504.4 ± 66.6	H = 29.246; p < 0.001	ED vs. MF: p < 0.001; r = –0.446 ED vs. FW: p = 0.011; r = –0.215 MF vs. FW: p < 0.001; r = –0.278
Substitutes	464.5 ± 124.6	585.9 ± 73.0	498.0 ± 76.8	H = 2.845; p = 0.241	ED vs. MF: p < 0.001; r = –0.416 ED vs. FW: p = 0.253; r = –0.149 MF vs. FW: p < 0.001; r = –0.499
Wilcoxon test for the within-playing position differences	Z = –0.454; p = 0.650	Z = –1.761; p = 0.078	Z = –0.221; p = 0.825		
HSR distance at 5-min epoch (m)					
Starters	18.8 ± 5.8	23.3 ± 10.9	24.7 ± 11.1	H = 12.153; p = 0.002	ED vs. MF: p = 0.176; r = –0.123 ED vs. FW: p = 0.100; r = –0.139 MF vs. FW: p = 0.678; r = –0.030
Substitutes	24.2 ± 12.8	31.2 ± 15.9	25.5 ± 14.4	H = 27.892; p < 0.001	ED vs. MF: p = 0.218; r = –0.155 ED vs. FW: p = 0.621; r = –0.064 MF vs. FW: p = 0.212; r = –0.127
Wilcoxon test for the within-playing position differences	Z = –1.293; p = 0.196	Z = –2.567; p = 0.010	Z = –0.295; p = 0.768		
Sprint distance at 5-min epoch (m)					
Starters	1.3 ± 3.4	1.8 ± 3.9	6.1 ± 6.9	H = 2.384; p = 0.304	ED vs. MF: p = 0.694; r = –0.036 ED vs. FW: p = 0.006; r = –0.232 MF vs. FW: p = 0.004; r = –0.211
Substitutes	4.7 ± 7.8	2.8 ± 4.8	7.3 ± 8.9	H = 15.879; p < 0.001	ED vs. MF: p = 0.253; r = –0.144 ED vs. FW: p = 0.100; r = –0.214 MF vs. FW: p < 0.001; r = –0.402
Wilcoxon test for the within-playing position differences	Z = –1.599; p = 0.110	Z = –1.372; p = 0.170	Z = –0.514; p = 0.607		

performance fully. However, the group differences become more evident when the game reaches its final stages. This is similar to a previous study that reported higher sprinting and high-intensity running actions of substitutes compared to starters during the last 15 minutes of the game [19]. These differences were evident onlyin sprinting actions which are considered key to achieve success in soccer [20–22]. Therefore, having substitute players available at final stages of a match can prove to be advantageous for the team and can increase the probability of winning, especially considering an expected drop in physical performance during the match [23]. In summary, the current study indicates the relevance of substitutes and their role in keeping (or even improving) team's performance during matches [24].

There were no differences in the physical demands between the substitutes that entered the game at different moments (considering 15-minute intervals). It could be expected that relativizing the variables for effective playing time would indicate a growing trend (i.e. higher responses for players entering the game at last stages) as players would be less exposed to fatigue effects, which was not observed. Two reasons can explain the current findings. First of all, previous studies showed no differences in physical responses of starters considering the 15-minute intervals during the first half of match

play [19, 25]. Similar results were also observed in technical actions (e.g., passes, dribles), which do not seem to change over the first half for starters when considering different game periods [26]. Therefore, it seems that it takes longer than half-match (the time interval investigated in the current study) for between-player differences to emerge. Moreover, there is a growing number of substitutions over the second half of a match [27]. Along with other actions (injuries, winning teams stalling, and others), this recurrent game stop might reduce the physical advantage of a player recently called from the bench by giving the other players more time to recover. Therefore, time-related differences might be less evident, as shown in the current results. Finally, it is worth noting that previous research has indicated that substitutes, despite their relative physical impact, are unable to replicate or exceed the peak physical demands experienced by players who participate in the entire duration of a match [28]. This finding highlights the unique challenges and demands placed on players who are on the field for the full duration of a game.

Previous studies showed playing position-related differences in soccer, although not considering the role of a starter or a substitute was not considered. Interestingly, external defenders are among the most demanded players in official matches, presenting higher values of total distance and high-intensity actions than central midfielders and forwards [29], higher values of sprinting distance than central midfielders [30], and higher values of total distance and distance at the highest speed thresholds than central midfielders and forwards [31]. This trend, however, was not observed in the current research, as external defenders did not outperform other playing positions and, in fact, underperformed in total distance and sprinting. The main reason for this might be related to the characteristics of substitutions in elite soccer. Specifically, most substitutions have offensive purposes [16], and there is preference for replacing forwards. Therefore, external defenders might not be expected to be replaced in most matches, which might require them to save energy for the whole match, even unconsciously. On the other hand, forwards can adopt an all-out strategy when they believe they will be replaced in the second half. Consequently, the workload might be higher for them when considering the whole match, which explains the current results. To support this rationale, future studies should test whether providing players with explicit information about the substitutions before they occur during simulated matches will impact players' match-related physical outcomes.

This study provides the first comprehensive assessment of the differences between starters and substitute players considering the time period and playing positions. Despite its strengths, such as the novelty, the size of the dataset, and the exciting results, there are some limitations that must be considered. First, teams are still adapting to the change of the rules relating to the number of substitutions allowed per match (from three to five). This implies that the current results might represent a transition between one and the other state, calling for follow-up designs to be confirmed. Moreover, the characteristics of the game model might influence the profile of the substitutions, which requires more contexts (e.g., leagues and clubs) to be investigated. The contextual factors (such as a match score, red cards, injuries, a match venue, and others) that might influence coaches' decisions to replace a player were not considered and remained a gap in the literature. Finally, it is important to note that the effective playing time was not taken into consideration in this study, which may have an impact on the final outcomes [32]. Future research should address this limitation by focusing on analyzing the effective locomotor demands during the actual playing time, excluding pauses and breaks.

Future research is necessary to address the aforementioned limitations and further expand our understanding in this research area. To enhance the existing knowledge base, it is recommended that future studies compare different epoch periods, taking into consideration variations in playing positions and the effects of entering the field at different periods of the match. Additionally, incorporating more detailed tactical information rather than solely contextual information can provide a more comprehensive analysis and help elucidate the factors influencing variations in locomotor performance.

Despite the limitations of our study, it is worth noting (with caution and acknowledging the low certainty of evidence) that being substitute or starting the match does not appear to have a significant impact on locomotor performance. However, it is important to recognize that confounding variables, such as situational variables, tactical behavior, and team dynamics, can heavily influence locomotor responses. On the other hand, there is greater certainty in affirming that playing position significantly influences locomotor responses, and therefore, training interventions should consider this factor to adequately prepare players for the specific demands of their positions, irrespective of whether they are starters or substitutes.

CONCLUSIONS

The substitutes out performed starters in sprinting, though the differences were observed only in the last time priod (90–95 m) The current study also compared the locomotor demands between the moments of entering the match (45–60, 60–75 and 75–90 minutes). The comparison showed that, the moment in which the substitution took place did not influence the dependent variables. Within-position differences were observed only for HSR among midfielders, with higher values for the substitutes than for the starters. Finally, between-position differences were observed in the current study. The starter midfielders out performed external defenders and forwards in total distance, which was also higher for the starter forwards compared to starter defenders. Moreover, the substitute forwards out performed substitute midfielders in sprinting.

Ethics approval and consent to participate

Medical Ethic Commity in Gdańsk (Decision number 62/2022). All participants and their legal guardians were informed about the study and signed free consent.

Consent for publication

Not applicable.

Availability of Data and Materials

All data generated or analyzed during this study are available at the request of the corresponding author.

Competing interests

The authors declare that they have no competing interests

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Technical and tactical evolution of the offensive team sequences in LaLiga between 2008 and 2021. Is Spanish football now a more associative game?

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ABSTRACT: The aim of this investigation was to study the technical and tactical evolution of the offensive team sequences in the Spanish football teams from 2008/09 to 2020/21. A comparative analysis including twelve variables related to the development of offensive sequences in 4940 matches was performed from 2008/09 to 2020/21 seasons of the Spanish professional football league (LaLiga). All match observations were recorded using a validated video tracking system. Multilevel linear mixed models were used to examine the differences across seasons, considering the effects of contextual variables. The number of passes per sequence (2.4 [CI: 2.2–2.5] vs 3.2 [CI: 3.0–3.4]; +33.3%), the passing accuracy (72.1 [CI: 70.6–73.5] vs 76.9 [CI: 75.4–78.3]%; +6.8%) and the average duration of the team sequences (6.4 [CI: 5.9–6.8] vs 8.3 [CI: 7.8–8.7] seconds; +25.76%) showed a small increasing trend over the seasons ($P < 0.05$). In contrast, variables such as the direct speed of progression (2.2 [CI: 2.1–2.3] vs 1.6 [CI: 1.5–1.7] metres/second; -24.5%), key passes (8.1 [CI: 7.6–8.5] vs 6.8 [CI: 6.3–7.2]; -15.8%), and the sequences that ended in the attacking third (64.8 [CI: 62,7–66.8] vs 57.1 [CI: 55.1–59.2]; -11.7%) or in a shot (13.0 [CI: 12.4–13.6] vs 10.2 [CI: 9.6–10.8]; -21.6%) showed a small decreasing trend from 2008/09 to 2020/21 ($P < 0.05$). Spanish professional football teams slightly evolved technically and tactically towards a more associative style of play that includes longer passing sequences. This evolution also involved a decreasing speed of progression and fewer technical actions such as through balls, key passes and shots.

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INTRODUCTION

Match analysis in football (soccer) has exponentially grown in the last decades [1] and currently football is one of the most scrutinized team sports. The development of multiple technological systems (GPS devices, multi-camera tracking systems, etc.) has led to the collection of a high quantity of data during football matches even in real time [2]. This has allowed researchers and practitioners to have more opportunities to evaluate the activity of the players in relation to the technical, tactical, physical, and psychological match performance more accurately [3]. In this developing context, recent investigations have explored the technical, tactical and physical evolution of professional football competitions such as the Spanish *LaLiga* [4, 5], the English Premier League [6, 7], the German *Bundesliga* [8] and the UEFA Champions League [9]. Mainly, these investigations have been focused on the analysis of the evolution of players' performance indicators such as the distance covered at different speeds and the number of technical actions per match. Considering that some differences can be found among competitions [10, 11], these studies revealed that high-speed running, the number of sprints and the number of passes seem to be increasing, while the total distance covered, the number of shots, crosses, tackles and clearances are showing a progressive descending trend over the seasons. In summary, these findings suggest that teams of the European football leagues are currently displaying a more possession-oriented style of play with more intense physical efforts.

Despite the relevant contribution of the above-mentioned studies, their findings are mainly based on individual technical and physical parameters, while no collective indicators were considered. In light of the necessity of exploring more interactive and representative parameters in football [12], the analysis of offensive team sequences offers key information of the collective connection of players in order to build an attack, such as number of passes per sequence, speed of progression or the final outcome of the

team sequence, which can capture the team technical and tactical behaviour and performance when in possession of the ball [13]. For instance, the analysis of offensive team sequences has been a key field of study since the beginning of football performance analysis [14]. Since then, a constant debate has been carried out by researchers about the convenience and offensive effectiveness of long versus short passing sequences [15, 16]. Furthermore, the analysis of passing sequences has also been explored through other techniques such as network analysis [17] and positional data [18].

However, although there has been a constant evolution of training methods [19, 20], match strategies [21] and match analysis methods [1, 22] in the last decade that have provided insightful scientific literature and practical applications, there is still limited evidence about how the game of football has evolved over the years. Thus, it seems necessary and very interesting to explore how offensive team sequences are evolving technically and tactically to explore the effects of training and competition methods. In this analysis, the evaluation of the offensive moment could capture key technical and tactical variables related to the speed of the ball when progressing to the opponent's goal, the duration and number of skilled actions such as passes, as well as the location of ball possession and player's movements, which are key to evaluate styles of play [23] and to achieve football success [24].

Therefore, the aim of this investigation was to study the longitudinal technical and tactical evolution of the offensive team sequences in the Spanish *LaLiga* football teams from the 2008/09 to 2020/21 seasons.

MATERIALS AND METHODS

Sample

The study sample consisted of 4940 match observations in 38 professional football teams that competed in the professional Spanish football league (*LaLiga*) in any of the thirteen seasons under investigation (from 2008/09 to 2020/21). During this period, data of offensive sequences for a total of 9880 sets of data were obtained (i.e., two sets of data per match, corresponding to the two teams competing). Data were obtained from *LaLiga*, which authorized the analysis of the variables included in this investigation and the publication of results with a scientific objective. In accordance with the ethical guidelines of *LaLiga*, this investigation does not include information that identifies football players.

Procedure

This investigation is a descriptive and comparative analysis to determine the evolution of offensive team sequences in the first division of Spanish football for thirteen seasons. The technical and tactical characteristics of each offensive team sequence were collected by

TABLE 1. Description and definition of the technical and tactical variables evaluated in the present study.

Dimension	Variables	Description
Characteristics of team sequences	Number of sequences	Technical variable that quantifies the total number of offensive team sequences registered per team in a match.
	Sequence width	Tactical variable that quantifies the average distance between the leftmost point and the rightmost point reached by the ball in the offensive sequence, in metres.
	Sequence length	Tactical variable that quantifies the average distance that the ball travelled forward during each sequence per team and match, in metres.
	Sequence time	Tactical variable that quantifies the average duration of the offensive team sequences per match, in seconds.
Passing performance	Total passes	Technical variable that quantifies the total number of passes performed per team during the match.
	Passing accuracy (%)	Technical variable that quantifies the percentage of passes that are completed over the total number of passes per match.
	Passes per sequence	Tactical variable that quantifies the average number of passes performed per team during the team offensive sequences.
	Direct speed	Tactical variable that quantifies the average distance that the ball moved towards the opponent goal line during the sequence per second, in metres per second (m/s).
Offensive performance	Through balls	Technical variable that quantifies the total number of passes that penetrated through the opposing defensive line per team during the match.
	Key passes	Technical variable that quantifies the total number of passes that allow the recipient of the ball to directly shoot at goal per team during the match.
	Sequences that end in the attacking third	Tactical variable that quantifies the total number of offensive team sequences that enter and finish in the final third of the field during the match.
	Sequences that end in a shot	Tactical variable that quantifies the total number of offensive team sequences that lead to producing a shot during the match.

TABLE 2. Comparative analysis of tactical variables between the seasons 2008/2009 (reference) and 2020/2021.

Tactical variables	Coefficient	SE	*P*	ES
Number of sequences	-19.203	0.94	< 0.001	*Large*
Sequence width	0.016	0.13	0.908	*Trivial*
Sequence length	-1.236	0.15	< 0.001	*Large*
Sequence time	1.856	0.09	< 0.001	*Small*
Total passes	67.071	4.57	< 0.001	*Small*
Passing accuracy (%)	4.839	0.35	< 0.001	*Small*
Passes per sequence	0.814	0.03	< 0.001	*Small*
Direct speed	-0.530	0.02	< 0.001	*Small*
Through balls	-3.197	0.12	< 0.001	*Small*
Key passes	-1.278	0.19	< 0.001	*Small*
Sequences that end in the attacking third	-7.614	0.81	< 0.001	*Small*
Sequences that end in a shot	-2.762	0.24	< 0.001	*Medium*

SE = standard error; P = generalized mixed linear model. ES=*effect size (F^2 statistic).*

Mediacoach, a video tracking system that can validly assess teams' match statistics during match play [25]. Mediacoach collects data by using information from OPTA Sportsdata and then organizes the information to facilitate the analysis of variables for professional football teams. Recent investigations have presented the analysis of several of these data such as match statistics [26] and offensive and defensive playing style variables [27]. With this system, all the team sequences produced in each *LaLiga* match are automatically recorded and analyzed. Operationally, an offensive team sequence is defined as a passage of play in possession of the ball that belongs to one team and is ended by defensive actions, stoppages in play or a shot. For each offensive team sequence, a total of twelve technical and tactical variables were analyzed to describe the general characteristics of the team sequences, as well as the passing and the offensive performance (see Table 1). On one hand, this analysis includes collective tactical variables such as number of sequences per match, sequence width, length, duration, passes per sequence, direct speed, sequences that end in the attacking third and sequences that end in a shot. On the other hand, technical variables such as number of passes per match, passing accuracy, key passes and through passes are also included in the study.

Statistical analysis

The data were transferred from Mediacoach to a .csv database which was organized in Microsoft Excel. All statistical analyses were carried out using the software IBM SPSS Statistics Version 27.0. Due to the hierarchical structure of teams' performance in football (each team has its own tactical style), a multilevel mixed model [28] was performed to cluster the collective performance (level 2) into teams (level 1). With this organization of the data, a generalized linear model was carried out to explore the longitudinal effect of the season (fixed effects) on the different tactical variables evaluated in this study, considering the effect of the team (random effects). Thus, the "team effects" represented unobserved team characteristics that influence the collective performance and account for the non-independence of the data [29]. To consider the possible contextual effects [30], the model included as fixed effects other four contextual variables: match location (home versus away), match outcome (win versus draw vs lose), ranking of the opponent in quartiles (first, second, third and fourth) and ranking of the team in quartiles (first, second, third and fourth). The Cohen F^2 statistic was calculated as the effect size of the fixed effects [31]. The Cohen F^2 effect size is a measure of the proportion of variance in the outcome explained by the fixed effects included in the model. In this regard, it was considered a trivial effect at a value lower than 0.02, small at a value of 0.02, medium at a value of 0.15 and large at a value of 0.35 [32].

Graphic charts with the predicted means and confidence intervals were displayed to show the longitudinal evolution of the different tactical variables throughout the seasons according to the generalized mixed linear model. Pairwise comparisons of the estimated means were performed through Fisher's least significant test. The significance level was set to $P < 0.050$.

RESULTS

Table 2 shows the comparison of data between the 2008/2009 and 2020/2021 seasons. All variables, except for "sequence width" were different between the seasons ($P < 0.05$). Particularly, the number of sequences largely decreased over the seasons ($P < 0.05$), while the sequences length moderately decreased ($P < 0.05$) and the sequence time had a small increase over the years ($P < 0.05$).

Regarding the passing performance, the total number of passes, passing accuracy and passes per sequences showed a small increase in the last years ($P < 0.05$), while the direct speed increased (small size effect, $P < 0.05$). As for the offensive indicators, the number of through balls, key passes and sequences that end in the attacking third and in a shot significantly decreased from 2008/2009 to 2020/2021.

Figure 1 depicts the longitudinal evolution of the number of sequences per match, sequence width, length, and duration. In comparison to the 2008/09 season, the number of sequences started to decrease in the 2016/17 season ($p < 0.05$) and remained lower in the last five seasons under investigation. The sequence width remained stable through the seasons, rounding to the average of 34 metres, while the length of the sequences showed a descending trend over the seasons, initiating this decline in the 2014/15 season ($p < 0.05$). Regarding the average duration of teams' sequences, a significant increasing trend was found ($P < 0.05$), especially from the season 2014/15 onwards, when the average time of team sequences changed from 6.4 seconds to 8.3 seconds (+33.3%).

Figure 2 shows that total passes per match (+18.7%), passing accuracy (+6.8%) and the number of passes per sequence (+34.4%) registered an increasing and constant trend over the seasons ($P < 0.05$). Otherwise, the direct speed of progression showed a decreasing trend, especially from the 2013/2014 season onwards ($P < 0.05$).

Regarding the offensive indicators, the number of key passes significantly decreased from the 2014/15 season ($P < 0.05$), while the number of through balls started a decreasing process from the 2011/12 season onwards ($P < 0.05$). Finally, the number of sequences that ended in the attacking third gradually decreased ($P < 0.05$). Also,

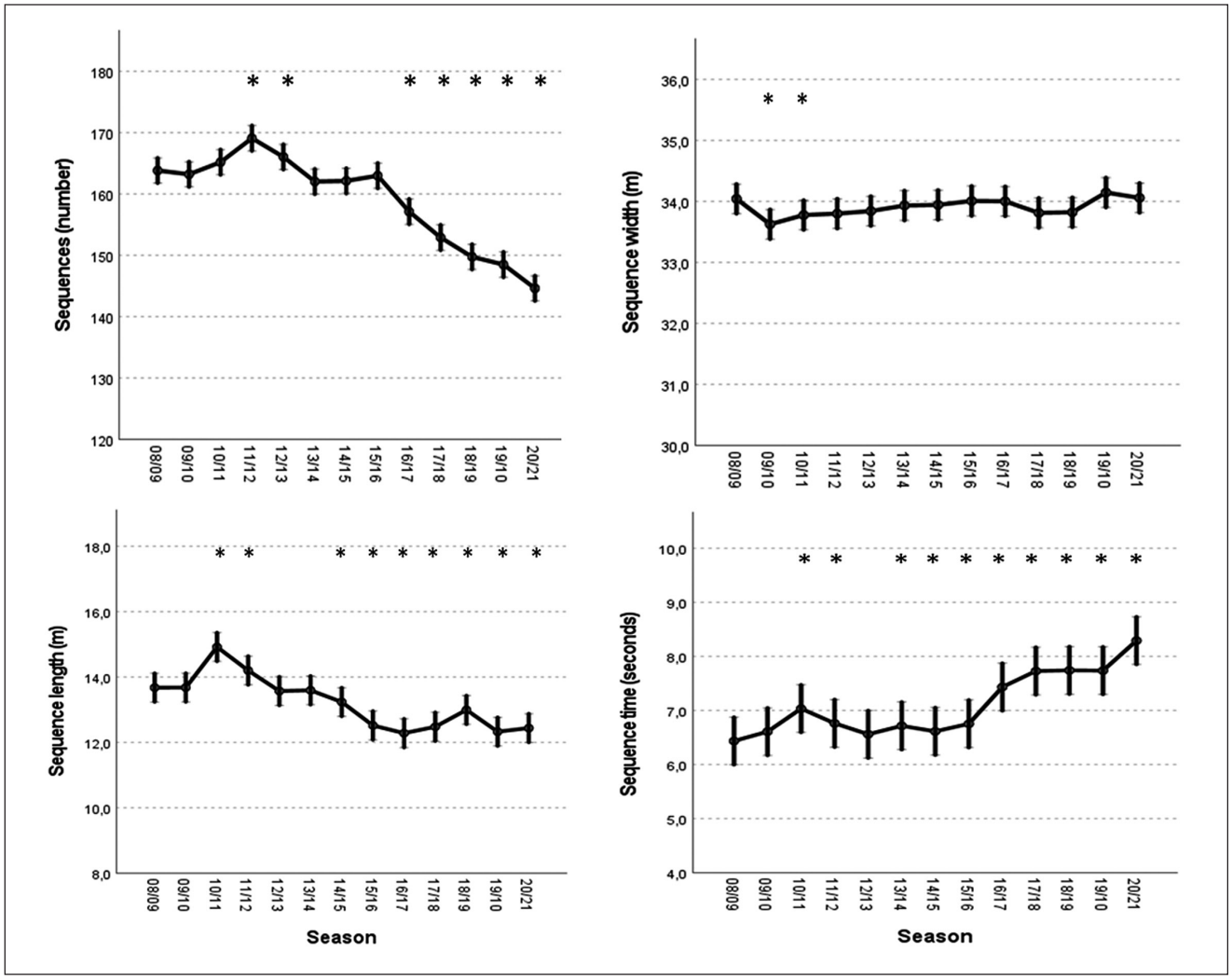


FIG. 1. Predicted means and 95% confidence intervals for the variables "sequences", "sequence width", "sequence length" and "sequence time" in *LaLiga* from 2008/09 to 2020/21 according to the generalized mixed linear model.
* = Significantly different from the 2008/2009 season (Fisher's least significant test) ($p < 0.05$).

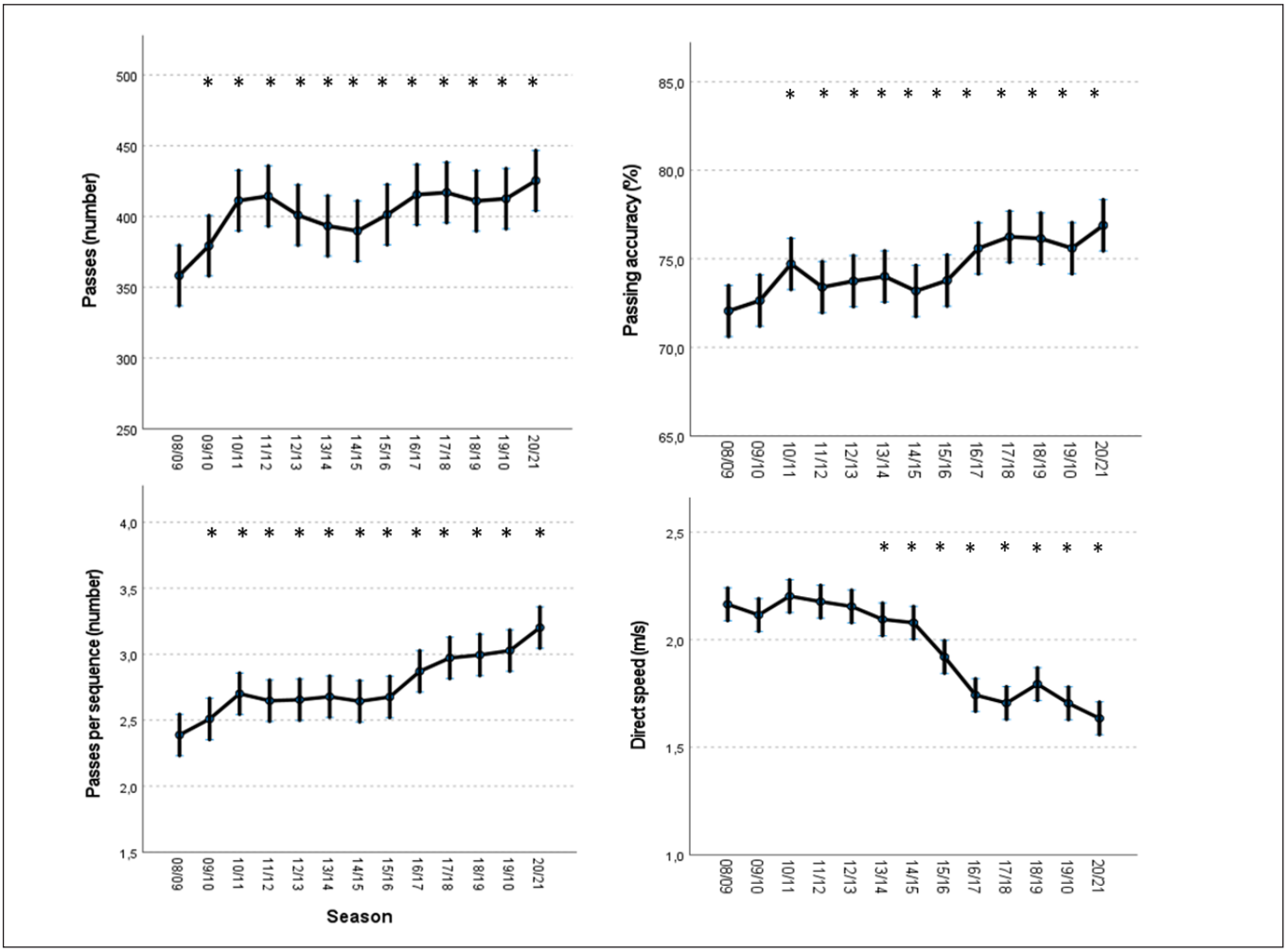


FIG. 2. Predicted means and 95% confidence intervals for the variables "passes", "passing accuracy", "passes per sequence" and "direct speed" in *LaLiga* from 2008/09 to 2020/21 according to the generalized mixed linear model.
*= Significantly different from the 2008/2009 season (Fisher's least significant test) ($p < 0.05$).

the number of sequences that ended in a shot decreased ($p < 0.05$) with statistically significant differences between the 2008/09 season and all the seasons after the 2011/12 season (Figure 3).

DISCUSSION

The aim of this investigation was to study the technical and tactical evolution of the team offensive sequences in *LaLiga* football teams from the 2008/09 to the 2020/21 seasons. The main outcome of this investigation was that offensive team sequences in *LaLiga* have significantly and slightly changed towards a lower number but longer offensive sequences, including a small increase in passes, accuracy, and duration. However, the number of sequences that ended in the attacking third or ended in a shot, as well as the number of technical actions such as through balls and key balls, progressively and slightly decreased. Collectively, this information indicates that *LaLiga* football teams evolved tactically towards a more associative style of play that includes longer passing sequences at the cost of decreasing speed of progression during the game. These results also suggest that football teams now produce more elaborate sequences until the opportunity to attack is present, as the number of goals has not changed [4], despite there being a lower number of sequences that end in the attacking third and those ending in a shot.

Our study revealed that the number of passes per match, the number of passes per sequence and the average duration of each sequence showed a small increasing trend over the seasons. Although existing literature has reported that the Spanish *LaLiga* is characterized by a more combinative style of play in comparison to other leagues [10, 33], this study confirms that this style is the result of a constant evolution, at least since the 2008/09 season. In line with these results and focusing on the role of specific playing positions, Lago-Peñas et al. [5] found that, in *LaLiga*, central backs significantly increased the number of total passes and the number of long passes from 2012 to

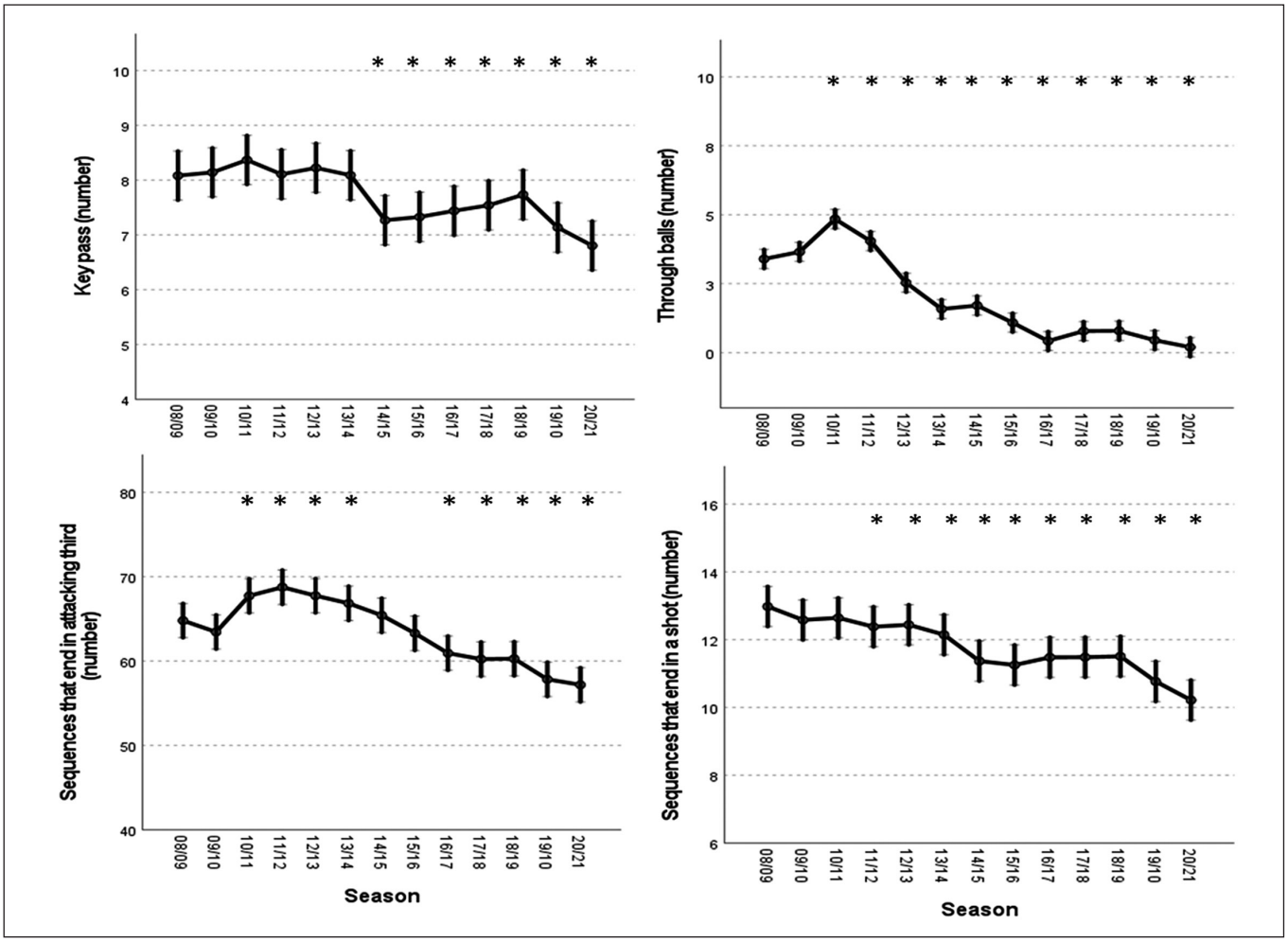


FIG. 3. Predicted means and 95% confidence intervals for the variables "key pass", "through balls", "sequences that end in the attacking third" and "sequences that end in a shot" in *LaLiga* from 2008/09 to 2020/21 according to the generalized mixed linear model.
*= Significantly different from the 2008/2009 season (Fisher's least significant test) ($p < 0.05$).

2019. This indicates that central backs are now more involved in the build-up phase of the game and suggests that offensive sequences are now formed further from the opposing team's goal. Additionally, the direct speed, a variable that measures the capacity of the team to progress in each sequence, as the average speed of ball movement towards the opponent's goal line during the sequence, has decreased, suggesting that sequences are now more horizontal, reducing the use of direct play game styles.

In this regard, Yi et al. [9] recently observed that teams in European leagues are now more focused on the control of match play by increasing passing frequency and accuracy, which coincides with the outcomes of the current investigation. Other studies have also reported an increasing passing performance in English Premier League from 2006 to 2013 [7] and the German *Bundesliga* from 2014 to 2017 [8]. In contrast, Zhou et al. [34] did not find any increase in the passing performance in the Chinese Super League from 2012 to 2017. Although these results reflect the different technical and tactical development of each domestic competition according to cultural, economic, and social dimensions, it seems that there is an international tendency for a higher passing frequency in elite football teams, converting football today into a more combinative sport than it was a decade ago.

In the Spanish context, this technical and tactical evolution towards a more associative game seems to have had a small boost during the 2010/11 season. In this season, the number of passes per match increased by 8.4% from the previous season (Figure 2), with 8.0% more passes per sequence. It is interesting to mention that this season took place just after the Spanish National team won the 2010 World Cup tournament in South Africa with a very popular possession-oriented style of play [35, 36]. Also, the 2010/11 season was played two years after Pep Guardiola took over FC Barcelona in a very successful period that started by winning one

Champions League title and two consecutive *LaLiga* trophies with a style of play characterized by making a great number of passes and creating constant interactions and connections between players [37]. Thus, the possible effect of these two successful teams that implemented possession-oriented styles of play may have influenced the rest of the Spanish teams to adopt more associative passing sequences in the next years to try to achieve higher success.

Despite the small increase in the number of passes and time per sequence, our study found that the length of the sequences and the speed of progression showed a small descending trend over the seasons. From a practical perspective, these two changes suggest that teams progressed a shorter distance than before, despite using longer sequences. Furthermore, the number of through balls and key passes also showed a descending trend. Although further research is needed to understand the current attributes of passing in football, these results do not indicate that passing tempo or ball speed has decreased, but that teams now decide to produce more elaborate plays with more horizontal and backward passes until a favorable moment to attack and progress is obtained. Additionally, these findings suggest that teams could have strengthened their defensive organization [4], and pressure on the ball, which would make it more difficult to penetrate through the opposing lines, forcing attacking teams to display more passing combinations to overcome the defensive teams. This tactical scenario with both a more offensive combination and defensive organization could reduce the opportunities for the teams to have moments of transitions from defense to attack to recover the ball, which is very effective to create goal-scoring opportunities [38, 39]. Similar to these results, Konefal et al. [8] suggested that the evolution of football seems to be directed towards more collective and organized tactics, reflecting a better understanding of the tactical roles of players. These technical and tactical features could have a physical impact so that this better collective organization could help players to optimize their physical efforts and save energy to perform higher-intensity actions during the game, which is another new feature of modern football [40]. In line with this interpretation, the recent study by Lago-Peñas et al. [5] recorded a reduction in the total distance covered by *LaLiga* players, while the number of efforts made at high-intensity running increased in recent seasons.

As for the offensive performance, our data revealed that those sequences that ended in the attacking third or achieved a shot showed a descending trend over the seasons. Our results support the findings of Errekagorri et al. [4], which demonstrated how Spanish teams produced a smaller number of technical actions such as shots and crosses in recent seasons. These facts are surely aligned with the previous findings that indicated fewer through balls and key passes, which is probably due to the better protection of key spaces by the defensive teams. These technical and tactical characteristics seem to create a more controlled and predictable offensive context where penetrating into the final third of the pitch or achieving a shot could become more difficult than several years ago.

This study is not exempt from limitations. Firstly, it is crucial to mention that this investigation was performed following a static approach [41], where data are collected per match without considering the contextual variables that change throughout the match. Additionally, the current study was carried out with data from a national football league of elite male players and the results of the investigation should not be extrapolated to other leagues, other categories, or to women's football.

CONCLUSIONS

The current data show that football teams competing in *LaLiga* have slightly evolved technically and tactically towards a more associative and combinative style of play that includes longer passing sequences both in time and quantity of passes. This technical and tactical evolution also presents a decreasing speed of progression and distance progressed in each sequence, as well as a small decrease in the number of through balls, key passes, and shots.

As practical applications for football coaches and sporting directors, it seems that football tactics are in a slight evolution towards a more associative style of play with less offensive verticality and penetration, which requires coaches not only to train players to be accurate in their actions to possess the ball but also to be able to acquire excellent dribbling or passing skills to disrupt the defensive organization of the opposing team. Additionally, as the number of through balls, key passes, and shots has been decreasing in recent years, having players with attributes to produce these actions may be key to having a successful football squad. The knowledge provided by this study encourages football coaches and analysts to reflect on the actual development of football tactics.

Disclosure of interest

The authors report no conflict of interest.

Data availability statement

The data that support the findings of this study are available from *LaLiga*. Restrictions apply to the availability of these data, which were used under license for this study. Data are available from the authors with the permission of *LaLiga*.

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Is muscle stiffness a determinant for range of motion in the leg muscles?

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ABSTRACT: Previous training studies with comprehensive stretching durations have reported that an increase in range of motion (ROM) can be related to decreases in muscle stiffness. Therefore, the purpose of this study was to analyze the association between the passive muscle stiffness of three muscle groups (triceps surae, quadriceps, hamstrings) to the respective joint ROM. Thirty-six healthy male soccer players volunteered in this study. After a standardized warm-up, the muscle stiffness was tested via shear wave elastography in six muscles (gastrocnemius medialis and lateralis, rectus femoris, semitendinosus, semimembranosus, and biceps femoris long head). The hip extension, hip flexion, and ankle dorsiflexion ROM were also assessed with a modified Thomas test, a sit and reach test, and a standing wall push test, respectively. We found significant moderate to large correlations between hip flexion ROM and muscle stiffness for the semimembranosus ($rP = –0.43$; $P = 0.01$), biceps femoris long head ($rP = –0.45$; $P = 0.01$), and overall hamstring stiffness ($rP = –0.50$; $P < 0.01$). No significant correlations were found for triceps surae ($rP = –0.12$; $P = 0.51$ to 0.67) and rectus femoris muscle stiffness ($rP = 0.25$; $P = 0.14$) with ankle dorsiflexion and hip extension ROM, respectively. We conclude that muscle stiffness is an important contributor to hip flexion ROM, but less important for hip extension or ankle joint ROM. Additional contributors to ROM might be tendon stiffness or stretch/pain tolerance.

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INTRODUCTION

Methods such as stretching and foam rolling can increase the range of motion (ROM) acutely (stretching [1–3], foam rolling [4, 5], or the combination of stretching and foam rolling [6, 7]]) as well as in the long term (stretching [8, 9], foam rolling [10]). Two major mechanisms have been reported to be involved in the changes in ROM. On the one hand, an increase in stretch tolerance (i.e., higher tolerated torque) seems to be the most common mechanism for ROM increases after both an acute stretch or foam rolling intervention [11, 12] and after long-term interventions with these modalities [13, 14]. On the other hand, a decrease in muscle stiffness has been reported to be another mechanism for an increase in ROM after acute static and proprioceptive neuromuscular facilitation stretching (but not after dynamic stretching) interventions of > 60 s, as well as after foam rolling [15, 16]. Such decreases in muscle stiffness have also been observed following several weeks of high-volume stretching (i.e., > 30 min a week per muscle group) [17], but not following long-term foam rolling [13]. These training-induced changes in muscle stiffness after stretching (acute and long term) and foam rolling (acute) indicate a causal correlation between changes in muscle stiffness and ROM. However, to date, it is not clear if joint ROM is related to stiffness of the surrounding muscle groups. While studies have reported a correlation between some leg muscle stiffness and ROM, this seems to depend on age, sex, and muscle [18–20]. More specifically, Hirata et al. [20] reported a significant correlation between gastrocnemius medialis and gastrocnemius lateralis muscle stiffness and ankle dorsiflexion ROM in young but not in older participants, measured in a 15° dorsiflexion position. In addition, Miyamoto et al. [18] reported such correlations at 0° ankle angle (gastrocnemius medialis + gastrocnemius lateralis to ankle dorsiflexion ROM) in young male participants but not in young female participants. Moreover, a correlation between the hamstring muscles (semimembranosus, semitendinosus, and biceps femoris long head) and hip flexion ROM was detected in young participants, but without analyzing sex-specific relationships [19]. This was in line with an earlier study which reported that hip flexion ROM is limited by hamstring muscle-tendon unit stiffness [21], without distinguishing between isolated muscle and tendon stiffness, respectively. However, to the best of our knowledge, no study to date has analyzed the association between rectus femoris muscle stiffness and hip extension ROM. Furthermore, no study to date has performed a correlation analysis of all joints and related muscles in the leg within one project.

Additionally, concerning soccer players it is well known that lower joint ROM [22] and higher muscle stiffness [23] can lead to

a higher injury prevalence. Thus, it would be important to understand the association between lower leg ROM to muscle stiffness especially in soccer players.

Therefore, the aim of this study was to investigate the correlations between the passive muscle stiffness of three muscle groups (triceps surae, quadriceps, and hamstrings) and the respective joint ROM in recreational soccer players. We hypothesized that local muscle stiffness would correlate with the respective joint ROM.

MATERIALS AND METHODS

Participants

An a priori power analysis, based on the results of Hirata et al. [20] revealed an optimal sample size of 27 participants (correlation: bivariate normal model, pH1 = 0.495, $\alpha = 0.05$, $\beta = 0.80$). Therefore, to account for dropout, we recruited 36 healthy male, recreational soccer players from 3rd to 6th Austrian league (training frequency: 3 to 4 times per week + 1 game at the weekend, age: 23.36 ± 4.11 years; height: 181.8 ± 5.2 cm; body mass: 81.2 ± 6.8 kg) to participate in this study. Minimum 6 month prior the study participants were free of any injuries or neuromuscular disorders. The participants were asked to avoid strenuous exercises 72 h prior to the test and should avoid physical training on the test day before the test. All participants signed a written informed consent form. The study was approved by the Ethics Committee of the University of Graz (approval code: GZ. 39/68/63 ex 2020/21) and was performed according to the Declaration of Helsinki.

Experimental design

Participants visited the laboratory on two separate days. The first appointment was to familiarize the participants with the test procedure. During the second appointment, the data acquisition in the dominant leg (used for kicking a ball) was undertaken. Prior to the measurements, each participant performed a 5-min warm-up on a stationary cycle ergometer (Monark, Ergomedic 874 E, Sweden) at a cadence of 60 rev/min [24] and a resistance of 60 W. Following the warm-up and after positioning the participant for the measurement (about 5 min in between warm up and start of the first measurement) shear wave elastography (SWE) of the dominant leg of six leg muscles (gastrocnemius medialis and gastrocnemius lateralis, rectus femoris, semitendinosus, semimembranosus, and biceps femoris long head) was performed to determine muscle shear modulus as an indicator for muscle stiffness. The ROM of ankle dorsiflexion (standing wall push), hip extension (modified Thomas test), and hip flexion (sit and reach test) was then tested. During the SWE measurements, the surface electromyography (sEMG) was visually monitored on one muscle of each of the three muscle groups of the leg (gastrocnemius lateralis, vastus lateralis, and biceps femoris long head), which allowed us to confirm that the participant was in a rested state.

Measurements

Shear wave elastography (SWE)

The SWE values were measured with an ultrasound scanner (Aixplorer V12.3, Supersonic Imaging, Aix-en-Provence, France) in combination with a linear transducer array (4–15 MHz, SuperLinear 10-2, Vermon, Tours, France) in the six leg muscles. The measurements were done by a qualified tester with ~4 years experience who tested all subjects. The scanner was used in SWE mode (musculoskeletal preset, penetration mode, smoothing level 5, persistence off, scale 0–300 kPa). Per muscle, 3 videos with 15 s each were obtained. The SWE values were analyzed with MATLAB R2017b (Math-Works, Natick USA) and the mean of five consecutive frames with the lowest SD within the range of interest within each video was calculated [25]. The final values for the muscle stiffness were calculated as the mean between the two closest values of the three videos and was divided by 3 to convert the shear wave speed to shear modulus [25]. A handheld technique without any stabilizing support or guiding rail was utilized during the measurements [26, 27]. The tester needed to keep the same probe position without any movement during the whole measurement duration.

To measure the shear modulus of the plantar flexor muscles, the participant was positioned prone in a dynamometer (CON-TREX MJ, CMV AG, Duebendorf, Switzerland) with the hip and knees fully extended (180°, respectively) and the ankle at neutral position (90°). The GM was first measured around the proximal third between the calcaneus and the popliteal fossa. The gastrocnemius lateralis was then measured at the same distance between the heel and knee but on the lateral side of the calf. For the SWE measurements in the rectus femoris, the participant was seated on a dynamometer, while the knee angle was set to 70° and the hip remained at 110° [28]. The rectus femoris was measured around the distal third of the distance between the proximal edge of the patella and the iliac spine [29]. For the shear modulus measurement of the hamstring muscles, the participant was positioned next to the dynamometer in a supine position with a hip angle of 90° and knee angle of 120° to achieve a slightly stretched position of the hamstring muscles [28]. The measuring position for the semitendinosus was distal to the tendinous insertion [25, 30] and the measurement of the biceps femoris long head was performed about half way between the popliteal fossa and the ischial tuberosity on the lateral side of the back thigh [25, 30]. The semimembranosus was measured more medial and more distal than the measuring position of the semitendinosus [25, 30].

The measurement position of the transducer for each muscle was determined during the familiarization session and was marked on a reusable foil [16]. The probe was aligned with fascicle orientation and kept in place for the whole measurement process [31]. Pressure on the skin was avoided to not influence tissue or muscle structure [32]. A conditioning procedure with passive stretches controlled in the dynamometer was performed prior to the SWE to guarantee the same muscle condition in all participants. The angle range of the conditioning was the same for all participants and was chosen

carefully to not stretch the tissue too much prior the SWE measurement. The range of interest (ROI) during the measuring process was set centrally and maximized as much as possible, but without including any aponeuroses. The participant was asked to relax completely and avoid any movement during the measurement. This was confirmed by sEMG, as values up to 5% of maximal isometric contraction activation were tolerated. For each muscle, three videos of 15 s each were recorded. For the analysis, the mean of five consecutive frames with the lowest standard deviation of the averaged shear modulus of the ROI within each video was considered. To calculate the mean passive stiffness of a muscle, the two closest mean values out of the three videos were taken [25]. The reliability of all the SWE assessments in any muscle was confirmed in previous experiments [4, 16, 33]. Furthermore, the mean SWE values of all the respective muscle groups where more than one muscle was assessed (i.e., plantar flexors (gastrocnemius medialis + gastrocnemius lateralis), hamstrings (semitendinosus + semimembranosus + biceps femoris long head)) were also calculated as a proxy for overall muscle group stiffness.

Range of motion (ROM)

The ROM measurements of the dorsiflexion and hip extension were tracked with a 3D motion capture system (Qualisys, Gothenburg, Sweden). Eight cameras were used, and reflective markers (diameter: 1 cm) were positioned on the participant's hip and dominant leg according to the Qualisys Gait module "CAST lower body marker set". Two additional markers were positioned on the right and left iliac crest to ensure proper tracking during the hip extension ROM in supine position. Firstly, the dorsiflexion ROM was tested with the standing wall push exercise. The exercise was repeated three times for 5 s each time. The starting position was standing upright in front of a wall. The hands were positioned on the wall at shoulder height and width. After the start command, the participant was asked to move the dominant leg behind the body as far as possible and position it with extended leg and the heel touching the ground. The toes of both legs were front facing. To reach the maximum dorsiflexion ROM at the point of discomfort in the stretched calf muscles of the dominant leg, the knee of the other leg could also be flexed. To test hip extension ROM, the participant was asked to perform three modified Thomas tests [34] with the dominant leg, each for 5 s (in the end position). In each test, the participant lay supine on a medical bed, with the gluteal fold right behind the edge of the bed, and the hip was flexed to 90° with knees fixed by hands with extended elbow joints. The extended elbows ensured the same positioning for each participant and also helped to maintain the contact of the lumbar spine with the medical bed during the test to avoid pelvic tilt during the movement [35]. The participant was asked to relax their legs completely. The contralateral leg was held in position with both hands while the dominant leg was lowered unassisted toward the floor until the end position in a relaxed state was reached. Moreover, to test hip flexion ROM, the participant performed three sit and reach tests with the help of a Sit n' Reach trunk flexibility box (Fabrication Enterprises; Baseline Model 12-1086, New York, USA). The participant was positioned sitting on the ground in front of the flexibility box with the whole sole of each foot touching the box and the knees fully extended and relaxed. For the starting position, the trunk was kept upright and the arms were held parallel to the ground. The task was to move the slider on top of the flexibility box slowly as far in the direction of the toes (and further) as possible. The knees were kept in a completely extended position during the forward bend procedure. Moreover, both hands were on top of each other during the pushing phase to minimize possible trunk rotation during the hip flexion. The value reached in the maximum forward bend position was noted.

The camera system was calibrated at the beginning of each test day and the data of each trial was controlled for completeness after the measurement. Only trials with clear visibility of all markers during the ROM movement were taken for analysis. If the data of a trial was not complete (i.e., markers were missing) one more trial was conducted. For the analyzing procedure, the data points of the reflective markers were labeled within Qualisys and then exported to Visual 3D, a biomechanical modeling software (Velamed GmbH – Science in Motion, Köln, Germany) to calculate the joint angles within the single ROM-tests. These joint angles were exported to a spreadsheet and the best attempt out of the three was then chosen for further analysis. If an evasive movement in any joint in any of the tests was detected, the attempt was repeated.

Surface electromyography (sEMG)

SEMG (Myon320, myon AG, Zurich, Switzerland) was used to monitor the muscle activation during SWE testing. Skin preparation and surface electrode positioning (BlueSensor N, Ambu, A/S, Ballerup, Denmark) were performed according to SENIAM recommendations [36] on the muscle belly of the vastus lateralis, biceps femoris long head, and gastrocnemius lateralis. The signal was sampled at 2000 Hz and normalized by a maximal voluntary isometric contraction. If any muscle activation was detected during the SWE assessments (exceeding 5% of maximal muscle contraction, [37]), the trial was repeated. The data were checked live during the SWE assessment. If any abnormality was found during the SWE assessment in the raw sEMG the data was further processed by performing a high-pass filtered (10 Hz Butterworth) and root-mean square (RMS, 50 ms window).

Statistics

For the statistical analysis, SPSS (version 28, SPSS Inc., Chicago, Illinois) was used and the normal distribution was tested with the Kolmogorov-Smirnov test. In the case of a normal distribution, Person's correlation coefficient (rP) was used to determine the correlations between the ROM and SWE variables of the respective joints. If the values showed no normal distribution (semitendinosus shear modulus data only), Spearman's rho (rS) was calculated. The effect size of the

correlation coefficients was assessed according to the suggestions of Hopkins [38], i.e., trivial (0–0.1), small (0.1–0.3), moderate (0.3–0.5), large (0.5–0.7), very large (0.7–0.9), and nearly perfect or perfect (0.9–1). The 95% confidence intervals (CIs) for the correlations were also calculated. The alpha level was set to 0.05.

RESULTS

Correlation analysis of plantar flexor muscle shear modulus (i.e., stiffness) and ankle dorsiflexion range of motion

The correlation analysis revealed no significant relationship between the muscle shear modulus of the gastrocnemius medialis (rP = –0.12; P = 0.51; 95% CI = –0.43 to 0.22) or gastrocnemius lateralis (rP = –0.07; P = 0.67; 95% CI = –0.39 to 0.26) and the dorsiflexion RoM.

Moreover, no correlation was detected between the mean shear modulus of the gastrocnemii (gastrocnemius medialis +gastrocnemius lateralis) and the dorsiflexion ROM (rP = –0.12; P = 0.51; 95% CI = –0.43 to 0.22).

Correlation analysis of rectus femoris muscle shear modulus (i.e., stiffness) and hip extension range of motion

The correlation analysis revealed no significant relationship between the muscle shear modulus of the rectus femoris (rP = 0.25; P = 0.14; 95% CI = –0.09 to 0.53) and the hip extension ROM.

Correlation analysis of hamstring muscle shear modulus (i.e., stiffness) and hip flexion range of motion

The correlation analysis revealed a significant moderate negative relationship between the shear modulus of the semimembranosus (rP = –0.43; P = 0.01; 95% CI = –0.67 to –0.12) and biceps femoris long head (rP = –0.45; P = 0.01; 95% CI = –0.68 to –0.14) and hip flexion ROM. However, there was no correlation between the semitendinosus (rS = –0.10; P = 0.57; 95% CI = –0.42 to 0.25) and hip flexion ROM.

Moreover, a significant large correlation was detected between the mean shear modulus of the hamstring muscles (semimembranosus + semitendinosus + biceps femoris long head) and the hip flexion ROM (rP = 0.50; P < 0.01; 95% CI = –0.71 to –0.21).

The scatter plots for all the correlations of the hamstring muscle SWE to hip flexion ROM are presented in Figure 1.

DISCUSSION

The purpose of this study was to investigate if passive muscle stiffness of three muscle groups (triceps surae, quadriceps, and hamstrings) is related to the respective joint ROM. We found a significant small to large negative correlation between hip flexion ROM and the stiffness of the semimembranosus (rP = –0.43), biceps femoris long head (rP = –0.45), and the overall hamstrings (rP = –0.50), which indicates that higher stiffness causes lower hip flexion ROM.

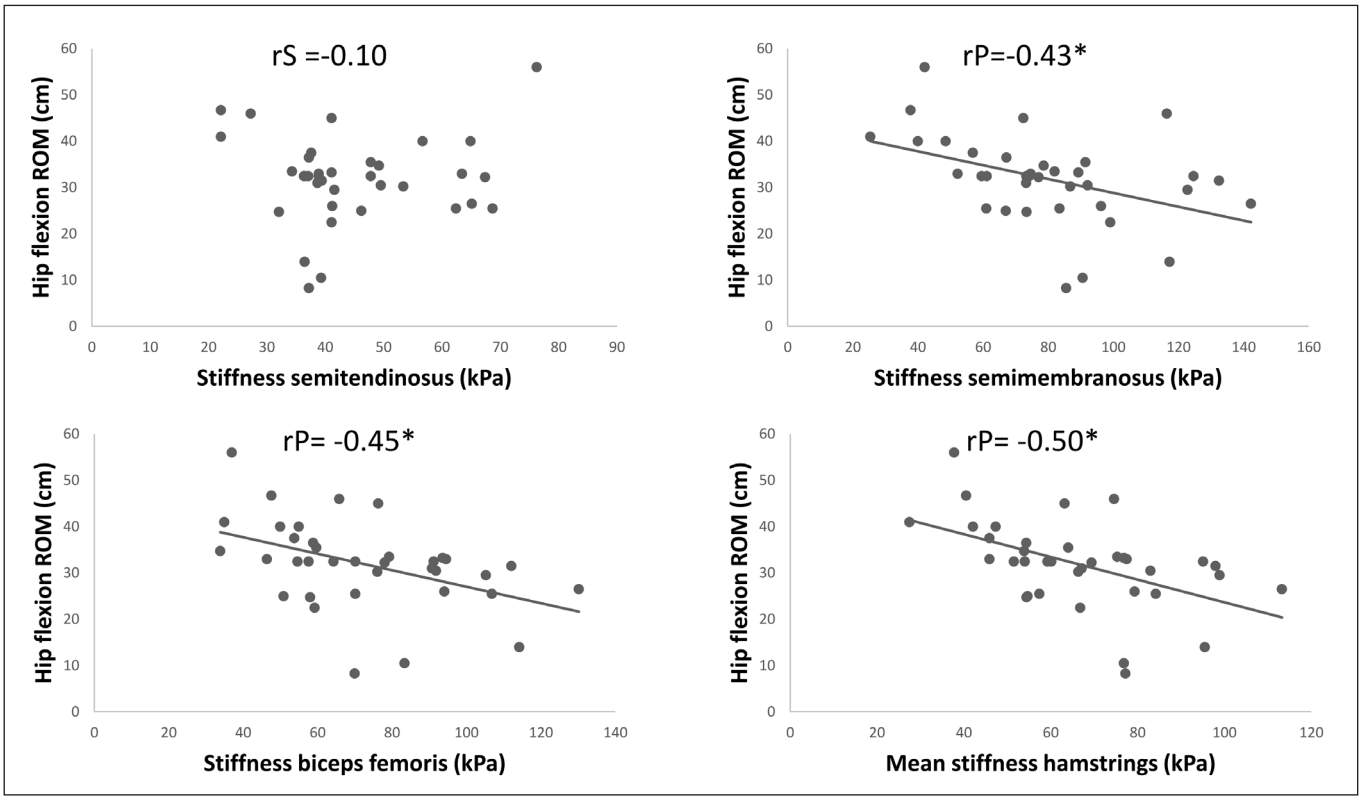


FIG. 1. Scatter plots of the correlation between hamstring muscles stiffness assessed with shear wave elastography and hip flexion range of motion. * indicates a significant correlation.

However, in the third hamstring muscle, the semitendinosus, we did not find such a correlation. Moreover, there was no significant correlation between ankle dorsiflexion ROM and gastrocnemius medialis or gastrocnemius lateralis stiffness. Similarly, there was no significant correlation between rectus femoris stiffness and hip extension ROM.

Since previous studies have reported a significant correlation between gastrocnemius medialis and gastrocnemius lateralis stiffness assessed via SWE and dorsiflexion ankle ROM in young men [18, 20], it was surprising that we could not confirm this result in the present study. Differences cannot be explained by the participants as all the studies included young males of a similar age (Hirata et al. [20] age = 22; Miyamoto et al. [18] age = 21.6; current study age = 23). However, the previous studies did not specify whether their participants were athletes or not. We recruited recreational soccer players of the 3rd to 6th Austrian league, and hence it can be assumed that, besides age [20] and sex [18], the training status of the participants may have also affected the correlation between gastrocnemius medialis or gastrocnemius lateralis stiffness and ankle dorsiflexion ROM. This was confirmed in previous studies which reported lower muscle stiffness in untrained participants compared to their athlete peers [39]. Although the muscle stiffness might be higher in athletes, a recent meta-analysis showed that regular strength training can increase the ROM of a joint [40]. Consequently, mechanisms other than muscle stiffness, such as stretch tolerance, may be responsible for the relatively high ankle dorsiflexion ROM found in our sample (36.99 ± 5.37) [41]. Another explanation for the difference in results may be the assessment of the gastrocnemius medialis and gastrocnemius lateralis stiffness, which was performed in a neutral ankle joint position in the present study. Miyamoto et al. [18] and Hirata et al. [20] assessed gastrocnemius medialis and gastrocnemius lateralis stiffness in a slightly stretched position (Miyamoto et al. [18] 14° ankle angle; Hirata et al. [20] 15° ankle angle). When Miyamoto et al. [18] assessed stiffness at a neutral position, the significant correlation with ROM only remained in the gastrocnemius lateralis but not in the GM. No significant correlation was observed below slack length. Consequently, it is likely that such a correlation is dependent on the muscle-tendon unit length.

For the hamstrings, we found significant correlation between hip flexion ROM and the stiffness of the semimembranosus (rP = –0.43), biceps femoris long head (rP = –0.45), and overall hamstrings (rP = –0.50), but not for the semitendinosus (rS = –0.10). In contrast, Miyamoto et al. [19] found a significant correlation between the sit and reach score and all three tested hamstring muscles (semimembranosus (rP = –0.25), biceps femoris long head (rP = –0.263), semitendinosus (rP = –0.299)) and overall hamstring stiffness (rP = –0.331). The slightly less pronounced correlation compared to the present study could be explained by the female participants included in the study by Miyamoto et al. [19]. Previous studies have reported that young males, but not females, showed a significant correlation between gastrocnemius medialis and gastrocnemius lateralis stiffness to ankle dorsiflexion [19]. Consequently, it can be speculated that the correlation between hamstring stiffness and hip flexion ROM might also be sex-dependent. However, Miyamoto et al. [19] did not distinguish between sex in their study, so this remains an open question Another possible explanation for the more pronounced correlations compared to Miyamoto et al. [19] could be that the participants in the current study were recreational male soccer players. All in all, the correlations found in the study of Miyamoto et al. [19] and in the current study range from 0.25 to 0.5, and hence the effect sizes can be considered as small to large. Thus, only 6% to 25% of the variation in ROM can be explained by the variation in muscle stiffness, according to these findings. Consequently, the remaining variation might be explained by other mechanisms, such as stretch tolerance, tendon stiffness, or nerve stiffness.

To the best of our knowledge, this study was the first to explore the correlation between rectus femoris stiffness and hip extension ROM. However, no significant correlation was found between those two variables. Although previous studies have found an increase in ROM following a single bout of foam rolling, no changes in rectus femoris elongation (i.e., indication for stiffness) were reported [42]. Consequently, due to this lack of correlation found by Vigotsky et al. [42], as well as the lack of correlations found in this study, other structures such as the iliopsoas muscle, ligaments, or the joint capsule rather than the rectus femoris muscle could likely explain hip extension ROM.

This study does have some limitations. Firstly, we did not assess tendon stiffness. Since it is not recommended to assess tendon stiffness with SWE, due to the technical restrictions of the device [43], this parameter was not included. It is likely that Achilles tendon stiffness and patellar tendon stiffness might be related to ankle dorsiflexion and hip extension ROM, respectively. Consequently, future studies should aim to assess tendon stiffness via force-elongation curves [24] or other reliable methods such as the use of a MyotonPro device [44]. Additionally, through pilot studies we recognized that it was not possible to assess muscle stiffness with SWE of deep lying muscles such as the iliopsoas as well as the soleus muscle with high reliability. Consequently, we decided not to include these muscles into that study. Furthermore, we did not assess stretch tolerance, which is another likely candidate for a correlation with ROM [21]. Finally, we did not include female participants. Since there have been differences reported in the correlation between ROM and muscle stiffness between males and females in a non-athlete population [18], future studies should take this into account.

CONCLUSIONS

It can be concluded that a small to large correlation exists between hip flexion ROM and the stiffness of the semimembranosus, biceps femoris long head, and overall hamstrings (but not in the semitendinosus). However, it has to be noted that a maximum of 25% of the variation in hip flexion ROM can be explained by muscle stiffness. Moreover, we did not find a significant correlation between ankle

dorsiflexion ROM and gastrocnemius medialis or gastrocnemius lateralis stiffness. In addition, there was no significant correlation between rectus femoris stiffness and hip extension ROM. Consequently, other structures such as tendon stiffness or stretch tolerance might be factors which can be related to ankle dorsiflexion ROM and hip extension ROM.

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Conflict of Interest declaration

The authors declare no conflict of interest.

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Comparison of NIRS exercise intensity thresholds with maximal lactate steady state, critical power and rowing performance

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ABSTRACT: This study aimed to compare the intensity of deoxygenated haemoglobin concentration ([HHb]) and tissue saturation index (TSI) breakpoints ([HHb]-BP and TSI-BP) with maximal lactate steady state (MLSS) and critical power (CP), and to describe their association with 2000-m rowing ergometer performance. Fourteen male rowers performed on a rowing ergometer: I) a discontinuous incremental test with 3-min stages (INC_3); II) a continuous incremental test with 1-min stages (INC_1); III) constant workload tests to determine MLSS; and IV) performance tests of 500 m, 1000 m, 2000 m and 6000 m to determine CP. CP (257 ± 39 W; 3.79 ± 4.1 L·min^{-1}) was higher than [HHb]-BP_3 (205 ± 26 W; 3.48 ± 2.9 L·min^{-1}), [HHb]-BP_1 (207 ± 27 W; 3.27 ± 3.2 L·min^{-1}), and TSI-BP_3 (218 ± 31 W; 3.51 ± 3.0 L·min^{-1}), but not higher than TSI-BP_1 (222 ± 34 W; 3.43 ± 3.2 L·min^{-1}). MLSS (187 ± 26 W; 3.33 ± 3.2 L·min^{-1}) was lower than TSI-BP_3 and TSI-BP_1 for power output, but not different in any comparison for $\dot{V}O_2$. The limits of agreement for power output and $\dot{V}O_2$ suggest poor agreement among these thresholds. The low level of agreement compromises the use of [HHb]-BP and TSI-BP for estimating MLSS and CP; therefore, these thresholds should not be considered interchangeable.

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INTRODUCTION

Maximal lactate steady state (MLSS) and critical power (CP) are the most utilized exercise intensity thresholds for heavy-severe domain boundary demarcation [1]. However, their determination is laborious, making their incorporation in the routine assessment of athletes difficult. As an alternative to time-consuming protocols, single-day exercise intensity thresholds have important applications to estimate MLSS and CP [2, 3]. For this purpose, there are several techniques based on blood lactate concentration (BLC), heart rate, and ventilatory responses determined in incremental exercise testing [2, 3]. However, these thresholds are protocol-dependent, present large intra-individual variability, and in most cases overestimate MLSS, especially during rowing exercise [3, 4].

In addition to these techniques, near-infrared spectroscopy (NIRS) has been proposed as a novel and non-invasive tool to estimate exercise thresholds in different exercise modes [5, 6, 7]. This technique determines exercise intensity thresholds through the changes in deoxygenated haemoglobin (HHb) and myoglobin concentrations [7] or the tissue saturation index (TSI) [5]. Changes in [HHb] and TSI provide a reliable estimate of the dynamic balance between O_2 supply and O_2 consumption, although the latter may be more influenced by cutaneous blood flow [8, 9]. In contrast, TSI is determined using a spatially resolved method (called multi-distance spectroscopy) and, therefore, reflects a greater volume of the muscle of interest. During incremental exercise tests, [HHb] and TSI signals exhibit a breakpoint (i.e., [HHb]-BP and TSI-BP, respectively) which represents an attenuation in the response and is reported as a possible exercise threshold [5, 7]. However, while the protocol dependence of exercise thresholds (e.g., based on BLC) is well-documented, the influence of the incremental test in [HHb]-BP and TSI-BP during rowing exercise is still uncertain.

Due to its relationship with the whole-body physiological responses, [HHb]-BP and TSI-BP have been compared to other physiological-derived exercise intensity thresholds, especially in cycling [6, 10–13] and running exercises [5]. For cycling, there were no mean differences for the $\dot{V}O_2$ at [HHb]-BP and MLSS [1, 10, 11] or [HHb]-BP and CP [1, 11]. On the other hand, power output was higher at [HHb]-BP when compared to MLSS and CP [1, 11]. During running exercise, MLSS and TSI-BP presented similar average speeds [5]. For rowing exercise, however, less evidence is available. Turnes et al. [7] reported that the power output associated with [HHb]-BP was not significantly different from the fixed BLC of 3.5 mmol·l^{-1} in trained rowers, which in turn overestimated MLSS in rowing [3]. Accordingly, comparisons of NIRS-derived thresholds with single-day thresholds derived from BLC, HR, or ventilatory

responses may not be informative because they overestimated MLSS [3, 4, 14] and underestimated CP [3] in rowing. Thus, a direct comparison of [HHb]-BP and TSI-BP with MLSS and CP remains to be performed in rowers.

Therefore, the aim of this study was twofold: 1) to determine the relationship and the agreement of [HHb]-BP and TSI-BP derived from two incremental tests with MLSS and CP, and 2) to verify the relationship between NIRS-derived thresholds with 2000-m rowing ergometer performance. Based on the outstanding difference between MLSS and CP during rowing exercise, it was hypothesized that NIRS-derived thresholds would overestimate and underestimate MLSS and CP, respectively.

MATERIALS AND METHODS

Participants

Fourteen regional- and national-level male rowers (mean ± standard deviation [SD]; age: 26 ± 13 years; height: 1.82 ± 0.05 m; body mass: 81.0 ± 7.6 kg; training experience: 4.3 ± 3.0 years) volunteered and provided written informed consent (or it was provided by a relevant parent, as one participant was under the age of 18) to participate in this study. The participants were scull (four) and sweep (five) rowers, five lightweight and four heavyweight. This study was approved by the Institutional Ethics Committee for Research on Human Subjects (number 3.191.968) and was performed according to the Declaration of Helsinki. Rowers trained 6 days a week, including 525 ± 183 min per week of on-water and indoor rowing training as well as resistance workouts. The study was performed during the season, in a training period of preparation for the national evaluation system.

Design

Each rower completed 8–10 testing sessions within 30 days. All tests were interspersed with ≥ 48 h of recovery. The rowers accomplished: I) a discontinuous step incremental test with 3-min exercise stages and 30-s recovery intervals (INC_3) and a continuous incremental test with 60-s exercise stages (INC_1); II) two to four visits to determine MLSS; and III) three to four maximal time-trial tests of 500 m, 1000 m, 2000 m, and 6000 m to determine CP and the finite work capacity above CP (*W'*). Each participant was always tested at the same time of day (± 2 h) in a temperature-controlled laboratory (22 ± 1 °C). Performance tests were conducted in the athletes' clubs following standardized protocols. Athletes were instructed to abstain from vigorous physical activity for 24 h before each test and maintain their usual routine during the testing period.

Materials

All tests were performed on an air-braked rowing ergometer (Concept 2E, Morrisville, VT, USA). Rowers individually and manually set the drag factor on the ergometer on their first visit (mean ± standard deviation: 125 ± 3 arbitrary units). Oxygen uptake ($\dot{V}O_2$) was monitored using an automated open-circuit breath-by-breath gas analyser (Quark CPET; Cosmed, Rome, Italy) calibrated with known concentration values of oxygen and carbon dioxide. Capillary blood samples (25 μL) were taken from the earlobe and analysed for BLC using enzyme electrode technology (YSI 2700, Yellow Springs, USA). The NIRS signals were obtained from the vastus lateralis muscle using portable multi-distance continuous wave spectroscopy (PortaMon; Artinis Medical Systems BV, Zetten, The Netherlands).

Exercise Tests

Incremental tests

On two separate days, rowers performed a continuous and a discontinuous incremental test until voluntary exhaustion to determine maximal oxygen uptake ($\dot{V}O_{2max}$) and NIRS-derived thresholds. During the discontinuous incremental test (INC_3), the initial workload was set at 130 W for 3 min and then increased by 30 W every 3 min [15]. At the end of each stage, a 30-s rest period was provided to collect blood samples. During the continuous incremental test (INC_1), after a 2-min baseline period at 115 W, the initial PO was set at 130 W for 1 min and then increased by 15 W every 1 min [16]. The peak power output (Ppeak) in the INC_3 and INC_1 was defined as the workload attained at exhaustion, when the test was completed at the end of the stage. If the test was concluded before the final stage had been completed, the Ppeak was calculated as previously described [17]. The $\dot{V}O_{2max}$ was taken as the highest 15-breath rolling average value reached by $\dot{V}O_2$ [18]. To analyse $\dot{V}O_2$ throughout the incremental tests, the average of the final 30 s of each stage was computed [7].

Maximal lactate steady state determination

For the determination of MLSS, 2 to 4 constant work rate submaximal tests lasting 30 min were performed on different days. Before each test, a moderate-intensity warm-up of 5 min was executed followed by a 5-min passive recovery period. The workload for the first trial corresponded to 70% Ppeak from the INC_3 and was then increased or reduced in 5% until MLSS determinarion [14]. Capillary blood samples were collected at the pre-test and the end of ten and thirty minutes, during a 30-s rest period. The MLSS was defined as the highest workload where BLC did not increase by more than 1 $mmol \cdot l^{-1}$ between the 10^{th} and 30^{th} minutes of the test [19]. The $\dot{V}O_2$ value of MLSS was calculated by the average value of the final minute of exercise.

Critical power determination

CP and *W'* were estimated from three to four predictive trials, which were performed in random order [3]. Time-trial performance tests of 500 m, 1000 m, 2000 m, and 6000 m were carried out [3]. One rower was unable to finish the tests, and 9 and 4 rowers completed the 500-, 1000-, and 2000-m and 500-, 2000- and 6000-m tests, respectively, following the distances selected by the schedule in their clubs.

CP and *W'* were calculated according to the linear work-time ($P = [W'/\text{time}] + CP$), linear inverse-of-time ($P = W' [1/\text{time}] + CP$),

and hyperbolic (time = $W'/[P - CP]$) models, then three values of CP and W' estimates for each subject were provided. Data from the linear work-time model were used for subsequent analyses due to a better fit of linear data ($R^2 = 0.998 \pm 0.002$) and the lowest standard error for CP (4.2% ± 2.6%). The $\dot{V}O_2$ value of the CP was estimated by linear regression analysis from the relationship between the power output and $\dot{V}O_2$ from the INC_3 ($R^2 = 0.945 \pm 0.033$).

Data Analysis

NIRS procedures

The NIRS apparatus was used to detect relative changes in local muscle [HHb] and TSI. The raw data were recorded at 1 Hz. The NIRS probe was placed on the vastus lateralis belly approximately halfway between the trochanter and knee joints after the skin area had been shaved and wiped. Skinfold thickness was measured at the site of NIRS application (12.8 ± 5.4 mm) using a Lange Skinfold Caliper (Incorporated Cambridge, Maryland, USA), to ensure that the adipose thickness did not substantially contaminate muscle NIRS signals [9]. The device was covered with a black light-absorbing cloth to prevent contamination from ambient light.

NIRS-derived thresholds

The average of the raw data from the final 10-s of each stage during the incremental tests was used to individually plot [HHb] and TSI as a function of the workload. The [HHb]-BP and TSI-BP were determined by fitting the following double-linear model to the data [7, 20]:

$$f = if[x < BP, g(x), h(x)]$$
$$g(x) = i_1 + (s_1 x)$$
$$i2 = i_1 + (s_1 BP)$$
$$h(x) = i_2 + [s_2(x - BP)]$$
$$fitfy$$

Where f is the double-linear function, $\times$ is the workload, and y is [HHb] or TSI, BP is the time coordinate corresponding to the interception of the two regression lines (i.e., [HHb]-BP and TSI-BP), i_1 and i_2 are the intercepts of the first and second linear function, respectively, and s_1 and s_2 are the slopes. After the fitting, the [HHb]-BP and TSI-BP were identified as the inflection point followed by a plateau, under visual inspection by two independent researchers. The fitting was employed to enable visual inspection and to improve objectivity in data analysis, as confirmed by no disagreement between investigators. The two thresholds were determined during the INC_3 ([HHb]-BP_3 and TSI-BP_3) and INC_1 ([HHb]-BP_1 and TSI-BP_1).

Statistical analysis

Descriptive data are presented as mean ± SD. Statistical parameters are reported as mean point estimates with 95% confidence intervals (95% CI). Paired-sample Student *t*-tests were used to compare incremental test variables. The relationships between NIRS-derived thresholds with MLSS, CP, and 2000-m performance time were evaluated through a simple linear regression analysis, reporting the Pearson's coefficient of correlation (r), and the typical error of the estimate (TEE) [21]. Agreements between NIRS-derived thresholds with MLSS and CP were analysed using Bland-Altman's graphical analysis [22].

Repeated measures mixed effects ANOVA models were used for comparisons between NIRS-derived thresholds with MLSS and CP (power output and $\dot{V}O_2$). Fixed effects were the exercise intensity thresholds and the random effect was the identity of each subject. When appropriate, Dunnett's post-hoc test was used to identify pairwise differences. Correlation coefficients were interpreted as follows: < 0.09 trivial, 0.10 to 0.29 small, 0.30 to 0.49 moderate, 0.50 to 0.69 large, 0.70 to 0.89 very large, 0.90 to 0.99 nearly perfect, and 1.0 perfect [21]. Statistical significance was set at $p < 0.05$.

RESULTS

During the incremental tests, there were no differences in Ppeak (INC_3: 308 ± 39; INC_1: 311 ± 37 W; p = 0.369), $\dot{V}O_{2max}$ (INC_3: 4.19 ± 4.0; INC_1: 4.09 ± 4.9 $mL \cdot min^{-1}$; p = 0.106), and the power output and $\dot{V}O_2$ at [HHb]-BP (p = 0.765 and 0.054, respectively), and TSI-BP (p = 0.910 and 0.174, respectively) (Figure 1A-B). In two participants, the TSI-BP was not detectable, as their profiles did not display any clear breakpoint.

Comparisons between the different NIRS-derived thresholds from the same INC revealed that [HHb]-BP_3 and TSI-BP_3 were significantly different for power output (p = 0.018; bias ± LoA = 5.5% ± 14.1%) but not for $\dot{V}O_2$ (p = 0.350; bias ± LoA = 1.2% ± 8.7%). There were no significant differences between [HHb]-BP_1 and TSI-BP_1 for power output (p = 0.182; bias ± LoA = 5.9% ± 29.5%) and $\dot{V}O_2$ (p = 0.148; bias ± LoA = 3.6% ± 16.3%). The correlation for power output of [HHb]-BP_3 with TSI-BP_3 was very large (r = 0.87; 0.61 to 0.96) and that for [HHb]-BP_1 with TSI-BP_1 was moderate (r = 0.45; -0.13 to 0.80). Regarding $\dot{V}O_2$, the correlation was very large (r = 0.87; 0.61 to 0.96) and large (r = 0.58; 0.04 to 0.86) between [HHb]-BP_3 with TSI-BP_3 and [HHb]-BP_1 with TSI-BP_1, respectively.

The means ± SD of MLSS, CP, and NIRS-derived thresholds for power output and $\dot{V}O_2$ are shown in Table 1, and individual data are attached as supplementary material. There were significant differences between NIRS-derived thresholds and MLSS for power output ($F_{(2.7,\ 33.4)} = 5.174$; p = 0.006), but not for $\dot{V}O_2$ ($F_{(2.7,\ 33.7)} = 2.068$; p = 0.130). The post hoc analysis revealed that power output at MLSS was significantly lower than power output at TSI-BP_3 and TSI-BP_1 (p = 0.018 and 0.003, respectively), but not at [HHb]-BP_3 and [HHb]-BP_1 (p = 0.090 and 0.053, respectively).

For CP, there were significant differences in power output ($F_{(2.6,\ 31.4)} = 8.342$; p = 0.001) and $\dot{V}O_2$ ($F_{(2.2,\ 27.4)} = 6.365$; p = 0.004). Power output and $\dot{V}O_2$ at CP were significantly higher than at [HHb]-BP_3 (p = 0.001 and 0.017, respectively), [HHb]-BP_1 (p = 0.007 and 0.014, respectively), and TSI-BP_3 (p = 0.008 and 0.027, respectively), but not significantly higher than at TSI-BP_1

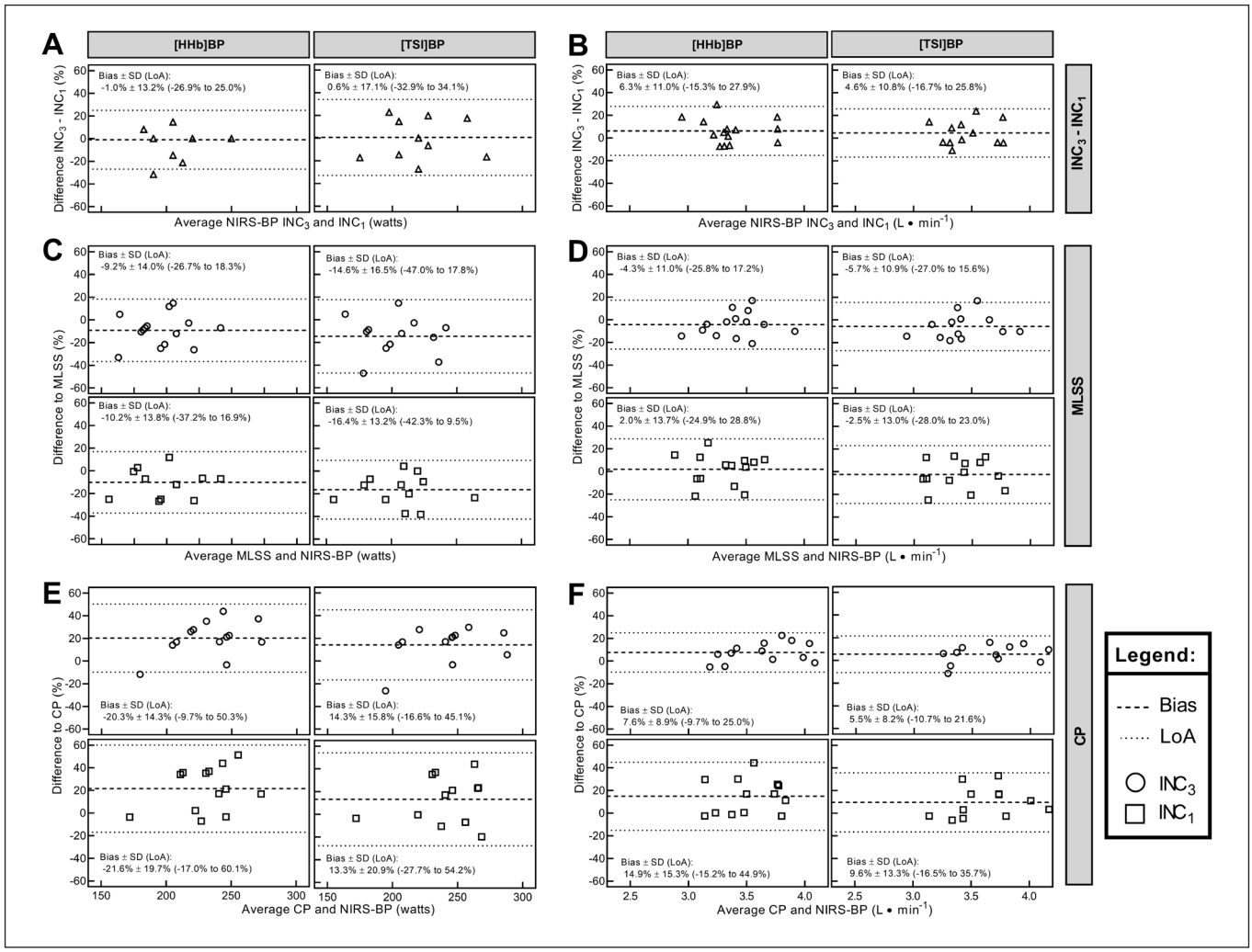


FIG. 1. Bias and limits of agreement between NIRS-derived thresholds determined in different incremental tests (A-B), maximal lactate steady state (C-D), and critical power (E-F).

TABLE. 1. Descriptive values of exercise intensity thresholds for power output and oxygen uptake.

	[HHb]-BP_1	[HHb]-BP_3	TSI-BP_1	TSI-BP_3	MLSS	CP
Power output (W)	207 ± 27	205 ± 26	222 ± 34	218 ± 31	187 ± 26	257 ± 39
Power output (%Ppeak)	67.2 ± 8.8	67.0 ± 6.5	71.4 ± 9.7	70.6 ± 8.9	60.9 ± 4.1*	82.6 ± 9.0*
$\dot{V}O_2$ ($L \cdot min^{-1}$)	3.27 ± 3.2	3.48 ± 2.9	3.43 ± 3.2	3.51 ± 3.0	3.33 ± 3.2	3.79 ± 4.1
$\dot{V}O_2$ (%$\dot{V}O_{2max}$)	80.9 ± 11.3	83.3 ± 8.0	83.7 ± 9.9	84.5 ± 7.4	79.7 ± 6.3*	89.7 ± 8.7*

Data are shown as mean ± SD. CP: critical power; MLSS: maximal lactate steady state; [HHb]-BP_1: deoxygenated haemoglobin breakpoint during incremental test with 1 min stages; [HHb]-BP_3: deoxygenated haemoglobin breakpoint during incremental test with 3 min stages; TSI-BP_1: tissue saturation index breakpoint during incremental test with 1 min stages; TSI-BP_3: tissue saturation index breakpoint during incremental test with 3 min stages; *: significant different from breakpoints determined during incremental test with 3 min stages ($p < 0.05$).

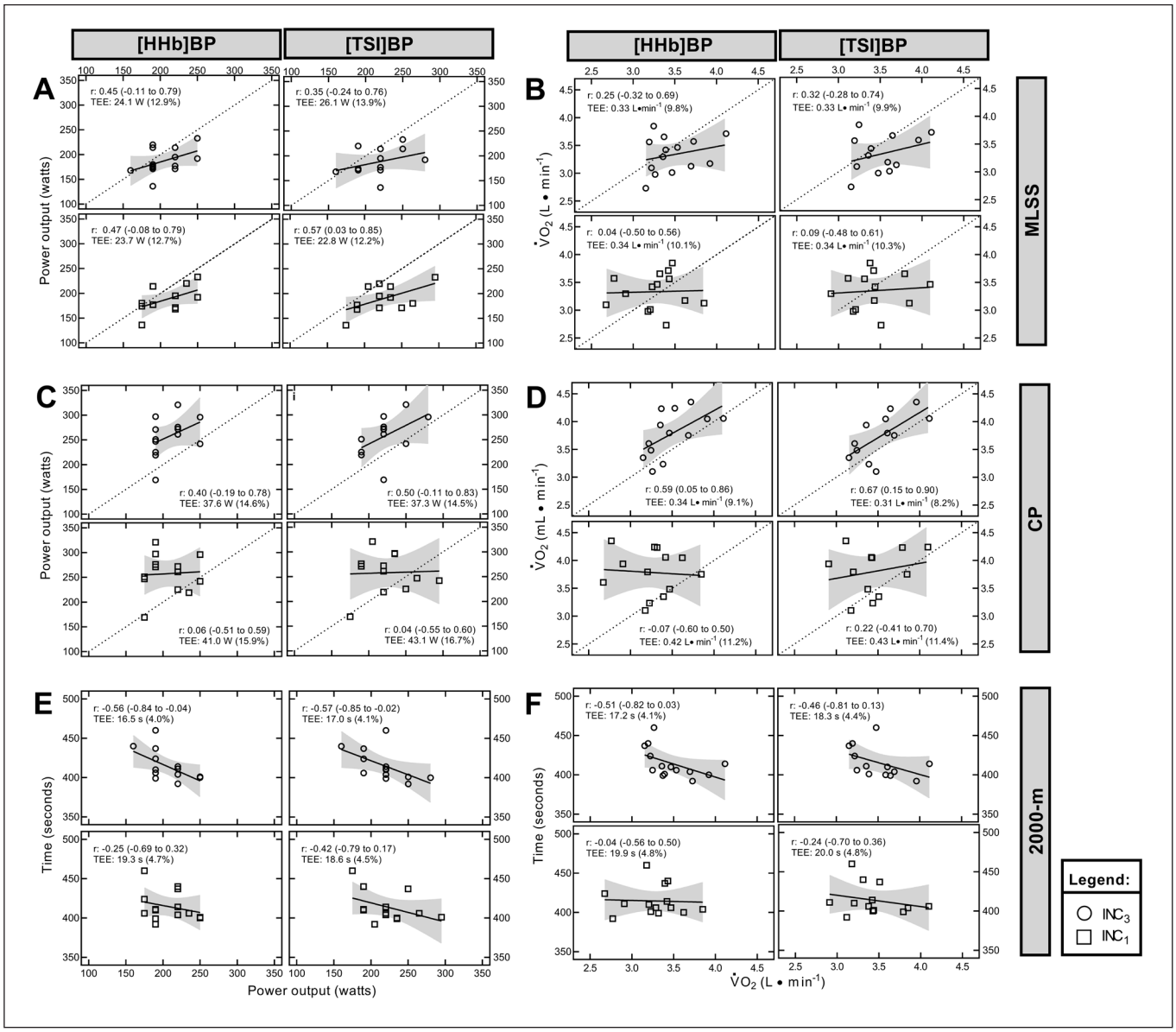


FIG. 2. Relationship between maximal lactate steady state (A-B), critical power (C-D), and 2000-m performance (E-F) with NIRS-derived thresholds.

(p = 0.101 and 0.069, respectively). The agreement analyses between MLSS/CP and NIRS-derived thresholds are presented in Figure 1C-F.

Figure 2 presents the linear regression between the power output and $\dot{V}O_2$ at MLSS (Panels A and B), CP (Panels C and D), and 2000-m performance time (panels E and F) with the power output and $\dot{V}O_2$ at NIRS-derived thresholds. Trivial to large correlations occurred among MLSS and CP with NIRS-derived thresholds. For 2000-m performance time, trivial to large correlations were observed.

DISCUSSION

In accordance with the primary hypothesis, poor agreement and an association of MLSS/CP with NIRS-derived exercise intensity thresholds were observed. Although such threshold was previously compared with the anaerobic threshold during rowing exercise [7], the novel aspect observed herein was the direct comparison with MLSS and CP. These two markers are important metabolic thresholds, with MLSS occurring at a lower intensity than CP, mainly in rowing exercise [3]. Despite this, the utilization of this novel approach for estimating MLSS and CP during rowing ergometer exercise is questionable because of the clear disagreement observed.

Regarding MLSS, no statistically significant differences in any NIRS-derived thresholds for $\dot{V}O_2$ and or both [HHb]-BPs for power output were found. Nevertheless, random errors expressed as LoA values were quite high and the association was low, which prevents us inferring that NIRS-derived thresholds are interchangeable with

MLSS. Previous investigations compared NIRS-derived thresholds and MLSS in running [5] and cycling [1, 10], the former using TSI-BP in the gastrocnemius [5] and the latter [HHb]-BP in the vastus lateralis [1, 10]. Likewise, the studies showed significant differences in the means and considerable errors of agreement between the running speed at MLSS and TSI-BP (bias ± LoA = ~3.5% ± 6.0%) [5] and the $\dot{V}O_2$ at MLSS and [HHb]-BP (bias ± LoA = ~-0.7% ± 22.6%) [10]. Although direct comparisons among studies should be carefully analysed because of differences in exercise modes, muscles involved, or methodological approaches, these findings demonstrate that TSI-BP and [HHb]-BP present high variability and should not be considered equivalent to MLSS.

With regard to CP, the present study verified that all NIRS-derived thresholds were lower than CP intensity, except for TSI-BP_1. In addition to the evident mean differences for power output and $\dot{V}O_2$, the LoA values suggest poor agreement between these thresholds and CP. As observed for MLSS, the comparison of NIRS-derived thresholds with CP is limited for cycling exercise and [HHb]-BP [1, 11, 23]. Comparisons with TSI-BP are still incipient. In healthy individuals, [HHb]-BP workload was higher than CP in two different studies [11, 23]. Of note, these studies estimated CP from the 3-parameter hyperbolic model [11] or 2-parameter best individual fit model before and after six weeks of training intervention [23]. In contrast, the present result indicated that [HHb]-BP_3 and [HHb]-BP_1 workloads were lower than CP for rowing. Despite the well-known protocol dependencies that influence the CP workload, it was previously observed that CP was ~30% higher than MLSS in rowing [3], a much higher difference than observed in cycling (~10%) [24], which in part may explain these contrasting findings. Finally, it is important to note that the predicted $\dot{V}O_2$ at CP from the INC_3 may still underestimate the real $\dot{V}O_2$ at CP due to the inherent $\dot{V}O_2$ slow component at this intensity. This would result in an even greater $\dot{V}O_2$ mean difference between NIRS-derived thresholds and CP than that reported in this study.

The rowing motor pattern is a complex task, which may make it difficult to determine the association between systemic and local responses due to the heterogeneity in the blood flow and O_2 extraction responses among muscles [25]. Therefore, as NIRS devices provide a signal of muscle oxygenation only at the level of a probe, and rowing exercise recruits several muscles associated with coordination of upper and lower limbs, caution is necessary when adopting [HHb]-BP or TSI-BP as an index presenting correspondence with different markers of systemic responses.

Some previous investigations verified the protocol dependence for workloads at thresholds derived from different incremental tests [2, 3, 4]. Conversely, the present study verified no differences, but high random error between NIRS-derived thresholds from the INC_3 and INC_1, suggesting that NIRS-derived thresholds cannot be used interchangeably. Interestingly, the determination of NIRS-derived thresholds has been assessed mainly in ramp incremental tests [1, 11, 23], which is not feasible to perform on a rowing ergometer. In an attempt to get closer to a ramp incremental test, the present study used a short step test (i.e., INC_1). However, the 30 seconds rest in INC_3 allows a partial recovery, which probably could influence the physiological pattern of NIRS-variables compared to INC_1. Furthermore, the associations between [HHb]-BP and TSI-BP and between NIRS-derived thresholds and MLSS and 2000-m performance were much lower when using the NIRS-derived thresholds from the INC_1. Thus, the use of the INC_3 is suggested in future studies in order to analyse the physiological relevance of NIRS-derived thresholds in rowing.

For cycling, running and swimming the mean differences between MLSS and CP (or critical velocity) ranged from 4% to 16%, and recently Galán-Rioja et al. [24] reported a mean difference of ~11% in cycling exercise, from a meta-analysis. Thus, it is reasonable to assume that CP occurs at greater intensities than MLSS at least for trained individuals. Furthermore, the substantial mean difference of approximately 30% between MLSS and CP reported before [3] is probably the largest difference found in the literature. The possible reason is the lower relative values of the MLSS in rowers [4, 14, 26] due to a different pattern of blood lactate dynamics. Indeed, Beneke et al. [27] verified that MLSS seems to decrease with the increase of primarily engaged muscle mass, which may influence the results considering the motor pattern of rowing and the muscle mass engaged. Therefore, the previous findings highlighted that CP and MLSS occur at different physiological intensities and cannot be considered analogous. Finally, the novelty of the present study was the comparison between NIRS-derived thresholds and both MLSS and CP in trained rowers.

The NIRS device can deliver real-time feedback to athletes and monitoring the [HHb] and TSI may be interesting during a training session in order to adjust the intensity, quantify the workload, and provide specific information about the working muscles [5, 28, 29, 30]. This could be even more relevant for on-water rowing, in which monitoring of training is more complex than indoor rowing. The small difference of [HHb]-BP with the fixed BLC of 3.5 $mmol \cdot l^{-1}$ and the good association with 2000-m rowing ergometer performance initially suggested that the local index may distinguish rower performance [7]. In the present study, [HHb]-BP and TSI-BP had a large and very large correlation with 2000-m rowing ergometer performance, respectively. The difference between the magnitude of correlation may be related to the increased tissue volume under consideration in the TSI compared to [HHb], considering that the vastus lateralis volume largely explained the variance in rowing ergometer performance [31]. In contrast, the TSI-BP was not detectable in all athletes and only captures a small portion of the selected muscle. Therefore, caution is required when establishing a link between NIRS-exercise thresholds and systemic responses. To date, regarding the performance prediction, the incremental test peak power output remains the most important predictor of 2000-m rowing performance at different training levels [3, 15, 32, 33], which may represent a more practical and easier marker than the NIRS-derived thresholds.

CONCLUSIONS

The NIRS-derived thresholds presented poor agreement with MLSS and CP, compromising their interchangeable use for estimating the markers of exercise intensity domains. Thus, the NIRS-derived thresholds are not useful indices for rowers.

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Disclosure Statement

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Road to Paris 2024: force-velocity profile in different speed climbers' abilities

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ABSTRACT: Speed climbing will be a new discipline in Paris 2024. The physical requirements of speed climbing are different from the other climbing modalities due to the short event time requiring higher level of strength and power. These parameters have been measured through the Force-Velocity (F-V) profile in different climbing disciplines. However, there are no known results evaluating different speed climbing abilities to establish whether F-V relationship is a determining factor between performance levels. The purpose of this study was to evaluate the upper and lower limbs F-V profile in different speed climbing abilities considering sex. Twenty-six speed climbers were divided into two groups based on their level of performance: international level (men n = 7 and women n = 2) and national level (men n = 8 and women n = 7). Participants performed pull-ups and squat incremental tests and F-V profile variables [Maximum theorical values of force (F_0), velocity (V_0) and power (P_{max})], one-repetition maximum value (1RM) and %1RM where peak power was expressed were collected using a linear encoder. There were significant differences in F_0, relative force, %1RM where peak power was expressed, and 1RM in pull-ups ($p < 0.05$) between groups. However, there were not significant differences between groups in squat variables. No significant sex differences were found in any variable. There were moderate-strong correlations between running time and 1RM (pull-ups and squat), F_0 and FV-slope (pull-ups) ($p < 0.05$) analyzed in the whole group. In conclusion, F_0 and 1RM in pull-ups were significantly higher in international climbers. Therefore, national climbers should focus their training on improving force by training with heavy loads. Additionally, squat F-V profile variables do not seem to be as important as in the pull-up for performance.

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INTRODUCTION

The 2024 Paris Olympics will once again feature sport climbing. In the previous event (Tokyo, 2020), the three disciplines (lead, bouldering and speed climbing) were included as one combined competition, not allowing the specialization of the climbers in a specific discipline [1]. This time, the Olympic program will separate sport climbing into two disciplines, speed climbing and the combined (lead and boulder). In fact, speed climbing has gained significant attention [1] as many climbers have started to specialize in this discipline in preparation for the Paris 2024 games. This discipline consists of climbing a standardized wall of 15.5 meters high and 5º inclination with 20 hand holds and 11-foot holds in the shortest time possible after a start signal of 3 tones separated by one second [2].

Speed climbers require higher levels of strength, anaerobic power, and speed compared to climbers of other disciplines [3–5]. Traditionally, climbing training programs have primarily focused on developing upper limb capabilities [6]. However, recent studies have highlighted the significant influence of the lower body in speed climbing, as it demands different abilities compared to other climbing disciplines, founding strong correlations between running time and leg power output and hypertrophy [7–9].

A key factor in power-based sports is the Force-Velocity (F-V) relationship [10]. This linear relationship is explained as the ability to produce force at different velocities of motion that are inversely related to each other [11]. For instance, two speed climbers can achieve the same power with two distinct F-V profiles, differing in the amount of force or the amount of velocity. Thus, the F-V profile can be used by coaches to orientate the strength training according to the estimated value of maximum force (F_0), the estimated value of maximum velocity (V_0) and the resulting estimated value of maximum power (P_{max}) [10].

The F-V profile can vary based on the physical requirements of the sport [12]. To the best of our knowledge, there is only one study analyzing the pull-up F-V profile in speed climbers [13]. Levernier et al. [13] compared climbers from the three disciplines obtaining significant differences in several F-V profile variables. However, this information is no longer relevant as sport climbing will not be a combined event anymore. Additionally, there is a lack of research comparing the squat F-V profile characteristics across different climbing performance levels and between sexes. Therefore, the aim of the current study was to determine the F-V profile in speed climbers in the

upper (pull-up) and lower limbs (squat) in different climbing abilities (international and national level) considering sex. We hypothesize that there will be differences in the F-V profile depending on the performance level of the climbers and depending on the sex with international men showing better results than women and national climbers.[14]

MATERIALS AND METHODS

Participants

A total of 26 speed climbers (women n = 11 and men n = 15) from different countries (Spain, Italy, Austria and Ecuador) participated in this study. They were divided into two groups based on the classification criteria established by Mckay et al. [14]: International level (n = 9;7 men and 2 women) where 3 climbers obtained a medal in an international event at least once in their life and national level (n = 17; 8 men and 7 women). Detailed information about the participants can be found in Table 1. None of them had been injured in the last year and they signed an informed consent form before the test which explained the procedure and the risks involved. The study was performed in accordance with the principles of the Declaration of Helsinki (October 2008, Seoul), and the experimental protocols were approved by the local ethics committee (CEIC).

Instruments

Incremental tests in both exercises were performed using a validated linear encoder (ADR encoder, Toledo, Spain) [15, 16]. This device uses linear motion translation technology to measure the mean propulsive velocity, which is defined as the mean velocity from the beginning of the concentric phase until the acceleration of the bar is less than gravity [17]. The encoder is fitted with a cable that is attached to the bar (in the case of squats) or to the back part of the harness (in the case of pull-ups) which makes possible to measure the linear displacement during the exercise.

Procedures

This study is a comparative descriptive design in which the F-V profile of international and national level speed climbers was analyzed and compared. All participants were instructed not to consume caffeine in the previous 12 hours before the test [18]. The menstrual cycle of the female climbers was not considered. However, recently it has been shown that force, velocity and power output remain very similar in all menstrual cycle phases [19] and particularly in the squat exercise [20]. The protocol was adapted from Muñoz-López et al. [21] although not exactly the same one as we analyzed loads under 70%1RM, and we did not get to the 1RM. Participants performed a standardized warm-up of 15 minutes which consisted of preparatory exercises (i.e., scapular retractions and glenohumeral joint external rotations) and one set of 5 unweighted pull ups (instructed to go "as fast as possible") before the first test. The first test carried out was incremental pull ups test in which participants started with a load of 0 kg extra-weight. Afterward, they increased the load by 5 kg or 10 kg (based on the drop of velocity from the previous load) until they reached an execution velocity of approximately 0.35 $m \cdot s^{-1}$. They performed two repetitions per load, where the one with a higher mean propulsive velocity was recorded. Participants started to do only one repetition when the mean propulsive velocity was under 0.5 $m \cdot s^{-1}$. The recovery time between each load attend was 3 minutes [22]. Concerning the body position, the climbers performed pull-ups placing their hands in pronation and at a distance of 1.5 their biacromial breadth (cm) following the posture protocol established by Levernier et al. [13]. The pull-up was considered valid if the chin went above the bar and if the participants did not intentionally use the legs to help themselves going up. Additionally, every repetition started with both arms fully extended and the exercise was performed linearly and controlling trunk movements in order to provide accurate distance measurements when using the linear encoder.

Between pull-up and squat tests, they were allowed to rest for 15 minutes. Before starting the squat incremental test, they were instructed to conduct again a standardized warm up of 10 minutes which consisted of hip mobility exercises and one set of squats at a velocity faster than 1 $m \cdot s^{-1}$ until 20% of velocity loss. The protocol for squat test was adapted from Bachero-Mena et al. [23]. The test started with a load of 20 kg and from this point on, they increased by 10 kg until they reached a velocity close to 0.45 $m \cdot s^{-1}$. They performed two repetitions with each load until they lifted the

TABLE 1. Participants characteristics.

Level	n	Age (years)	Body weight (Kg)	Best time (s)
Men international	7	17.7 ± 7.41	67.1 ± 3.57	6.72 ± 0.72
Women international	2	26.0 ± 0.00	61.1 ± 3.82	8.11 ± 0.71
Men national	8	16.0 ± 2.00	63.9 ± 7.74	8.83 ± 1.15
Women national	9	18.9 ± 7.41	63.1 ± 9.49	11.70 ± 1.69

International group: Climbers who have participated in international championships. National group: Climbers who perform at national competitions in their country.

load to a velocity of less than 0.7 $m \cdot s^{-1}$, from here on they lifted each load one time. The recovery time was 3 minutes between trials [22]. The squat was back squat and parallel (when the femur is parallel to the ground), so we adjusted the structure's supports to make the barbell touch the supports when the participant's thigh was parallel to the ground. The climbers were instructed to descend in a controlled manner, touch the supports with the bar for approximately half a second and to lift the load as fast as possible. In the pull-up test, a range between 58.8 ± 7.8% – 81.7 ± 5.9% considering F_0 was analyzed while in the squat exercise the range was from 53.6 ± 6.9% – 77.3 ± 6.3%.

In order to correlate pull-up and squat performances with the speed climbing ability we used an official running time (official wall and chronometer) performed in an official competition within seven days before or after pull-up and squat tests day.

Data analyses

The F-V relationship was determined by least-squares regression analysis. Tests with a correlation lower than $r^2 = 0.95$ between loads and velocities were discarded. Thus, four pull-up tests (One from international group and three from national group) and six squat tests were removed from the sample (One from international group and five from national group). We obtained F_0 and V_0 from the intercepts of the F-V curve regression with the axes X and Y. P_{max} was obtained through the following formula: $(F_0 \cdot V_0) / 4$ [24], while peak power was the highest power expressed during the test. F_0 and P_{max} were analyzed considering body weight. In order to estimate 1RM values, we considered the velocity proposed by Muñoz-López et al. [21] for pull-ups (0.26 $m \cdot s^{-1}$) and Sánchez-Medina et al. [25] for squat 1RM (0.32 $m \cdot s^{-1}$).

Statistical analyses

All data are presented as mean ± standard deviation (SD). The normality was checked before any analyses using Shapiro-Wilk test and data followed a normal distribution ($p > 0.05$). A significant level of $p < 0.05$ was adopted. A Two-way ANOVA test was conducted to examine differences in each dependent variable considering sex and level of performance. Pearson correlation test was carried out to analyze if there was a correlation between any of the dependent variables with running time. The statistical tests were processed with the use of the software Jamovi 2.3.18.0 for Windows.

RESULTS

No significant differences ($p > 0.05$) were found in any of the dependent variables between sexes. There were significant differences ($p < 0.05$) in the 1RM, relative force and F_0 in pull-ups between the international and national groups. In the squat exercise, no differences were found in any of the independent variables (Table 2).

TABLE 2. ANOVA between performance levels in pull-up and squat exercises.

	LEVEL	1RM (Kg)	Relative Force ($Kg \cdot BW^{-1}$)	F_0 ($N \cdot Kg^{-1}$)	V_0 ($m \cdot s^{-1}$)	P_{max} ($W \cdot Kg^{-1}$)	FV slope ($N \cdot (m \cdot s)^{-1}$)	%1RM (Peak power)
Pull-ups	International	47.80 ± 13.1**	0.68 ± 0.12**	19.50 ± 2.44*	1.93 ± 0.29	9.35 ± 1.07	-690 ± 206	59.70 ± 4.22*
	National	27.00 ± 8.25**	0.43 ± 0.13**	16.20 ± 2.13*	2.07 ± 0.35	8.33 ± 1.20	-511 ± 137	70.60 ± 6.33*
Squat	International	111 ± 22.2	1.60 ± 0.25	29.50 ± 4.23	1.93 ± 0.36	14.00 ± 1.46	-1090 ± 356	44.40 ± 9.57
	National	89.80 ± 20.1	1.40 ± 0.22	27.40 ± 3.20	2.21 ± 0.47	14.80 ± 1.90	-837 ± 285	48.50 ± 10.70

Note. 1RM = One repetition maximum; Relative Force = 1RM kg / bodyweight kg; F_0 = Maximal estimated force/ bodyweight Kg; V_0 = Maximal estimated velocity; P_{max} = Maximum estimated power/ bodyweight Kg; FV slope = Force-velocity slope; %1RM (Peak power) = Percentage of 1RM in which peak power is expressed; * $p < 0.05$; ** $p < 0.001$

TABLE 3. Significant Pearson correlations between running time and pull-up and squat variables.

Dependent variable	Correlation with running time
1RM pull-up	-0.74
1RM squat	-0.50
F_0 pull-up	-0.70
FV slope pull-up	0.61

Note. 1RM = One repetition maximum; F_0 = Maximal theorical force; FV slope = Force-velocity slope.

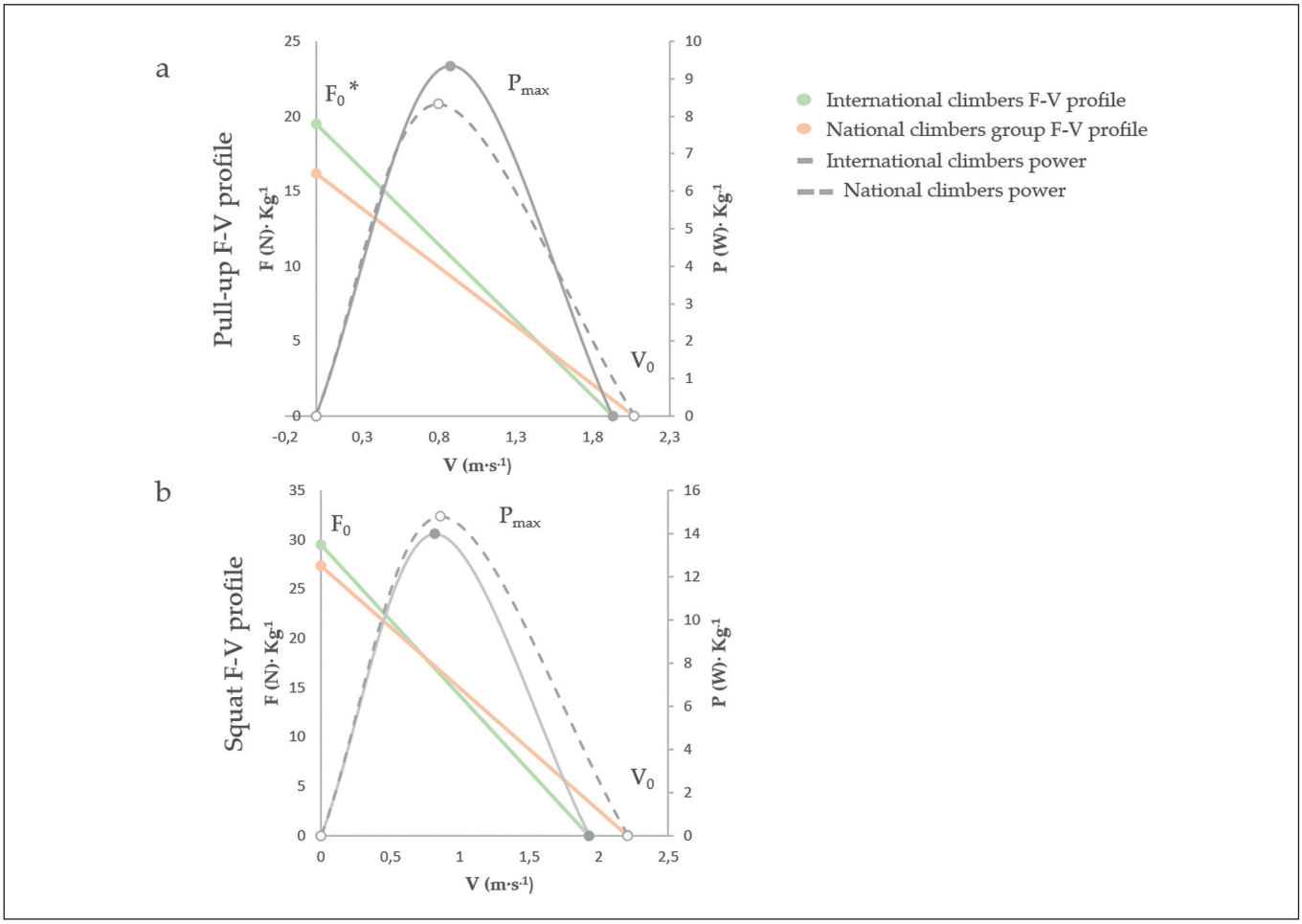


FIG. 1. a. (Pull-up F-V profile) b. (Squat F-V profile)
$F \cdot kg^{-1}$ = Force · bodyweight^{-1}; V = Velocity; $P \cdot kg^{-1}$ = Power · bodyweight^{-1}; F_0 = Maximal estimated force · bodyweight^{-1}; V_0 = Maximal estimated velocity; P_{max} = Maximal estimated power · bodyweight^{-1}; * $p < 0.05$.

These results have been graphically expressed in the figure 1.a and 1.b, showing the comparison between national and international level climbers regarding average values of F_0, V_0 and P_{max}.

Analyzing the percentage of 1RM at which participants reached peak power, we found significant differences in the pull-up exercise, with international level athletes reaching it at 59.7 ± 4.22% of 1RM and national level athletes reaching it at 70.6 ± 6.33% of 1RM, although 88% of climbers achieved this with their own body weight. In the squat we found no significant difference between levels in the percentage of 1RM in which they express their peak power where international level climbers obtain it at 44.4 ± 9.57%1RM and national level climbers at 48.5 ± 10.7%1RM.

No sex differences were found in any variable except from squat P_{max} (relative to bodyweight). Male speed climbers obtained significantly higher values ($p < 0.05$) than female climbers.

In addition, we analyzed the correlation between the different variables obtained from both tests and the time (s) spent on the wall (running time) where we found significant and strong correlations between this time and 1RM (pull-up and squat), F_0 (pull-up) and FV slope (pull-up) (Table 3).

DISCUSSION

The aim of this study was to determine the F-V profile in speed climbers in upper (pull-up) and lower limbs (squat) in different climbing abilities (international and national level climbers) and sexes. In relation to our hypothesis, there were significant differences in F_0, relative force, 1RM and percentage of 1RM where peak power is reached in pull-ups between groups. However, there were not significant differences between groups in squat F-V profile variables. In addition, no differences were observed between sexes in the pull-up and squat exercises.

Strength and power characteristics have been previously analyzed in national level speed climbers [5] and international-elite speed climbers [3, 7, 26]; however, the F-V profile has barely been

investigated and differences between performance levels have not been analyzed yet in speed climbing. Moreover, previous studies examined a small sample size and were conducted only with elite climbers, leaving a gap of knowledge on the different characteristics of climbers who perform at national level and who are on their way to high performance. For this reason, in our study, climbers from different countries were tested to obtain data from a bigger group who performed at different levels and therefore a larger sample that would provide us with a broader view.

Upper body exercises including pull-up exercise have been widely studied in sport and rock climbing [27]. Speed climbers have usually practiced other climbing disciplines and perform it in their training. However, not knowing the F-V profile of an athlete implies difficulties in orienting training towards more speed work or more strength work within strength training. Levernier et al. [13] compared the pull-up F-V profile in elite climbers of the three disciplines and found significant differences between boulder and speed climbers in V_0 and P_{max} (where boulder climbers had higher values) but did not find significant differences in F_0. Therefore, the F-V profile of speed climbers was not less force oriented than in other disciplines. Anyhow, we cannot compare these results with our study as we did not analyze climbers from different disciplines.

Our study presented a significant difference between levels in pull-up F-V profile, where F_0 was higher in international level. This could mean that pull-up training in speed climbers, especially in national level, might be oriented to maximize the force component (training with heavy loads) instead of performing ballistics movements (velocity component) [28]. In addition, 1RM and relative force values were significantly higher in international climbers.

Relations between power, force and velocity in lower limbs have previously been studied, where the correlation between running time and velocity component has been found to be stronger than running time and force component [7]. Nevertheless, this correlation was made analyzing only one jump (countermovement jump (CMJ)) per participant and not through an incremental test with different loads (F-V profile). Regarding our results, no differences were found between performance levels in the F-V profile variables in the squat exercise.

Nonetheless, previous research has shown the importance of lower limbs in speed climbing [4, 5, 7]. Strong correlations have been confirmed between the height of the jump in countermovement jump or vertical jump and the level of performance in the climbing wall [4, 26, 29], although in some of these studies the correlations were not significant. In our research we found a moderate correlation ($r \geq 0.5$; $p < 0.05$) between running time and squat 1RM, however correlations between pull-up variables and running time were greatly stronger (Table 3). Therefore, in the squat exercise it resembles that the most important variable is to have a high 1RM value regardless of whether it is through velocity or force component. The parallel squat exercise may not adequately display the climbers' lower limb skills. It has been found that the dynamic movements during the climbing route present a pattern of movement and a force-time profile very similar to that of the squat jump [26]. Future studies could investigate the correlation between the F-V profile of squat jump exercise and the performance on the wall.

The range of 1RM percentage at which the peak power is expressed in different exercises has been studied by several authors [30, 31] proposing that training with this percentage can provide greater benefits to improve power performance. However, studies analyzing this percentage in the pull-up exercise are scarce. Muñoz-López et al. [21] found that the load with which they expressed the greatest power was 70% of the 1RM and this amount usually coincided with the first load of the test, which was their own body weight. However, they concluded that this load was not light enough to find peak power and that this would be found by using a resistance that allows the body weight to be reduced (i.e., assisted training using elastic bands or conditioning machines). In our study, we obtained similar results, since most of the climbers obtained their peak power with the first load (body weight), except for some of the participants with the best 1RM who obtained it with a load of 10 kg. Likewise, Muñoz-López et al. [21], national level climbers obtained peak power in a range close to 70%1RM, however, international level climbers expressed it in a significantly lower percentage close to 60%1RM. This could be because international level climbers had a higher 1RM than national level climbers and the participants in Muñoz-López et al. [21].

On the other hand, the squat exercise has been more studied. It has been found that elite athletes express their peak power in the squat exercise between 30–70% of the 1RM [30]. Our results showed that national and international level climbers expressed the highest power value in this range, although generally closer to 30% than 70% of 1RM. In fact, there is no significant difference between the percentages of 1RM at which they obtained peak power between performance levels.

Regarding to sex differences, distinctions [32, 33] and similarities [34] in F-V profile characteristics have been evaluated in different physical exercises. One of the factors that can influence the F-V profile sex discrepancies could be the body fat-free mass [35]. Although we took body weight into account in the calculation of the F-V profile variables, body composition data were not considered. This might be considered a research limitation and we suggest measuring body composition values in future studies of this topic to evaluate possible differences between sexes. However, there were significant sex differences in squat power despite no sex differences in force or velocity components. Similarly, previous studies have reported results in other speed related sports, obtaining greater values for men in peak power [36]. This can be explained as commonly, women have less muscle volume than men in this kind of sports and the among of muscle mass is the major determinant of joint torque [37].

In addition, other limitations were found in this study. Previous studies have shown that a doble-hyperbolic equation between force and velocity fits more properly than the common linear one. In fact, using a linear equation can provide invalid and underestimated data

respecting V_0 and P_{max} [11]. However, the practical application of the linear equation may reduce the limitations in specific contexts, principally when data collected is above 45% F_0 as in the present study. Furthermore, multi-joint exercises, such as those carried out in our research, may present a decrease in the curvature magnitude compared to single-joint exercises [11].

This study showed the abilities differences in F-V profile in pull-ups and squats in speed climbers where pull-up F_0 was found significantly higher in international speed climbers. The information provided could be used by coaches and sport scientists in order to improve speed climbers' performance. In addition, further studies could consider our findings and keep on studying this new Olympic discipline which its scientific background is scarce.

CONCLUSIONS

The pull-up F-V profile displayed different between performance levels due to a higher F_0 value in international climbers. Therefore, the training of pull-ups in national level climbers could be considered to be focused on increasing the force component through heavy loads training. The squat F-V profile stayed similar at both performance levels although it is possible that this exercise does not adequately represents the lower body capabilities of speed climbers. Forthcoming studies should analyze F-V in squat jump as it has been shown that this exercise expresses climbers' skills more adequately [26]. In terms of peak power, national level climbers obtained it in a higher percentage of 1RM than the international level climbers in the pull-up exercise. Additionally, there were not significant sex differences in any F-V profile variables except from squat P_{max}, however fat-free mass should be considered in future research. Nevertheless, the science in this new Olympic discipline remains very young and more scientific studies are needed to provide vital information for the sake of adequate and specific training programs.

Data availability statement

The data that support the finding of this study are available from the corresponding author upon reasonable request.

Conflict of interest disclosure

VMC, VRC and JMGR have no conflicts of interest relevant to the content of this article.

Ethics approval statement

The experimental protocols were approved by the local ethics committee.

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Comparison of the effects of different foam rolling durations on knee extensors function

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ABSTRACT: Foam rolling (FR) intervention has recently attracted attention in sports and rehabilitation settings. However, the effects of FR using different rolling durations have not been fully clarified. Thus, this study focused on FR durations and examined the acute and prolonged (i.e., 20-min; 40-min, 60-min) effects of different FR intervention durations on maximal voluntary concentric contractions (MVC-CON), knee flexion range of motion (ROM), pain pressure threshold (PPT), and tissue hardness. The participants were 10 male university students (22.5 ± 1.0 years), and the target muscles were the dominant leg knee extensors. Three sets of 60-seconds FR interventions were performed in the randomized crossover trials in each condition. The three intervention conditions were fast (1 rolling/2 s, 30-repetition × 3 sets, 90 repetitions), medium (1 rolling/6 s, 10-repetition × 3 sets, 30 repetitions), and slow speed (1 rolling/12 s, 5-repetition × 3 sets, 15 repetitions). Before as well as immediately, 20-min, 40-min, and 60-min after the interventions, MVC-CON, ROM PPT, and tissue hardness were measured. The results showed no interaction effect in the acute effect but a main effect of time for all variables ($p < 0.05$). Also, no interaction was observed in prolonged effect, but main effects of time were observed in knee flexion ROM, PPT, and tissue hardness ($p < 0.01$) but not for MVC-CON. Post-hoc tests showed significant PPT ($p < 0.05$) and knee flexion ROM ($p < 0.01$) increases up to 20- and 60-minutes respectively after all interventions. Tissue hardness was significantly ($p < 0.01$) decreased up to 60-minutes after all interventions. This study showed that the FR intervention changed ROM, PPT, tissue hardness, and MVC-CON regardless of rolling duration and that the effects persisted up to 20–60 minutes.

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INTRODUCTION

Previous studies showed that foam rolling (FR) could increase the range of motion (ROM) without decreasing muscle strength or performance [1–3]. Furthermore, FR could decrease tissue hardness [1]. For these reasons, FR has attracted attention as a warm-up method. In addition, previous studies showed FR interventions could decrease pain associated with delayed onset muscle soreness [4–6]. Moreover, FR interventions could decrease pain in post-operative patients [7, 8]. Therefore, FR is applied in both sports and rehabilitation.

Although many previous studies have shown the effectiveness of FR interventions, detailed data on the use of FR are sparse. A previous study examining optimal FR intensities showed that ROM increases were possible regardless of low (rating of perceived pain (RPP): 3.9/10), moderate (RPP: 6.2/10), or high (RPP: 8.2/10) rolling pressures were applied without negatively affecting muscle strength or performance [9]. Soft, medium, and hard (EVA foam) FR densities all showed increased knee flexion ROM and PPT regardless of FR densities [10]. Moreover, the minimum duration of FR intervention to increase ROM might differ between target muscles. Nakamura et al. [23] reported that FR of the calf muscles for at least 90-seconds could be expected to increase dorsiflexion ROM. On the other hand, Sullivan et al. [21] reported that a 5-second FR intervention on the knee flexors significantly increased sit and reach ROM.

In FR intervention, individual rolling duration (speed) is considered an essential factor in addition to intensity (pressure), total FR session time, and density. Still, only a few previous studies have focused on the FR durations. Wilke et al. [11] examined the effects of

different intervention duration on the anterior thigh with an intervention time of 45 seconds × 4 sets and an intervention intensity of Numerical Rating Scale (NRS) 6–7. The three rolling duration conditions were compared: a fast intervention condition of 1 roll/2-seconds, a slow intervention condition of 1 roll/10-seconds, and a control condition. The results showed no increase in knee flexion ROM in any conditions. On the other hand, they reported a significant decrease in tissue stiffness after 5- and 10-minutes in the fast intervention condition and after 10-minutes in the slow intervention. A commentary by Behm et al. [13] suggested that a rolling duration of 2–4-seconds per direction is optimal for increasing ROM. However, they used regression equations from a prior study data to predict optimal responses rather than conducting a research study to directly compare rolling durations (speeds). However, to our knowledge, the effects of different rolling durations in FR interventions have not been fully investigated. In addition, the previous study by Wilke et al. [11] only examined the effects of FR at different durations up to 10-minutes post-rolling. In our previous study [1], a 180-second FR intervention on knee extensors significantly increased ROM at least 30-minutes after the intervention. However, the duration of increased ROM after FR is unknown, and hence, studies applying longer testing periods than 30 minutes are needed to investigate the prolonged effects of FR. Therefore, this study's objectives were the following: The first was to compare and examine the acute effects of different FR intervention durations (speeds) on maximal voluntary concentric contractions (MVC-CON), range of motion (ROM), pain pressure threshold (PPT), and tissue hardness of the knee extensors. The second was to compare the prolonged effect of different FR intervention durations (speeds). Behm et al. [13] reported that the effect of FR on increasing ROM was greater at an intervention duration of 2–4 seconds per direction. In addition, our previous study [1] found a significant increase in ROM at an intervention duration of 1 second per direction. It has also been reported that FR does not decrease muscle strength and performance [12, 13]. Therefore, in this study, we hypothesized that the effect of increasing ROM is larger the fast and smaller the later. In addition, we considered that muscle strength and performance would not change regardless of the FR duration (speed).

MATERIALS AND METHODS

Experimental set-up

A repeated randomized measures experimental design was used to compare differences in the duration of FR intervention. The participants were instructed to visit the laboratory three times with a ≥ 48 h break. They were exposed to the following three conditions: FR-Fast, FR-Medium, and FR-Slow in a random order (Figure 1). For FR-Fast, FR-Medium, and FR-Slow, rolling interventions were performed from proximal to distal and back to proximal of the dominant (preferred to kick a ball) knee extensors in 2-, 6-, and 12-seconds, respectively. One set was 60-seconds for each condition, and three sets were performed (total of 180-seconds). The measurement periods were before the intervention (PRE), immediately after (POST), 20-, 40-, and 60-minutes after the intervention. The measurements were tissue hardness, PPT, knee flexion ROM, and MVC-CON, and these were assessed in this order. Since knee flexion ROM and MVC-COM measurements may influence PPT and tissue hardness measurements, measurements were performed in this order.

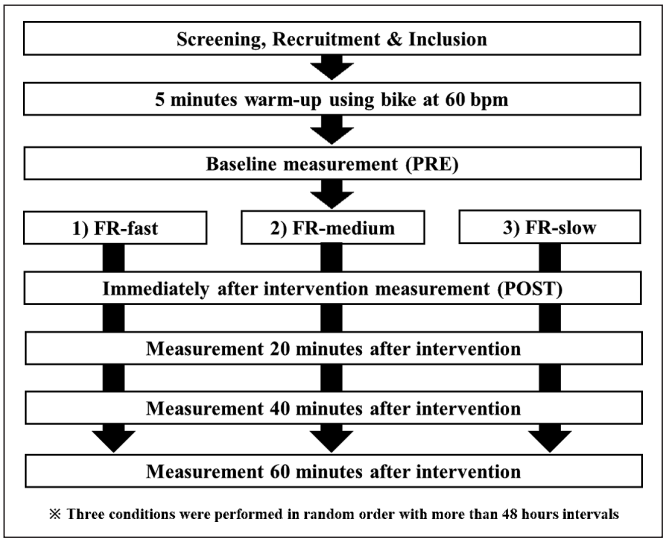


FIG. 1. The experimental set-up for foam rolling (FR) intervention with three durations: FR-Fast, FR-Medium, and FR-Slow. For FR-Fast, FR-Medium, and FR-Slow, rolling interventions were performed from proximal to distal and back to proximal of the dominant (preferred to kick a ball) knee extensors in 2-, 6-, and 12-seconds, respectively. One set was 60-seconds for each condition, and three sets were performed (total of 180-seconds). The measured parameters were maximal voluntary concentric contraction torque, knee flexion range of motion, pain pressure threshold and tissue hardness in all time intervals.

Participants

Ten healthy, recreationally active males were enrolled (mean ± SD: age, 22.5 ± 1.0 years; height, 170.1 ± 3.5 cm; weight, 69.8 ± 8.7 kg). The participants completed the three conditions described above in random order. Individuals with a history of neuromuscular disease and musculoskeletal injury involving the lower extremities were excluded. The required sample size for a repeated-measures two-way analysis of variance (ANOVA) (effect size = 0.25 [large when considering interaction effects for 2-way ANOVAs], αerror = 0.05, and power = 0.80) based on our previous study's ROM results [14] using G* power 3.1 software (Heinrich Heine University, Dusseldorf, Germany) was more than eight participants.

For the study, participants were fully informed about the procedure and aims, after which they provided written informed consent. The study complied with the requirements of the Declaration of

Helsinki and was approved by the Ethics Committee of the Niigata University of Health and Welfare, Niigata, Japan (Procedure #18615).

Outcome assessment

Knee flexion ROM

Each participant was placed in a side-lying position on a massage bed with the hips as well as the knee of the non-dominant leg flexed at 90° to prevent pelvic movements [1, 15]. A licensed physical therapist, the investigator, brought the dominant leg to full knee flexion with the hip joint in a neutral position. A goniometer (MMI universal goniometer Todai 300 mm, Muranaka Medical Instruments, Co., Ltd., Osaka, Japan) was used to measure knee flexion. ROM was measured three times in each measurement period, and the average value at each measurement period was used for analysis.

Pain pressure threshold (PPT)

PPT measurements were conducted in the supine position using an algometer (NUTONE TAM-22(BT10); TRY-ALL, Chiba, Japan). The measurement location was set at the midway point of the distance between the anterior superior iliac spine and the dominant side's superior border of the patella for the rectus femoris muscle. With continuously increasing pressure, the soft tissue in the measurement area was compressed with the metal rod of the algometer. The participants were instructed to immediately press a trigger when pain, rather than just pressure, was experienced. The value read from the device at this time point (kilograms per square centimeter) corresponded to the PPT. In each condition, PPT was measured three times at each measurement period, and the mean value at each measurement period was used for further analysis.

Tissue hardness

Tissue hardness was measured using a portable tissue hardness meter (NEUTONE TDM-N1; TRY-ALL Corp., Chiba, Japan). The participant's measurement position and posture were similar to PPT measurements. This tissue hardness meter measured the penetration distance until a 14.71 N (1.5 kgf) pressure was reached [16]. The participants were instructed to relax while tissue hardness was measured three times at each measurement period, and the mean value at each measurement period was used for further analysis.

Maximal Voluntary Concentric Contractions (MVC-CON)

In accordance with previous studies [17], MVC-CON of the dominant leg's knee extensors was measured at an angular velocity of $60°.s^{-1}$ between 20° and 110° knee flexion using an isokinetic dynamometer (BIODEX System 3.0, Biodex Medical System Inc. Shirley, NY, USA). The participants sat on the dynamometer chair adopting an 80° hip flexion angle, with adjusted Velcro straps fixed over the exercised limb's trunk, pelvis, and thigh. Three trials were performed at each measurement period, and the highest value was analyzed. In all trials, strong verbal encouragement was given to elicit maximum effort.

Foam rolling (FR) Intervention

A physical therapist instructed the participants how to use the foam roller (Stretch Roll SR-002, Dream Factory, Umeda, Japan). For familiarization, they were allowed to practice using the foam roller three to five times on the non-dominant leg (non-intervention leg) immediately before the FR intervention to verify that the participants were able to perform the FR intervention at the specified duration (speed) and location. FR was performed using three sets of 60-seconds with a 30-seconds rest between sets. One cycle of FR was defined as one distal rolling movement followed by one proximal rolling movement. FR-fast was performed in 2-seconds (30 repetitions × 3 sets, 90 repetitions), FR-medium in 6-seconds (10 repetitions × 3 sets, 30 repetitions), and FR-slow in 12-seconds (5 repetitions × 3 sets, 15 repetitions) for one cycle. A metronome (Smart Metronome; Tomohiro Ihara, Japan) was used for control. The participants were asked to place as much body mass on the roller as tolerable. All FR interventions were supervised by one well-trained physical therapist.

Statistical analysis

SPSS (version 28.0; IBM Corp., Armonk, NY, USA) was used for the statistical analysis. We calculated the coefficient of variation (CV) and intraclass correlation coefficient (ICC) from PRE data in three conditions to check the test-retest reliability. To verify the consistency of PRE values, PRE values were tested among all conditions using a one-way ANOVA. To clarify the difference with the acute effects of different rolling duration, two-way 2 × 3 repeated measures ANOVA using two factors (test time [PRE vs. POST] and condition [FR-fast vs. FR-medium vs. FR-slow]) was analyzed for interactions and main effects. For the prolonged effect, a two-way 3 × 3 repeated measures ANOVA using two factors (test duration [PRE vs 20 min vs 40 min vs 60 min] and condition [FR-fast vs FR-medium vs FR-slow]) was analyzed for interactions and main effects. Classification of effect size (ES) was set where $\eta_p^2 < 0.01$ was considered small, 0.02–0.1 was considered medium, and more than 0.1 was considered to be a large effect size [18]. As post-hoc tests, a paired t-test with Bonferroni correction was used for the acute effect, and a multiple comparison test with Bonferroni correction was used for the prolonged effect. Additionally, we calculated the Cohen's d as effect size between PRE and POST, 20-, 40-, 60-minutes in each condition, respectively, distinguishing trivial (d = 0–0.19), small (d = 0.20 to 0.49), medium (0.50 to 0.79) or large (≥ 0.80 or higher) effects [18]. The significance level was set to 5%, and all the results are shown as mean ± SD.

RESULTS

Comparison between PRE values among the three conditions

There were no significant differences in all PRE variables between the three conditions. The CVs of measurements for MVC-CON, knee ROM, PPT, and tissue hardness were 6.0 ± 2.8%, 1.5 ± 1.2%, 12.7 ± 7.6%, and 9.6 ± 4.3%, respectively, and the ICC (1,3) for

measurements were 0.929, 0.563, 0.735, and 0.854, respectively.

Acute effects in MVC-CON, knee flexion ROM, PPT, and tissue hardness

Table 1 shows the changes in MVC-CON, knee flexion ROM, PPT, and tissue hardness, before (PRE) and immediately following (POST) the three durations of FR intervention. There were no significant interaction effects for all variables (MVC-CON: $F = 0.1$, $p = 0.93$, $\eta_p^2 = 0.01$, knee flexion ROM: $F = 0.3$, $p = 0.71$, $\eta_p^2 = 0.03$, PPT: $F = 0.2$, $p = 0.84$, $\eta_p^2 = 0.01$, tissue hardness: $F = 0.4$, $p = 0.70$, $\eta_p^2 = 0.03$). However, there were significant main effects of test time with all variables (MVC-CON: $F = 5.7$, $p < 0.05$, $\eta_p^2 = 0.18$, knee flexion ROM: $F = 87.2$, $p < 0.01$, $\eta_p^2 = 0.76$, PPT: $F = 36.5$, $p < 0.01$, $\eta_p^2 = 0.58$, tissue hardness: $F = 44.9$, $p < 0.01$, $\eta_p^2 = 0.62$). The post-hoc test results showed that MVC-CON ($p < 0.05$), knee flexion ROM ($p < 0.01$), PPT ($p < 0.01$) were significantly higher at POST and tissue hardness ($p < 0.01$) was lower at POST.

Prolonged effects in MVC-CON, knee flexion ROM, PPT, and tissue hardness

Table 2 illustrates MVC-CON, knee flexion ROM, PPT, and tissue hardness values, PRE, 20-min, 40-min, and 60-min after three

TABLE 1. The acute changes (mean ± SD) in MVC-CON torques, knee flexion range of motion (ROM), pain pressure threshold (PPT), and tissue hardness before (PRE) and immediately after (POST) the intervention.

	FR-fast		FR-medium		FR-slow	
	PRE	**POST**	**PRE**	**POST**	**PRE**	**POST**
MVC-CON (Nm)	184.4 ± 28.4	192.5 ± 26.2*	189.2 ± 40.5	194.9 ± 27.5*	188.1 ± 22.3	194.1 ± 23.0*
	d = 0.30		d = 0.17		d = 0.27	
Knee flexion ROM (deg)	137.5 ± 2.1	140.5 ± 2.6*	137.1 ± 4.7	140.2 ± 4.0*	137.7 ± 3.9	141.3 ± 4.0*
	d = 1.27		d = 0.72		d = 0.92	
PPT (kg)	4.2 ± 1.2	5.1 ± 2.1*	3.9 ± 0.8	5.0 ± 1.4*	3.5 ± 0.8	4.4 ± 1.1*
	d = 0.54		d = 1.01		d = 0.92	
Tissue hardness (N)	18.3 ± 4.1	16.0 ± 3.4*	17.9 ± 3.8	16.2 ± 3.0*	19.3 ± 3.6	17.5 ± 4.2*
	d = -0.61		d = -0.51		d = -0.47	

*: Significant difference ($p < 0.05$) from PRE.

TABLE 2. The prolonged changes (mean ± SD) in maximal voluntary concentric contraction (MVC-CON) torques, knee flexion range of motion (ROM), pain pressure threshold (PPT), tissue hardness PRE and after 20-, 40-, and 60-minutes after the interventions.

	FR-fast				FR-medium				FR-slow			
	PRE	**20 min**	**40 min**	**60 min**	**PRE**	**20 min**	**40 min**	**60 min**	**PRE**	**20 min**	**40 min**	**60 min**
MVC-CON (Nm)	184.4 ± 28.4	192.5 ± 26.2	185.6 ± 25.9	183.5 ± 22.2	189.2 ± 40.5	188.8 ± 32.4	187.0 ± 33.2	182.5 ± 30.5	188.1 ± 2.3	192.8 ± 20.7	189.9 ± 20.6	188.1 ± 17.0
	d =	0.19	0.04	-0.04	d =	-0.01	-0.06	-0.19	d =	0.22	0.08	0.00
Knee flexion ROM (deg)	137.5 ± 2.1	139.7 ± 2.7*	138.7 ± 2.5*†	138.4 ± 2.3*†‡	137.1 ± 4.7	139.0 ± 4.4*	138.3 ± 4.7*†	137.5 ± 4.7*†‡	137.7 ± 3.9	140.3 ± 4.2*	138.6 ± 3.7*†	138.2 ± 3.6*†‡
	d =	0.91	0.52	0.39	d =	0.42	0.26	0.09	d =	0.64	0.24	0.12
PPT (kg)	4.2 ± 1.2	5.1 ± 2.1*	4.7 ± 1.9	4.6 ± 1.8	3.9 ± 0.8	4.4 ± 1.4*	4.2 ± 1.2	3.8 ± 0.7	3.5 ± 0.8	4.0 ± 1.2*	4.0 ± 1.2	3.9 ± 1.1
	d =	0.30	0.25	0.05	d =	0.39	0.26	-0.13	d =	0.49	0.44	0.37
Tissue hardness (N)	18.3 ± 4.1	16.8 ± 3.3*	16.7 ± 3.5*	17.2 ± 3.8*†‡	17.9 ± 3.8	16.3 ± 3.3*	16.4 ± 3.0*	17.2 ± 3.2*†‡	19.3 ± 3.6	17.5 ± 3.9*	17.8 ± 3.4*	18.7 ± 3.5*†‡
	d =	-0.41	-0.42	-0.27	d =	-0.45	-0.46	-0.22	d =	-0.49	-0.43	-0.18

*: Significant difference ($p < 0.05$) from PRE; †: Significant difference ($p < 0.05$) from 20 minutes after the intervention; ‡: Significant difference ($p < 0.05$) from 40 minutes after the intervention.

durations of FR intervention. There were no significant interaction effects for all variables (MVC-CON: $F = 0.3$, $p = 0.95$, $\eta_p^2 = 0.02$, knee flexion ROM: $F = 1.25$, $p = 0.29$, $\eta_p^2 = 0.09$, PPT: $F = 0.5$, $p = 0.82$, $\eta_p^2 = 0.04$, tissue hardness: $F = 0.3$, $p = 0.92$, $\eta_p^2 = 0.02$). However, there were significant main effects of test time with knee flexion ROM, PPT, and tissue hardness (knee flexion ROM: $F = 58.3$, $p < 0.01$, $\eta_p^2 = 0.68$, PPT: $F = 6.4$, $p < 0.01$, $\eta_p^2 = 0.19$, tissue hardness: $F = 22.6$, $p < 0.01$, $\eta_p^2 = 0.46$). There were no prolonged effects with MVC-CON. The post-hoc tests showed that knee flexion ROM ($p < 0.01$), PPT ($p < 0.01$) were significantly higher at POST and tissue hardness ($p < 0.01$) was lower at POST. Post-hoc test results showed that knee flexion ROM was significantly ($p < 0.01$) higher 20-, 40-, and 60-minutes after the intervention compared with PRE. The values at 20-minutes were significantly ($p < 0.01$) higher than at 40- and 60-minutes, and the values at 40-minutes were significantly ($p < 0.01$) higher than at 60-minutes. PPT was significantly ($p < 0.05$) higher at 20-minutes compared to PRE. Tissue hardness was significantly ($p < 0.01$) lower 20-, 40-, and 60-minutes after the intervention compared with PRE. The values at 60-minutes were significantly ($p < 0.01$) higher than at 20- and 40-minutes.

DISCUSSION

The positive effects of an acute bout of FR were not duration specific (from 2 to 12 seconds per rolling direction). This study shows that 180-seconds of FR increased ROM, PPT, MVC-CON and decreased tissue hardness immediately post-test but there were no prolonged MVC-CON effects (20–60-minutes post-intervention). However, PPT increased for up to 20-minutes whereas both ROM increased, and tissue hardness decreased for up to 60-minutes after the FR intervention regardless of rolling duration (rolling speeds).

FR has been reported to be effective in increasing ROM [3, 13, 19]. The lack of difference in effectiveness with different FR durations (speeds), support the results of Wilke et al. [11]. However, while knee flexion ROM did not significantly change after 45 seconds × 4 sets of FR in the Wilke et al. study, the present study showed a significant increase in knee flexion ROM at all measured time points. This result must be interpreted through a lens of a moderate (ICC: 0.563) reliability coefficient [20]. This discrepancy may be due to differences in knee flexion ROM measurements, where Wilke et al. [11] employed active knee flexion measurements, this study used passive knee flexion measurements. The influence of FR on the difference between active and passive ROM measurement needs further investigation. Furthermore, whereas individuals seem to focus on or emphasize hip flexion (hamstrings) flexibility, less emphasis and time is typically placed on knee flexion (quadriceps) flexibility. Hence, this lower familiarization with knee flexion ROM may have contributed to the moderate reliability values and the increasing ROM with successive ROM tests at all time points. Still, Wilke et al. [11] reported a significant reduction in tissue stiffness after 5- and 10-minutes of FR in the fast speed condition and only after 10-minutes in the slow speed condition suggesting that the FR effect may be greater with the fast versus slow speed conditions. Behm et al. [13] in their commentary recommended a rolling time of 2–4 seconds per direction to increase ROM. In our previous study [1], FR for 180-s increased ROM up to 30-minutes with FR durations of 1-second per direction. However, in the present study, a significant increase in ROM was also observed in the slow duration condition (12-seconds per 1 roll). Wilke et al. [11] also stated that their study examined acute effects and that prolonged effects need to be examined. In this study, the prolonged effect was examined up to 60 minutes after the intervention, and as with the acute effect, there were no differences between the groups. Although the total duration of the FR intervention in Wilke et al. [11] and the present study were identical, differences in the intervention duration per set and the number of sets might cause differences in the effects of intervention duration. Furthermore, the commentary by Behm et al. [13] recommends 30–120 seconds × 1–3 sets of FR intervention when aiming to increase ROM. Moreover, FR has been suggested to have a volume-response relationship [5, 21]. This suggests that increasing the intervention time may have different effects depending on the intervention duration (speed).

In this study, PPT was significantly increased up to 20-minutes, and knee flexion ROM was significantly increased up to 60-minutes. Previous studies have suggested that increases in stretch tolerance (pain sensation) are involved with the increase in ROM after FR and vibration foam rolling interventions [19, 22–24]. FR may reduce pain by activating either neural-gating mechanisms [25, 26] or releasing endorphins and enkephalins as theorized with the diffuse noxious inhibitory control mechanism [27]. In addition, there are reports of increased PPT after FR intervention [1, 28–30]. Although the detailed relationship between increased PPT and increased ROM is not clear, it is possible that FR intervention altered stretch tolerance (pain sensation), resulting in increased ROM. However, ROM remained elevated for 60-minutes, hence, increased pain or stretch tolerance cannot be the primary or sole factor in this increase.

Tissue hardness was significantly decreased up to 60-minutes after the intervention regardless of rolling duration, suggesting that the decrease in tissue hardness may be involved in the maintenance of increased ROM. A systematic review and meta-analysis [31] reported that FR intervention decreases tissue hardness in the quadriceps muscle. FR may decrease tissue hardness through thixotropic changes [32] and increase tissue perfusion [33]. The same mechanism is likely responsible for the significant decrease in the present study.

In this study, regardless of FR duration, MVC-CON was increased immediately following the FR intervention. However, there were no significant prolonged (20–60-minutes post-intervention) changes in MVC-CON regardless of FR duration. A meta-analysis [34] reported that FR did not affect muscle strength or performance. The results of the present study are consistent with a systematic review and meta-analysis by Glänzel et al. [31], suggesting that FR of the knee extensors can significantly increases MVC-CON. The mechanism of

FR-induced increase in muscle strength is thought to be due to an increase in local blood flow. Warm-up effects such as increased blood flow are thought to increase muscle temperature and induce nitric oxide release [33, 35] promoting vasodilation and phosphate creatine replenishment. In addition, the thixotropic effect of FR may allow for restful movement and that the decrease in pain sensitivity may allow for higher contractile strength. The effects of FR interventions on muscle strength and performance have not been consistently reported, and much further study is needed.

This study has several limitations: first, the maximum prolonged effect of the FR intervention is unknown. Secondly, since this study was conducted on healthy male university students, it is not known whether the same effects can be obtained on athletes or female subjects. Third, the order of measurements in this study was not randomized; thus, the results may differ depending on the order of the measurements. However, since the order of the measurements was the same before and after the interventions, a potential effect of the order is likely negligible. The fourth limitation is the intervention method. In this study, FR was practiced on the non-dominant side. This may have caused a crossover effect. However, due to the short intervention time and the practice sessions that were standardized for all conditions, the effect of the practice sessions on this study was small. Furthermore, crossover effects or non-local muscle effects have been reported to be trivial when single discrete actions are observed [36]. Fifth, this study examined the effects of different FR intervention durations, but the effects compared to stretching are unknown. Kasahara et al. [17] showed that combined FR and stretching interventions had a cumulative effect, while Yuan et al. [37] did not. Therefore, future studies are needed to examine different combinations of rolling duration and stretching intervention to construct an effective protocol.

Practical implications

As a pre-exercise warm-up, short-term, i.e., 30-second FR or VFR intervention is recommended when the goal is to increase ROM while maintaining muscle strength. Also, this beneficial effect was prolonged for 10 or 15 minutes after interventions. Also, in short-term interventions, vibration function does not necessarily need to be added to FR as a warm-up routine.

CONCLUSIONS

When using FR intervention for warm-up, rolling duration (speed) has no effect when the goal is to immediately increase ROM, PPT, and MVC-CON torque and decrease tissue hardness. FR intervention can be expected to increase ROM and decrease tissue hardness up to 60 minutes after FR intervention, regardless of rolling duration (speed), so it can be incorporated into warm-up routines. It is possible to adjust the intervention rate for each subject based on the results of this study, which is important information because it indicates that FR may be applicable in many fields.

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Ethics Approval information

This study was approved by the Ethics Committee of the Niigata University of Health and Welfare, Niigata, Japan (Procedure #18615), and has complied with the requirements of the Declaration of Helsinki.

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Fitness and fatness in children and adolescents: investigating their role in the association between physical activity and cardiometabolic health

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ABSTRACT: To verify the role of the combination of fitness and fatness in the relationship between physical activity (PA) and cardiometabolic risk in children and adolescents. This is a cross-sectional study performed with 2786 children and adolescents (6 to 17 years). Fitness was determined by the cardiorespiratory fitness (CRF) six-minute walking and running test. Waist circumference (WC) was considered a fatness indicator. A self-reported questionnaire was used to determine PA practice, whereas the clustered cardiometabolic risk score (cMetS) was calculated by summing z-scores of triglycerides, total cholesterol/HDL-C ratio, systolic blood pressure, glucose, and WC. Considering the combination of CRF (fitness) and WC (fatness), the following phenotypes were created: Fit/Unfat, Fit/Fat, Unfit/Unfat and Unfit/Fat. Moderation analyses were tested using linear regression models. Significant interactions were found between PA and Unfit/Fat category ($\beta = -0.001$; $p = 0.001$) only for adolescents. The interaction observed in the Unfit/Fat phenotype indicated that adolescents who practise PA for 330 minutes per week presented lower cMetS compared to those who do not practise or practise for 60 minutes respectively. The combination of fitness and fatness moderates the relationship between PA and cardiometabolic risk, suggesting that adolescents, particularly those who are less fit and present high adiposity, should be encouraged to engage in regular PA to improve their metabolic health.

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INTRODUCTION

The onset of cardiometabolic risk factors used to be observed only in adults; however, this scenario has changed, beginning to manifest already during childhood, increasing the risk of developing the cardiometabolic disease later in life [1]. In the context of lifestyle behaviour, the practice of physical activity (PA) brings significant benefits for cardiometabolic health in youth, including decreased low-density lipoprotein cholesterol, insulin resistance, and arterial blood pressure, as well as increased high-density lipoprotein cholesterol (HDL-C) [2, 3]. Similarly, cardiorespiratory fitness (CRF), which is mainly determined by PA habits, is an important health indicator and independent predictor of cardiometabolic risk [4, 5].

In an opposite direction, obesity is a chronic disease associated with the development of many cardiometabolic disorders [6]. The combination of both conditions, fitness and fatness, gives rise to the "fat-but-fit" paradox, which suggests that appropriate levels of CRF could attenuate the deleterious consequences for metabolic health related to an excess of fatness [7]. Therefore, the general belief that being normal weight is equal to being healthy could be wrong since studies have indicated that normal-weight/unfit subjects could be at a higher risk of developing cardiovascular disease than obese/fit subjects [7, 8]. Some studies reinforce the fat-but-fit paradigm, indicating that CRF could counteract the

effect of obesity in cardiometabolic risk factors in children [9], while others shows that both CRF and adiposity are important for having a low cardiometabolic risk [10, 11]. In this context, considering the role of sexual maturation, it is essential due to its influence on physical activity, fitness and cardiometabolic risk factors, in which evidence indicates that early sexual maturation was positively associated with obesity in both girls and boys and also that pubertal stage has an interaction effect on the association of cardiometabolic risk with body mass index [12–14].

Recent studies have suggested that adiposity mediates the association between CRF and cardiometabolic risk [15, 16], which could be related to the correlation between body weight and the computation of CRF. Also, it has been suggested that CRF could play a moderator role in the association between PA and cardiometabolic risk factors [17]. Indeed, these indicators seem to influence the development of cardiometabolic risk in children and adolescents, as well as in the PA levels. However, it is not yet clear how CRF and adiposity interact in this relationship. Based on these assumptions and the strong link among PA, CRF and adiposity, we hypothesized that the combination of fitness and fatness would moderate the association between PA and cardiometabolic risk factors in Brazilian children and adolescents.

Thus, the authors intend to address new evidence in the literature from the perspective of exploring how the combination of fitness and fatness is capable of changing the relationship between PA and cardiometabolic risk factors. Consequently, the present study aimed to verify the role of the combination of fitness and fatness in the relationship between PA and cardiometabolic risk in children and adolescents.

MATERIALS AND METHODS

Study design and sample

This cross-sectional study was developed with 2786 children (n = 1170) and adolescents, aged between six and 17 years (mean age 12.17 ± 2.75), from public and private schools from a city located in southern Brazil. Data from the present study belong to a cohort in which the initial sample was recruited in 2004. Students of all regions of the city were considered to calculate the population density of students to be included in the research. Twenty-five schools were randomly selected from fifty schools with 20,380 schoolchildren, considering schools of different regions of the municipality to form a representative sample of this city. All students from the 25 schools were invited to participate in the cohort, which were organized as follows: Phase I (2004–2005), Phase II (2007–2009), Phase III (2011–2012), Phase IV (2014–2015), and Phase V (2016–2017). Data from Phase IV and V were used for the present study. The age range to classify children and adolescents was set according to the Brazilian recommendations [18].

Procedures

The present study was conducted in accordance with Resolution 466/2012 of the National Council of Health in Brazil, and approved by the research ethics committee at the University of Santa Cruz do Sul (no. 4.278.679). The schoolchildren's parents or legal guardians signed free and informed consent forms. Data were collected between 2014 and 2017 in the facilities of the University of Santa Cruz do Sul by trained researchers, including physical activity professors, a technical professional in nursing and a pharmacist.

Evaluations

CRF was assessed by the six-minute walking and running test following the procedures of *Projeto Esporte Brasil* [19]. Schoolchildren should achieve the greatest number of turns, running or walking, in a six-minute period. The test was performed on an outdoor athletic track with demarcations every 10 m to indicate the exact distance (in metres) that was covered. The number of laps completed and the additional distance achieved for the ones unable to complete a full lap at the end of the test were calculated. Thus, CRF was determined by multiplying the number of laps by the metres covered. The six-minute walking and running test is commonly used for the evaluation of Brazilian youth due to ease of application, low cost of material, and the opportunity to evaluate a large number of participants simultaneously [20, 21]. Children and adolescents were classified as Fit and Unfit according to the cut-off points proposed by *Projeto Esporte Brasil* for sex and age [19].

Waist circumference (WC) was evaluated using an inelastic tape with a resolution of 1 mm (Cardiomed), placed at the mid-point between the lower ribs and the iliac crest [23]. WC was classified as Fat or Unfat according to the cut-off points by age and sex proposed by Taylor (2000) [22]. WC was considered the fatness indicator as it is a well-accepted indicator of central adiposity [23]. In addition, it has been suggested that WC presents a stronger association with cardiometabolic risk compared to other anthropometric indicators, such as waist-to-height ratio [24].

Considering the combination of CRF (fitness) and WC (fatness) we created the following categories: Fit/Unfat, Fit/Fat, Unfit/ Unfat and Unfit/Fat.

Weight and height were measured using an anthropometric scale with a coupled stadiometer (Filizola). To determine body mass index (BMI), weight (in kilograms) was divided by height (in square meters).

Self-reported questions were used to determine skin colour and PA practice. The parents of the children aged between 6 and 10 years helped them to fill in the questionnaire. Concerning skin colour, participants were required to indicate one of the following options: white, black, brown/mulatto, indigenous and yellow, as indicated in Brazilian population censuses [25]. PA was evaluated through the following questions: "Do you usually practise any sport/physical activity?" (yes, or no); "How many times a week and hours/minutes per day do you practise this sport/physical activity?" Subsequently, the total minutes per day spent practising sports/physical activities were summed.

Blood samples were collected after 12 hours of fasting to determine triglycerides (TG), total cholesterol (TC), HDL-C, and glucose.

Serum samples and commercial kits were used (DiaSys Diagnostic Systems, Germany), performed on Miura 200 automated equipment (I.S.E., Rome, Italy). Systolic blood pressure (SBP) was evaluated by the auscultatory method, using a sphygmomanometer and a stethoscope according to standard procedures [26]. The children/adolescents were supposed to be resting for five minutes prior to the measurement, which was made early in the morning. Each device had three different sized cuffs so that researchers could select the most suitable for each arm circumference. Two measurements on the right arm were made, and the lowest blood pressure was recorded.

The cardiometabolic risk was assessed using a clustered cardiometabolic risk score (cMetS), which was calculated by summing z-scores of TG, TC/HDL-C ratio, SBP, and glucose, and dividing the sum by five. Sex- and age-specific standardized z-scores were calculated using international references for each risk factor with the following equation: z-score = ([X – □]/SD), where X is the continuous value observed for the risk factor, □ is the predicted mean calculated for the risk factor using regression equations, and standard deviation (SD) is the standard deviation of the international reference [27]. Before analysis, skewed variables (TC/HDL-C ratio, TG, and WC) were transformed by the natural logarithm.

Sexual maturation was determined according to Tanner's criteria [28]. The researcher explained the pictures with the different stages of maturation and the schoolchildren were required to indicate the image accordingly to their current stage, considering genital development for boys, breast development for girls and pubic hair for both. Thus, five stages of sexual maturation were considered and subsequently categorized into four: pre-pubertal (stage I), initial development (stage II), continuous maturation (stages III and IV), and matured (stage V). Socioeconomic status was evaluated according to Brazilian Association of Research Companies [29], which considers the head of the household's educational level and the number of certain items they have (bathrooms, washing machines, car, among others). Each answer was scored and the sum of these scores was used as a measure of the family social class. Subsequently, individuals were classified into three distinct economic classes: low (D-E), medium (C) and high (A-B).

Statistical Analysis

Descriptive data were presented as mean and standard deviation (continuous variables) and frequencies (categorical variables). All variables were checked to test normality distribution through an exploratory analysis according to box-plot visual inspection and scatter-dot graphs to verify the behaviour of variables concerning linearity. Independent two-tailed *t* tests or chi-squared tests were used to examine differences between children and adolescents

Moderation analyses were tested through multiple linear regression models using the PROCESS macro for SPSS version 23.0 (IBM Corp). The models were tested as follows: a) Associations between the combination of fitness and fatness categories with cMetS, adjusted for PA; b) Associations between PA and cMetS, adjusted for the combination of fitness and fatness categories; and c) Interactions between the combination of fitness and fatness categories and PA, which consists of the result of the multiplication between the effect of the independent variable (PA) and moderator variable (combination of fitness and fatness categories) in the dependent variable (cMetS).

The PA minutes were categorized into tertiles, provided by the moderation analysis. To test the differences in cardiometabolic risk score according to the combination of fitness and fatness categories in adolescents who practise ≥ 330 minutes of PA a week the ANOVA was used.

All analyses were adjusted for sex, pubertal status, socioeconomic level and skin colour. The probability value $p < 0.05$ was considered to be significant for all analyses.

Multiple linear regression was used as a statistical test for post-hoc sample calculation in the G* Power 3.1 program (Heinrich-Heine-Universität), considering the following parameters: a significance level of $\alpha = 0.05$, and effect size of 0.02. The number of predictors considered was seven, with a sample size of 2786 children, and test power $(1\text{-}\beta) = 0.99$.

RESULTS

A total of 1,170 children (54.0% female) and 1,616 adolescents (59.0% female) participated in the study. Adolescents presented higher mean values of weight, height, BMI, WC, PA, systolic blood pressure, total cholesterol, triglycerides and cMetS compared to children. In addition, 73.6% and 16% of the adolescents were classified in the unhealthy zone for CRF and presented overweight/obesity respectively, while for children 45.6% were classified as unhealthy and 26% were overweight/obese. Concerning the combination of fitness and fatness, 17.8% of the children and 14.1% of the adolescents were classified in the Unfit/Fat category (Table 1).

The combination of fitness and fatness in the relationship between PA and cardiometabolic risk in children and adolescents is presented in Table 2. In children, the Fit/Fat and Unfit/Fat categories were positively associated with cMetS; however, no interactions were found. In adolescents, the Fit/Fat, Unfit/Unfat and Unfit/Fat categories were positively associated with cMetS. Also, significant interactions were found between PA and the Unfit/Fat category, indicating that this phenotype is a moderator in the relationship between PA and cMetS.

For a better comprehension of the interaction observed in adolescents, we present a graphic representation of the moderating role of the combination of fitness and fatness in the relationship between PA and cardiometabolic risk (Figure 1). The interaction observed for Unfit/Fat indicated that for the adolescents who practise PA for 330 minutes per week, the cMetS was lower compared to 0 minutes and 60 minutes of practice. For the other phenotypes, no interactions were found.

In addition, we sought to identify the differences in the cardiometabolic risk score according to the combination of fitness and fatness categories in adolescents who practise ≥ 330 minutes of

TABLE 1. Characteristics of the sample

	All	Children	Adolescents
	n = 2786	n = 1170	n = 1616
		Mean (SD)	
Age (years)	12.17 (2.75)	9.46 (1.35)	14.13 (1.59)*
Weight (kg)	47.35 (15.11)	36.40 (10.14)	55.28 (13.01)*
Height (meters)	1.51 (0.15)	1.38 (0.10)	1.61 (0.10)*
Body mass index (kg/m^2)	20.28 (3.98)	18.85 (3.60)	21.32 (3.93)*
PA (minutes/week)	130.14 (188.72)	105.24 (162.51)	148.18 (203.78)*
Systolic blood pressure (mmHg)	105.84 (13.92)	99.39 (12.47)	110.51 (13.03)*
Glucose (md/dL)	88.66 (9.07)	87.22 (9.16)	89.70 (8.86)*
Total cholesterol (md/dL)	161.05 (31.92)	164.61 (31.44)	158.48 (32.02)*
High-density lipoprotein cholesterol (md/dL)	59.20 (11.23)	61.04 (11.40)	57.88 (10.91)*
Total cholesterol/ High-density lipoprotein cholesterol	2.79 (0.66)	2.77 (0.65)	2.81 (0.67)
Triglycerides (md/dL)	74.49 (171.33)	66.98 (30.50)	79.93 (223.33)*
cMetS (z-score)	-0.08 (0.68)	-0.17 (0.70)	-0.02 (-0.67)*
	n (%)	n (%)	n (%)
Sex			
Male	1200 (43.1)	538 (46.0)	662 (41.0)*
Female	1586 (56.9)	632 (54.0)	954 (59.0)
Cardiorespiratory fitness			
Healthy	1064 (38.2)	637 (54.4)	427 (26.4)*
Unhealthy	1722 (61.8)	533 (45.6)	1189 (73.6)
Waist circumference			
Normal weight	2223 (79.8)	866 (74.0)	1357 (84.0)*
Overweight/obese	563 (20.2)	304 (26.0)	259 (16.0)
Combination of fitness and fatness categories			
Fit/Unfat	937 (33.6)	541 (46.20)	396 (24.5)
Fit/Fat	127 (4.6)	96 (8.2)	31 (1.9)
Unfit/Unfat	1286 (46.2)	325 (27.8)	961 (59.5)
Unfit/Fat	436 (15.6)	208 (17.8)	228 (14.1)*
Skin color			
White	2211 (79.4)	970 (82.9)	1241 (76.8)*
Black	203 (7.3)	75 (6.4)	128 (7.9)
Brown/mulatto	335 (12.0)	105 (9.8)	220 (13.6)
Indigenous/yellow	37 (1.3)	10 (0.9)	27 (1.7)
Pubertal status			
Pre-pubertal	583 (20.9)	526 (45.0)	57 (3.5)*
Initial development	639 (22.9)	399 (34.1)	240 (14.9)
Continuous maturation (stage III and IV)	1310 (47.0)	223 (19.1)	1087 (67.3)
Maturated	254 (9.1)	22 (1.9)	232 (14.4)
Socioeconomic status			
Low	1199 (43.1)	488 (41.8)	711 (44)
Medium	1449 (52.0)	624 (53.3)	825 (51.1)
High	138 (5.0)	58 (5.0)	80 (5.0)

SD: Standard deviation; PA: Physical activity; cMetS: Cardiometabolic risk score*Independent t-test or chi-square for differences between children and adolescents ($p < 0.05$).

TABLE 2. Combination of fitness and fatness in the relationship between physical activity and cardiometabolic risk in children and adolescents.

	β	CI (95%)	P
		cMetS	
		Children	
Fit/Unfat	1		
Fit/Fat	0.473	0.292; 0.654	0.001
Unfit/Unfat	0.005	-0.101; 0.110	0.928
Unfit/Fat	0.700	0.577; 0.823	0.001
PA	0.001	-0.001; 0.001	0.630
PA × Fit/Unfat	1		
PA × Fit/Fat	-0.001	-0.001; 0.001	0.813
PA × Unfit/Unfat	-0.001	-0.001; 0.001	0.835
PA × Unfit/Fat	-0.001	-0.001; 0.001	0.113
		Adolescents	
Fit/Unfat	1		
Fit/Fat	0.377	0.080; 0.674	0.013
Unfit/Unfat	0.190	0.091; 0.290	0.001
Unfit/Fat	0.764	0.636; 0.893	0.001
PA	0.001	-0.001; 0.001	0.154
PA × Fit/Unfat	1		
PA × Fit/Fat	0.001	-0.001; 0.001	0.954
PA × Unfit/Unfat	-0.001	-0.001; 0.001	0.370
PA × Unfit/Fat	-0.001	-0.002; -0.001	0.001

PA. Physical activity; cMetS. Cardiometabolic risk score. All analyses were adjusted for sex, pubertal status, socioeconomic level, skin color.

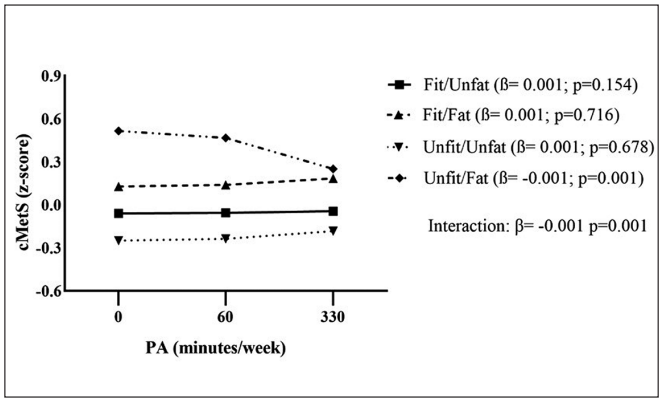


FIG. 1. Moderating role of the combination of fitness and fatness in the relationship between physical activity and cardiometabolic rosk in adolescents.
Note: PA. Physical activity; cMetS. Cardiometabolic risk score.

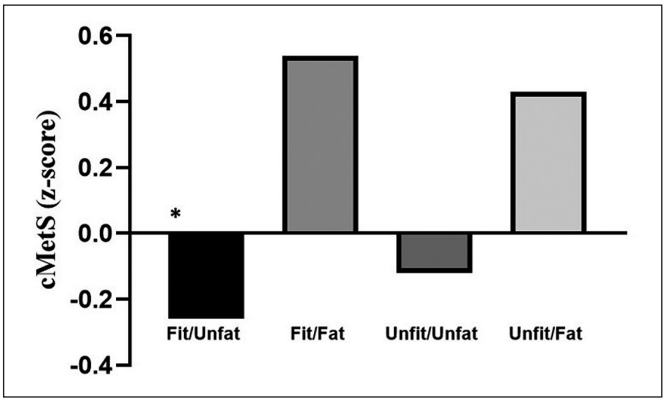


FIG. 2. Cardiometabolic risk score according to the combination of fitness and fatness categories in adolescents who practice ≥ 330 minutes of physical activity a week. Note: * Indicates significant compared to Fit/Fat and Unfit/Fat categories.

physical activity a week (Figure 2). It was observed that the Fit/Unfat category presented the lowest cMetS compared to Fit/Fat ($p = 0.004$) and Unfit/Fat ($p < 0.001$). Also, there was no significant difference between the categories Fit/Unfat and Unfit/Unfat ($p = 0.607$).

DISCUSSION

The main findings of the present study indicated that the combination of fitness and fatness is a moderator in the relationship between PA and cardiometabolic risk only in adolescents and not in children. It was observed that for the adolescents with an unhealthy profile,

represented by low levels of CRF and high adiposity, the practice of PA for 330 minutes a week may attenuate the cardiometabolic risk score. In addition, for these active adolescents who present a normal weight, being fit does not exert an additional benefit for cardiometabolic health.

There is extensive evidence in the literature concerning the fit-but-fat paradox and its association with cardiometabolic health, indicating controversial results. Some of the studies are in agreement with the paradox [8, 30, 31], while others indicated that CRF is not able to neutralize the negative influence of adiposity on cardiometabolic risk factors [32, 33], showing that CRF is a partial mediator in the association between fatness and cardiometabolic risk factors in European adolescents, although it is not able to completely counteract the negative influence of adiposity on cardiometabolic health. In addition, CRF is considered a prognostic marker of cardiovascular disease, once engaging in regular PA practice is crucial for metabolic health because exercise serves as a powerful stimulator, activating multiple functions at the molecular and cellular levels. This activation can significantly enhance the overall functional capacity of the general population [34]. In obese individuals, the CRF is improved through the regular practice of exercise and demonstrates benefits to metabolic health, independent of weight loss [35]. A recent longitudinal study developed with Brazilian children and adolescents indicated that healthy body composition may exert a stronger protective effect on cardiometabolic health than high levels of CRF [36]. On the other hand, it has been suggested that fitness is a better predictor of cardiovascular disease risk than fatness in adolescents [36, 37].

In this context of modifiable behavioural risk factors, the influence of PA practice must be taken into account. Therefore, the present study sought to go further by determining how PA interacted with the combination of fitness and fatness to exert an influence on a cardiometabolic risk score. To the authors' knowledge, previous studies have investigated the role of CRF in modifying the association between PA and cardiometabolic risk [17, 38], but these did not investigate the influence of adiposity. The role of adiposity must be taken into account, as healthy body fat seems to be as important as high CRF for metabolic health [36, 37]. In addition, high PA is associated with lower adiposity [39, 40]. Thus, it seems that having low physical fitness, even having a normal weight, may be related to a lower metabolic risk, when the adolescents practised 330 min of PA. Therefore, the ones who present an unhealthy profile but comply with the recommendations of PA practice for Brazilian youth [40] may achieve benefits in cardiometabolic health.

The findings of the present study are partially in agreement with those reported by Brage et al. [38], who found that PA was inversely associated with metabolic risk and interactively associated with physical fitness, indicating that the potential beneficial effect of PA may be more pronounced in children with lower CRF. Similarly, CRF moderated the association between PA with cardiometabolic outcomes; that is, a lower cardiometabolic risk score was observed in children with low CRF, while no associations were observed for children with high levels of CRF [17]. Another study also pointed out that it is necessary to maintain adequate physical fitness and to be physically active for the reduction of abdominal adiposity in overweight/obese children [41].

Regarding obesity and PA, it was observed that PA and adiposity are independently associated with cardiometabolic risk factors, in which individuals with better PA levels and low body fat percentage presented lower cardiometabolic risk. However, when analysing the combination of PA/weight, individuals who are obese and physically active presented higher cardiometabolic risk compared to normal weight and physically inactive individuals, suggesting that adiposity is more important than adequate PA levels for cardiometabolic health [2]. In contrast, another study found that PA practice is essential for health in children and adolescents, especially in overweight/obese individuals, in whom there is a higher risk of developing chronic diseases in adulthood [42]. In general, obese adolescents tend to have lower physical fitness and a lower level of PA compared to non-obese peers [43]. It was also observed that in children and adolescents with low physical conditioning, PA seems to exert a fundamental role in abdominal adiposity [44]. These aspects are associated with many lifestyle behaviours including inadequate sleep, excessive time spent in sedentary behaviour, such as watching television and using electronic devices, unhealthy eating, and less active commuting [45, 46]. It is well known that PA is essential for healthy development in youth, being one important strategy for the prevention and treatment of obesity and its metabolic complications [47]. Moreover, a PA intervention programme is important to improve physical fitness and cardiometabolic risk factors in individuals with overweight and obesity [48]. Indeed, this evidence supports the present findings highlighting the importance of PA, which may attenuate the cardiometabolic risk score in the presence of low levels of CRF and high adiposity. Therefore, the favourable effects on cardiometabolic risk observed in the present study might be explained by the practice of PA, rather than a high CRF per se, as habitual PA is weakly associated with CRF in youth [49].

This study has some important strengths, such as the large sample size composed of Brazilian children and adolescents. It presents new findings regarding the influence of the fat/fit paradox in the association between PA and cardiometabolic risk score, while literature evidence is still controversial about the role of this paradox in cardiometabolic risk. Also, the present study reinforces the importance for unfit/fat adolescents to be physically active, to minimize the risk of developing cardiometabolic disorders in adulthood. However, some limitations should be mentioned. The findings should be cautiously interpreted due to the cross-sectional design which precludes confirmation of cause and effect. The evaluation of PA practice through self-reported questionnaires may lead to underestimating or overestimating data because at the moment it was not possible to evaluate this sample using accelerometry due to the high cost. In addition, we considered only leisure PA, but did not include PA time

during physical education classes, and we did not estimate PA intensity. Also, CRF and fatness were assessed using field measurements. Finally, although important potential confounding factors were considered, behavioural factors such as nutritional habits could further confound the observed relationship.

CONCLUSIONS

In conclusion, this study indicates that the combination of fitness and fatness moderates the relationship between PA and cardiometabolic risk in adolescents. As practical application, the present study suggests that adolescents, particularly those who are less fit and present high adiposity, should be encouraged to engage in regular PA to improve their metabolic health. Thus, it is recommended that future research take into account the difference in ages, due to adolescents being more exposed to the deleterious effects of obesity and low CRF levels on metabolic health.

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Conflict of interest declaration

The authors declare no conflict of interest.

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Evaluation of measurement reliability for selected indices of postural stability based on data from the GYKO Inertial Sensor System

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ABSTRACT: The main aim of this study is to use comprehensive statistical analyses to evaluate measurement reliability of selected variables that characterize postural stability. The study examined twenty-nine healthy non-athlete students. The examinations were performed twice, with a one-week interval. The Microgate GYKO inertial sensor system was used to evaluate the reliability of variables that characterize postural stability. The relative reliability of the repeated test was evaluated using the intraclass correlation coefficient (ICC) with 95% confidence interval (95% CI). Next, the standard error of measurement (SEM) and minimal detectable change (MDC) were computed. Relative reliability of the repeated test for all analysed variables of ICC ranged from 0.31 to 0.75. For four variables, ICC values were ca. 0.7, i.e., they can be considered as good. For four other variables, ICC ranged from 0.41 to 0.54, with these values considered fair. Satisfactory reproducibility of postural stability measurements using the GYKO inertial sensor system demonstrates that it can offer an inexpensive and efficient alternative to measurements that use force balance platforms.

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INTRODUCTION

In many sports (e.g., skiing, swimming, team games), balance is one of the leading coordination (neurophysiological) abilities. Therefore, monitoring the ability to maintain balance is critical for the comprehensive analysis of the level of athletes' motor preparation, and during recruitment and selection for teams [1, 2]. A high level of postural stability is also needed to perform everyday activities in a safe and independent manner and to reduce the risk of falling [3, 4].

Data from various types of balance platforms are most often used to measure various indicators of postural stability. The results obtained provide a benchmark for other studies ("gold standards"). They allow for evaluation of postural stability based on displacement of the COP (centre of pressure) on the supporting surface during relaxed standing (standing with both feet and one foot) [4, 5]. Unfortunately, these platforms are usually very big and expensive, while their installation and software are complex [6]. Another group of equipment for balance measurements is Wii Balance Boards [7, 8, 9]. In population studies, balance is examined by means of various motor coordination tests. Among them, the most popular is the Flamingo Balance test from the Eurofit Physical Fitness Test Battery [10]. Other tests have also been used for many years: the Stork Balance Stand Test, the Y Balance Test, the Stick Lengthwise Balance Test, the Balance Beam Test [11]. Due to the aforementioned limitations of posturographic platforms, postural stability is measured by means of various types of accelerometers. They are cheaper, more mobile, and easy to use in population studies [12, 13, 14]. The use of accelerometery [15, 16, 17, 18, 19] for recording body sway gained popularity when the costs of accelerometers with improved measurement parameters fell, and wireless technology became commonplace. ICC (intraclass correlation coefficient) values obtained during postural stability measurements performed by means of accelerometers are good, usually over 0.75 [20]. Studies have also demonstrated a strong correlation between results obtained from force platforms and accelerometer data. The location of accelerometers on the human body is also an important issue due to various links between the movements of individual body segments and COM (centre of mass) displacements used to maintain postural stability. In his research, Golema, based on correlation coefficients, determined the relationships between COM and body trunk displacements. The results demonstrated that body trunk displacements (displacements of a point located near the body trunk centre of gravity) can be considered the same as those of COM. This assumption applies only to maintaining postural stability in undisturbed conditions [21].

Therefore, in our study, we measured the variables that characterize postural stability by means of the GYKO inertial sensor system (Microgate, Bolzano, Italy) located on the body trunk of study participants.

The primary objective of this report was to evaluate the repeatability of measurements of selected variables characterizing postural stability.

MATERIALS AND METHODS

Study group

The study examined 29 healthy non-athlete students (age = 21.67 ± 0.88 years, body weight = 179.82 ± 6.87 cm, body mass = 75.55 ± 7.62 kg). The tests and anthropometric measurements were performed in accordance with the Declaration of Helsinki. The examinations were approved by the Bioethics Committee at the Regional Medical Chamber in Kraków, Poland (approval No. 159/KBL/OIL/2017). The tests were conducted within statutory research No. 106/BS/IS/2016.

Technical specifications of the GYKO device

GYKO is an inertial measurement unit (IMU), consisting of an accelerometer for recording the following variables: power and speed of lifting a weight and monitoring of postural stability (recording of several variables). It offers options for analysis of body sway during gait, running and jumping, and evaluation of postural stability. A detailed description of the technical specifications of the accelerometer can be found in the manual [22].

Testing protocol

The participants performed all the tests barefoot, in the same room, between 8 a.m. and 3 p.m. A test was performed for 30 s while standing on both feet, without moving, with eyes open and arms relaxed along the sides of the body. Feet were positioned hip width apart and the participant was asked to look at a point on the wall at a distance of ca. 2.5 m. The group of university students always performed the first and second series of examinations at the same time of the day. The same research team supervised both test series. The tests and retests were conducted with a one-week interval. This approach has often been used in studies examining test reliability [23, 24]. During the first examination, we determined the height at which the GYKO system was to be attached. According to the manufacturer's recommendations, this height should be set at the level of the T1-T2 thoracic vertebrae. During the second examination, the GYKO system was attached again at the same height. The tension of the GYKO attaching straps (chest circumference) was also adjusted to each student. The wireless transmission protocol was used to transfer data recorded by the GYKO inertial sensor to the laptop.

Scope of the study

We examined the following variables characterising postural stability [25, 26, 27]: area [mm^2], convex hull area [mm^2], length (ML) [mm], length (AP) [mm], mean distance (ML) [mm], mean distance (AP) [mm], RMS distance (ML), RMS distance [mm], (AP) [mm], mean velocity (ML) [mm/s], mean velocity (AP) [mm/s].

Statistical analysis

1. The Shapiro-Wilk test was used to assess variables for normal distribution, whereas Levene's test was employed to assess the equality of variances.
2. Basic descriptive statistics (arithmetic mean, standard deviation) were computed for all the variables in both series of examinations (test, retest) and for the material in total.
3. The relative reliability of the repeated test [28, 29] was evaluated using the intraclass correlation coefficient (ICC) with 95% confidence interval (95% CI).

TABLE 1. Basic statistical characteristics of the analysed postural stability indices

Variable [unit of measurement]	(Test + Retest)/2 ± SD	Test ± SD	Retest ± SD
Area [mm^2]	839.48 ± 380.63	824.83 ± 394.33	854.13 ± 372.81
Convex hull area [mm^2]	581.46 ± 309.36	611.92 ± 352.61	555.01 ± 262.11
Length (AP) [mm]	266.34 ± 51.03	276.78 ± 49.62	255.90 ± 51.12
Length (ML) [mm]	192.02 ± 42.16	196.60 ± 42.51	187.44 ± 42.06
Mean distance (AP) [mm]	9.17 ± 3.67	8.83 ± 3.77	9.50 ± 3.62
Mean distance (ML) [mm]	4.45 ± 1.88	4.73 ± 2.12	4.16 ± 1.60
RMS distance (AP) [mm]	11.30 ± 4.54	10.84 ± 4.85	11.76 ± .,25
RMS distance (ML) [mm]	5.57 ± 2.35	5.92 ± 2.67	5.21 ± 1.98
Mean velocity (AP) [mm/s]	8.80 ± 1.69	9.14 ± 1.64	8.45 ± 1.69
Mean velocity (ML) [mm/s]	6.34 ± 1.39	6.49 ± 1.40	6.19 ± 1.39

4. Apart from ICC, we also computed standard error of measurement (SEM), which is used to express absolute measurement reliability [29], according to the formula:

$$SEM = SD \sqrt{1 - ICC}$$

where:
SEM – standard error of measurement
SD – standard deviation from test 1 and 2
ICC – intraclass correlation coefficient

5. Next, SEM% values were computed as

$$SEM\% = (SEM / grand\ mean) \times 100$$

This method was used to express SEM as a percentage of the mean value from the test and retest for each variable to allow comparison of absolute reliability between the measurements obtained in various units [30, 31, 32, 33].

6. SEM was used to evaluate the minimal detectable change (MDC), interpreted as the lowest value of changes needed to exceed the error of measurement for two repeated tests at specific CI. Computations were performed for 95% CI using the formula:

$$MDC_{95} = SEM \times 1.96 \times \sqrt{2}$$

7. Next, MDC_{95} was expressed as the percentage of the mean from the test and retest, according to the formula:

$$MDC_{95\%} = (MDC_{95} / grand\ mean) \times 100$$

8. The differences between means from the test and retest used the repeated-measures Student's t-test. We used the non-parametric Wilcoxon pair test if the distribution of variables differed from normal. Furthermore, the Cohen effect was also computed for each variable [34].

The calculations were performed using STATISTICA software (v. 12, StatSoft, Krakow, Poland), and Excel (2016 MSO, Microsoft Corporation, Redmond, WA, USA). Furthermore, the effect size was determined by means of GPower 3.1 free software and available for Mac OS X and Windows, which is widely used in social studies [35].

RESULTS

Table 1 illustrates basic statistical characteristics for test and retest variables. They were used for computation of the standard error of measurement (SEM), expressed as a percentage mean from test and retest (SEM%) and minimal detectable change ($MDC_{95\%}$).

As mentioned in the Introduction section, the aim of this report was to evaluate reliability indices for selected variables that characterize postural stability. The values of intraclass correlation coefficient (ICC), standard error of measurement (SEM) and minimal detectable change (MDC_{95}) are presented in Table 2. Relative reliability of the repeated test for all analysed ICC variables ranged from 0.31 to 0.75. The lowest ICC values were obtained for mean frequency (AP), whereas the highest values were found for area. For four variables, ICC values were ca. 0.7, i.e., they can be considered as good to excellent. Furthermore, for four variables, ICC values ranged from 0.41 to 0.54 and can be considered fair. Furthermore, low ICC values were obtained for two variables: mean distance (AP) and RMS distance (AP). It should be emphasized that all low ICC values concerned anterior-posterior sway (AP).

Table 2 also presents standard error of measurement (SEM), which is used to express absolute measurement reliability. In general, SEM% for all analysed variables ranged from 11 to 30%. High ICC = 0.70 values at low standard error of ca. 11–12% were obtained for length (ML) and mean velocity (ML). Furthermore, the highest SEM% was

TABLE 2. Values of intraclass correlation coefficient (ICC), standard error of measurement (SEM) and minimal detectable change (MDC_{95}) for the analysed indices of postural stability.

Variable [unit of measurement]	ICC	ICC (95% CI)	SEM	SEM%	MDC_{95}	$MDC_{95\%}$
Area [mm^2]	0.75	0.53÷0.88	190.31	22.66	527.51	62.83
Convex hull area [mm^2]	0.68	0.42÷0.84	176.66	30.29	481.36	82.85
Length (AP) [mm]	0.46	0.11÷0.71	37.49	14.07	103.91	39.01
Length (ML) [mm]	0.70	0.45÷0.85	23.09	12.02	64.00	33.32
Mean distance (AP) [mm]	0.31	-0.06÷0.61	1.56	17.01	4.32	47.10
Mean distance (ML) [mm]	0.54	0.22÷0.76	1.27	28.53	3.52	79.10
RMS distance (AP) [mm]	0.35	-0.02÷0.63	1.89	16.72	5.23	46.28
RMS distance (ML) [mm]	0.41	0.05÷0.67	1.75	31.41	4.85	87.07
Mean velocity (AP) [mm/s]	0.46	0.11÷0.71	1.24	14.09	3.43	38.97
Mean velocity (ML) [mm/s]	0.70	0.45÷0.85	0.76	11.98	2.10	33.12

ICC – coefficient of intraclass correlation, 95% CI – confidence interval, SEM – standard error of measurement, SEM% – percentage standard error of measurement, MDC – minimal detectable change.

found for convex hull area, which was also characterized by the highest measurement reliability.

The SEM values were used to compute the minimal detectable change, MDC_{95}. For the analysed variables, the values ranged from 33% to 82%. The lowest values of $MDC_{95\%}$ were obtained for length (ML) and mean velocity (ML). The highest $MDC_{95\%}$ was again obtained for convex hull area. In general, analysis of the data shown in Table 2 concerning MDC_{95} and ICC revealed high values at good and satisfactory measurement reliability.

The study also analysed the statistical significance of differences between the results of individual variables obtained for test and retest (Table 3) and Cohen effect size. Statistically significant differences were found only for two variables: length (AP) and mean velocity (AP). It should be emphasized that the likelihood, $p = 0.040$, was close to the limit of the set level of statistical significance ($p \leq 0.05$). No statistically significant differences were found between other variables. As suggested by Cohen, the effect size for six variables was small (below 0.2), whereas a medium effect was observed for the remaining four variables (between 0.22 and 0.30).

DISCUSSION

The reliability of a diagnostic method is one of the most important factors that determine its usefulness. Determination of the reliability and validity of measurements with respect to the so-called gold standard has practical implications for athletes and coaches. The main aim of this study was to verify measurement reproducibility for selected variables that characterize postural stability using comprehensive statistical analyses. A mobile Microgate GYKO inertial sensor system was used in the study. We will refer the results of our study to only certain comparative materials. This methodological approach was adopted due to the high number of scientific studies on reliability of postural stability measurements (various measurement apparatus, tests during standing on one leg, two legs, on stable or unstable ground, with eyes open or closed, with hands on hips or crossed on the chest, tests for the population of healthy people and those with geriatric problems, tests of people of different ages).

There are no unequivocal criteria for the evaluation of measurement reliability in the literature. Landis and Koch [36] proposed adopting the following reliability criteria: 0.01 to 0.20 – slight; 0.21 to 0.40 – fair; 0.41 to 0.60 – moderate; 0.61 to 0.80 – substantial; 0.81 to 1.00 – almost perfect. These reliability ranges have been respected by many researchers, but they have also been rejected or modified by others. The following criteria for ICC have also been adopted in studies: values in the range of 0.80 to 1.00 are considered excellent reliability, values in the range of 0.60 to 0.80 represent good reliability, while an ICC lower than 0.60 means poor reliability [30, 31]. Munro proposed a popular classification of ICC: values between 0.00 to 0.25 reflect little correlation; 0.26 to 0.49 – low correlation; 0.50 to 0.69 – moderate correlation; 0.70 to 0.89 – high correlation; and 0.90 to 1.00 – very high correlation [37]. Given various methodological approaches to balance tests, the classification proposed by Fleiss et al. [38] used in many publications seems to be the most justified, with ICC values > 0.75 labelled excellent, > 0.40 – fair to good, and < 0.40 – poor. This interpretation of ICC was also adopted in our study. Analysis of the literature concerning this subject reveals that in comparative studies, ICCs ranged from average and poor to high [24, 39]. The results obtained in our study for ICC reliability coefficients support these findings. Most of the reliability indices calculated in our study (according to the adopted classification) can be considered as fair and high. The

TABLE 3. Results of the evaluation of the significance of differences in the analysed variables of postural stability between test and retest and Cohen's effect sizes.

Variable [unit of measurement]	t (W)	p	C^d
Area [mm^2]	-0.577729	0.568	0.056908
Convex hull area [mm^2]	0.746482	0.122	0.192026
Length (AP) [mm]	2.142798	0.040*	0.308932
Length (ML) [mm]	1.501903	0.144	0.161460
Mean distance (AP) [mm]	0.659508^W	0.509	0.235702
Mean distance (ML) [mm]	1.654175^W	0.098	0.309534
RMS distance (AP) [mm]	0.789247^W	0.429	0.176544
RMS distance (ML) [mm]	1.469692	0.152	0.224612
Mean velocity (AP) [mm/s]	2.145012	0.040*	0.150311
Mean velocity (ML) [mm/s]	1.503076	0.144	0.160290

Without marking – value of the Student t-statistic for dependent samples; W – value of the Wilcoxon test statistic; C – Cohen's effect size, d > 0.2 = small, > 0.5 = medium, > 0.8 = large. *Significant at $p \leq 0.05$.

ICC values were below 0.40 (poor) only for two variables i.e., mean distance (AP) and RMS distance (AP). In general, slightly lower values of ICC in our study were observed compared to the studies that used platform tests (ICC = 0.46–0.98 [40, 41] and Wii Balance Boards (ICC = 0.64–0.91 [7, 42, 43]. ICC reliability results of postural stability measurements obtained with different types of accelerometers are good and are usually around 0.7 [44, 45, 46]. Similar ICC values (from 0.62 to 0.70) to our own study, for the test performed on the dominant limb, were obtained by Jaworski et al. [47]. The GYKO accelerometer was also used in the cited study.

The interpretation of ICC values does not provide much insight into the actual changes in the variables that could be used for practical interpretation. Consequently, many authors [29, 31, 32] suggest that absolute values of measurement reliability should also be interpreted. For this purpose, it is necessary to compute SEM and MDC to enhance identification of the levels of errors for the analysed variables. It should be emphasized that with large interindividual variability, high values of ICC can be obtained even if the absolute reliability is low. Evaluation of the test result interpretation error is directly related to its reliability. This means that the higher the SEM is, the lower are the absolute test reliability and accuracy of the results [30]. In our study, SEM% for all most variables ranged from 10 to 20%. This reflects good absolute measurement reliability. SEM values were also used to compute the minimal detectable change (MDC). This index is defined as the minimum value of changes needed to distinguish the actual change from the change caused by the variability of results or errors of measurement [48]. Therefore, evaluation of MDC for each variable that characterizes postural stability provides information about the errors of measurements that should be taken into consideration during determination of the smallest important changes expected for two consecutive measurements [49]. We obtained relatively high MDC values at good and fair measurement reliability.

Limitation of the study

The authors are aware of the limitations of this report. Therefore, in future research, we propose to increase the size of the research group, and to conduct the tests in different chronological age groups of both sexes, attaching the GYKO device in the lumbar region of the spine (L3).

CONCLUSIONS

1. Values of the intraclass correlation coefficient (ICC) for the variables of postural stability should be considered as good and fair.
2. High reproducibility of postural stability measurements using the GYKO inertial sensor system demonstrates that it can offer an inexpensive and efficient alternative to measurements that use force balance platforms.

Author Contributions

Conceptualization, J.J., G.L., K.W., R.K., and P.P.; methodology, J.J., G.L., K.W., R.K., and P.P.; software, J.J., G.L., K.W., R.K., and P.P.; validation, J.J., G.L., K.W., R.K., and P.P.; formal analysis, J.J., G.L., K.W., R.K., and P.P.; investigation, J.J., G.L., K.W., R.K., and P.P.; resources, J.J., G.L., K.W., R.K., and P.P.; data curation, J.J., G.L., K.W., R.K., and P.P.; writing – original draft preparation, J.J., G.L., K.W., R.K., and P.P.; writing – review and editing, J.J., G.L., K.W., R.K., and P.P.; visualization, J.J., G.L., K.W., R.K., and P.P.; supervision, J.J., G.L., K.W., R.K., and P.P.; project administration, J.J., G.L., K.W., R.K., and P.P.; funding acquisition, J.J., G.L., K.W., R.K., and P.P. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Bioethics Committee at the Regional Medical Chamber in Kraków, Poland (approval No. 159/KBL/OIL/2017).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflict of interest.

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Investigation of the involvement of platelet-activating factor in the control of hypertension by aerobic training. A randomized controlled trial

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ABSTRACT: Although studies have demonstrated the effectiveness of exercise in controlling systemic arterial hypertension (SAH), the mechanisms involved in this effect are still poorly understood. Thus, this study investigated the impact of aerobic training on the relationship between platelet-activating factor (PAF) circulating levels and blood pressure in hypertensives. Seventy-seven hypertensive subjects were enrolled in this randomized controlled trial (age 66.51 ± 7.53 years, body mass 76.17 ± 14.19 kg). Participants were randomized to two groups: the intervention group (IG, n = 36), composed of hypertensive individuals submitted to an aerobic training protocol, and the control group (CG, n = 41), composed of non-exercised hypertensives. Body mass index, arterial blood pressure, quality of life, respiratory muscle strength, and functional capacity were assessed before and after 12 weeks. PAF and plasma cytokine levels were also evaluated respectively by liquid chromatography coupled with mass spectrometry and enzyme-linked immunosorbent assay. Aerobic training promoted a significant reduction in blood pressure while functional capacity, expiratory muscle strength, and quality of life, PAFC16:0 and PAFC18:1 plasma levels were increased in comparison to the CG ($p < 0.05$). In addition, multiple correlation analysis indicated a positive correlation [$F(3.19) = 6.322$; $p = 0.001$; R^2adjusted $= 0.499$] between PAFC16:0 levels and expiratory muscle strength after aerobic training. Taken together, our findings indicate that PAF may be involved in the indirect mechanisms that control SAH, being mainly associated with increased respiratory muscle strength in hypertensive subjects undergoing aerobic training.

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INTRODUCTION

More than 30% of the world population is diagnosed with systemic arterial hypertension (SAH). This disease is the main risk factor for the development of cardiovascular diseases, especially coronary artery disease and stroke [1]. Although controlling SAH is challenging, pharmacotherapy combined with lifestyle changes can reduce the morbidity and mortality associated with this chronic disease [1]. Considering the pharmacological strategies currently available, diuretics, angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), beta-blockers, and calcium channel blockers (CCBs) are the main drugs used to treat SAH. Furthermore, some patients require two or more antihypertensive drugs to reach their target blood pressure (BP), increasing the risk of side effects [1, 2]. Considering this limitation, there is evidence that the association of antihypertensive drugs with non-pharmacological interventions based on aerobic training can improve the clinical management of SAH [3]. In addition, exercise training promotes fewer adverse reactions, is economically viable, and can interact additively or synergistically with antihypertensive drugs [3].

Although aerobic training is effective to attenuate SAH, physiological mechanisms involved in this effect have not been elucidated. Vascular function adjustments are proposed as potential effects of aerobic exercise training, especially considering that endothelial dysfunction is a characteristic change in hypertensive subjects [4], who exhibit increased vasoconstrictor tone and peripheral vascular

resistance [5]. There is evidence that aerobic exercise training can attenuate endothelial dysfunction and up-regulate endothelial nitric oxide synthase and superoxide dismutase expression and activity, as well as improving vascular antioxidant capacity, effects that by stimulating vasodilation may help to mitigate SAH [6]. In addition, down-regulation of vasoconstrictor endothelin-1 gene expression and reduced protein bioavailability, as well as reduced levels of angiotensin II type 1 receptor (required for angiotensin II-mediated vasoconstriction), were also found after aerobic exercise training [6].

Platelet-activating factor (PAF) is an endogenous phospholipid released from different biological sources, including platelets, polymorphonuclear leukocytes, macrophages, endothelial cells, and vascular smooth muscle cells [7]. This factor is recognized for triggering a wide spectrum of biological activities, including pro-inflammatory and hypotensive effects [7]. A study found long-lasting PAF-induced hypotension, dose-dependent, in both normotensive and spontaneously hypertensive rats, guinea pigs, rabbits and dogs [8]. As PAF is a phospholipidic mediator synthesized by endothelial and smooth muscle cells, its hypotensive effect may be attributed to the dilation of resistance vessels, suggesting its involvement in modulating endothelial dysfunction and vascular smooth muscle tone [7]. Furthermore, Lopes-Martins et al. found that intravenous administration of the PAF receptor antagonist WEB 2086 significantly potentiated the increased systemic vascular resistance induced by noradrenaline or by central activation of the sympathetic nervous system with glutamate, demonstrating that PAF is involved in the modulation of vasomotor tone and, hence, arterial blood pressure by a negative feedback mechanism triggered by important increases in vascular tone [9]. In addition, fatty acids of the PAF family containing C16 (PAFC16) or C18 (PAFC18) carbon chains promoted renal vasodilation with a decrease in renal vascular resistance and systemic hypotension [10].

Thus, these early studies suggest that the hypotensive effect of PAF may be by the actions on the central or autonomic nervous systems, or mainly by direct actions on the peripheral blood vessels.

PAF is synthesized by two pathways: the "remodelling pathway" is mainly associated with PAF production by activated leukocytes. This pathway requires a tightly coupled reaction between phospholipase A2 and acetyl CoA: Ikyl-sn-glycero- hosphorylcholine 2-O-acetyltransferase [7]. The second mechanism is mediated by the "*de novo* pathway". This mechanism is primarily identified in the kidney and central nervous system and requires the synthesis of 1-O-alkyl-cetylglycerol, which is then converted to PAF by a specific dithiothreitol-insensitive CDP-choline: Ikyl- cetyl-snglycerol choline phosphotransferase [7].

Some studies have shown that PAF from both pathways is involved in the control of several pathologies. Jeong et al. [11] demonstrated that PAF treatment induced a protective effect against mortality from endotoxic shock in mice. This effect was associated with the inhibition of apoptosis by lymphocytes and correlated with significantly decreased production of inflammatory mediators such as TNF-α, IL-1β, IL-12, and IFN-γ, and increased production of the anti-inflammatory cytokine IL-10. In addition, another study found that TNF-α promotes intestinal mucosal repair in parallel to upregulation of the PAF receptors in the intestinal epithelium [12]. Furthermore, PAF receptors are involved in enhanced apoptosis, via activation of the factor nuclear kappa B (NF-κB) pathways, in the same type of cancer [13, 14].

Studies have also demonstrated the importance of the cardioprotective effect of PAF, especially at low levels, both in reducing the extent of myocardial injury after infarction and in improving the recovery of left ventricular developed pressure and left ventricular end-diastolic pressure after an ischaemic episode [15].

Studies investigating the potential effects of exercise training on PAF activation are scarce. Although increased plasma levels of PAF after acute eccentric exercise have been identified in a previous study [16], the impact of chronic aerobic exercise on the relationship between PAF and BP remains overlooked. Thus, we used a randomized clinical trial framework to investigate this relationship, especially considering the potential implications of PAF in aerobic training-induced hypotensive effects in subjects with SAH.

MATERIALS AND METHODS

Study design and sample

This randomized controlled trial was previously registered in the Clinical Trials Register (ID: RBR-2vk9t4, registered in June 2019). Data were collected from August 2019 to March 2020. Furthermore, this study was approved by the local Ethics Research Committee for Human Research (protocol 039/2015, CAAE: 09381018.6.0000 .5142), which was conducted by the Declaration of Helsinki and the CONSORT recommendations for non-pharmacological trials [17].

Hypertensive subjects referred to the local Cardiac Rehabilitation Centre were included in this study. Systemic arterial hypertension was diagnosed by a certified cardiologist according to well-established criteria from the European Society of Hypertension and the European Society of Cardiology [18]. Volunteers of both sexes, between 40 and 80 years old, able to perform aerobic physical activity and who did not perform regular physical activities in the previous three months, were included in the study. The following exclusion criteria were adopted: the presence of diseases that prevent or interfere with exercise practice; inflammatory or infectious disease; chronic pain; history of surgery or fractures in the lower limbs in the last six months; neurological diseases or sequelae, unstable angina, thrombophlebitis, recent thromboembolism, 3rd-degree atrioventricular block, uncontrolled cardiac arrhythmia, haemodynamic instability, and uncontrolled SAH.

All potentially eligible subjects were contacted. Those interested in participating were invited to attend the physical examination to assess the inclusion and exclusion criteria. All participants were informed about the study and provided informed consent. An evaluator blinded to treatment groups performed the physical examination. Demographic and anthropometric data, antihypertensive drug use, and date of SAH diagnosis were collected at baseline before randomization and after randomization by the same blinded evaluator.

Diastolic (DBP) and systolic (SBP) blood pressures and plasma PAF levels were the primary outcomes evaluated in this study. DBP and systolic SBP blood pressures were analysed in participants sitting at rest for 10 minutes. A calibrated aneroid sphygmomanometer coupled to an appropriately sized brachial blood pressure cuff was used. All measurements were collected on the left arm at the heart level. The average of two readings was recorded as the blood pressure value for each subject. Recommended procedures were used to select the position and sizes of sphygmomanometers [19].

The following secondary outcomes were adopted: functional capacity, human activity profile, quality of life, maximum inspiratory pressure (MIP), maximum expiratory pressure (MEP), and cytokine plasma levels. The six-minute walking test (6-MWT) was used to assess functional capacity, following the guidelines of the American Thoracic Society [20]. The test was conducted along a 3 eter indoor walkway marked every 5 m with non-slip coloured tape glued to the floor. Before and immediately after the test, the heart rate (HR), SBP, DBP, and perceived exertion scores were recorded. Using a wristwatch, resting HR was measured for 60 seconds by palpating the radial artery. This measurement was preceded by at least 5 minutes of sitting rest.

Human activity profile

Each participant answered the Human Activity Profile (HAP) questionnaire at the beginning of the study [21]. This instrument includes 94 items related to activities such as self-care, transportation, home maintenance, entertainment/social, and physical exercises. Three responses are possible for each item: "still doing the activity", "have stopped doing the activity", and "never did the activity". Then, an adjusted activity score (AAS) was calculated to determine the respondents' activity levels, which were classified into impaired (AAS < 53), moderately active (AAS between 53–74), and active (AAS > 74).

Quality of life

The SF-36 (Short Form Health Survey) questionnaire was used to assess the quality of life [22]. This tool comprises 36 items, which are mostly assigned to one of eight health domains covering aspects of physical and mental health, as follows: physical functioning (10 items), physical role functioning (4 items), bodily pain (2 items), general health perceptions (5 items), vitality (5 items), social role functioning (2 items), emotional role functioning (3 items), and mental health (5 items). Higher scores in each domain indicate better health status. Scores range from 0 to 100, so results closer to 0 are less favourable to health status and scores closer to 100 are more favourable. As the questionnaire was administered, each question was read and clarified by the evaluator. After the participants answered all the questions, the score was calculated using the $SF36^{+}$ software.

Respiratory muscle strength

Maximal inspiratory and expiratory pressures were evaluated as indicators of respiratory muscle strength. These parameters were evaluated with the individual in the sitting position using an analogue manovacuometer with a scale ranging from -120 to +120 cmH_2O. A silicone mouthpiece adapter with a distal hole, which was sealed during each respiratory manoeuvre, was used to facilitate pressure recording [23].

PAF biochemical assay

PAF was analysed according to the Bligh and Dyer method [24]. Briefly, a 4 mL plasma sample was obtained from the cubital vein and immediately placed in a Vacutainer tube (BD Biosciences, USA), transferred to another 2 mL tube containing methanol, and spiked with 10 μL of internal standard solution (IS). Samples were then homogenized for 3 minutes at 30 Hz (Bead Ruptor 96, Omni Inc), followed by chloroform addition, and homogenization for more 3 minutes at 30 Hz. The homogenate was transferred to a glass tube with 250 μL of chloroform and 500 μL of ultrapure water. The samples were vortexed for 10 minutes and centrifuged at 3000 g for 10 minutes at 4°C. The lower phase (organic phase) was collected. The upper phase was re-extracted with 250 μL of chloroform, and the lower phase was additionally collected. The lower phases were combined and dried in a vacuum centrifuge for 1 h at room temperature. Aliquots of this dried organic phase were re-suspended in 50 μL $MeOH/H_2O$ (7:3, v/v) for liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis.

LC-MS/MS analysis was conducted using a High-Performance Liquid Chromatography (HPLC) system (Nexera X2, Shimadzu-Kyoto, HO, Japan) coupled to a TripleTOF 5600+ mass spectrometer (Sciex-Foster, CA, USA) equipped with a Turbo-V IonSpray and a Calibrant Delivery System (CDS). The APCI Positive Calibration Solution (SCIEX) was used. The Ascentis C18 column (15 cm × 2.1 mm, 2.7 μm; Supelco) was used for chromatographic separation, according to the method described by Godzien et al. [25], with some modifications reported below. Briefly, elution was performed using 0.1% formic acid solution prepared in water (v/v) (phase A) and 0.1% formic acid prepared in methanol (v/v) (phase B), and specific gradient conditions as follows: 0 to 1.0 min, 30% B; 1.0 to 3.5 min, 80% B; 3.5 to 12.0 min, 100% B. The column temperature was maintained at 40°C, the flow rate at 0.5 mL/min, and the samples were maintained at 4°C in the auto-sampler. The parameters used in the mass spectrometer were: nebulizer gas (GS1), 50 psi; turbo-gas (GS2), 50 psi; curtain gas (CUR), 25 psi; electrospray voltage (ISVF), 4.5 kV; turbo ion spray source temperature, 500°C, dwell time, 100 ms, and mass resolution < 2 ppm. Data acquisition was performed using the Analyst software (Sciex-Foster, CA, USA). Data were processed using PeakView 2.1 and MultiQuant 3.0.2 software (Foster, CA, USA) for multiple fragment ions' extraction, generation of MRMHR channels, and quantitative analysis. The MRMHR target parameters used were: PAF-C16:0 (524.3678/180.0731) and PAF-C18:1 (550.3859/184.0729) – parent ion and precursor ion (m/z), respectively.

Cytokine immunoassay

In addition to its potential involvement in SAH pathophysiology, PAF is a lipid mediator with several inflammatory and non-inflammatory roles, so its production may occur through the "remodelling pathway" under inflammatory conditions. Thus, cytokine plasma levels were also quantified. For that, aliquots of the same plasma samples obtained to evaluate PAF were frozen at -80°C and later used to quantify circulating cytokines. Plasma was obtained after centrifuging the blood at 500 *xg* and 4°C in the absence of an anticoagulant. A Cytometric Bead Array (CBA) human cytokine kit (BD Biosciences, USA) was used to quantify IL-2, IL-4, IL-6, TNF-α, and IFN-$_\gamma$ serum levels following the manufacturer's instructions. The concentration (pg/mL) of each cytokine was calculated from a standard curve of recombinant molecules (CellQuest, BD Biosciences, USA) using CBA software. The FACSCalibur cytometer (BD Biosciences, USA) was used for all readings.

Aerobic training protocol

Before starting the aerobic training, eligible participants were randomized to the intervention (IG) or control (CG) group. A research physiotherapist carried out the recruitment and evaluation of the participants, another carried out the randomization, and two other physiotherapists carried out the intervention. The allocation of participants was done using simple randomization by flipping a coin. IG participants performed three 5 inute sessions of treadmill aerobic exercise training (Movement RT250, USA) per week, on alternate days (Monday, Wednesday, and Friday), for 12 weeks in a total of 36 sessions. In this group, the training intensity was kept within 60–70% of the HR (target zone), which was calculated according to the Karvonen formula, with HRmax based on the formula 22 ge [26]. Heart rate was continuously measured using a precise heart rate sensor attached to a chest strap (Polar, model H10, NY, USA), from which data were transferred to a tablet via Bluetooth. Aerobic training was based on a inute warm-up, 4 inute target-zone training, and a final inute cool-down [27]. Training intensity was determined by a score ranging from 5 to 7 on the modified Borg's perceived exertion scale for participants who did not achieve target HR [27]. Blood pressure, HR, and RPE were measured before, during (every 10 minutes), and after each exercise session. This exercise training was administered between 7:00 and 10:00 am. Participants in the CG group were instructed to perform only simple activities associated with daily living and were invited to participate in the cardiac rehabilitation programme after the study. All participants were reassessed twenty-four hours after the last training session or after 12 weeks for control volunteers. Blood samples for PAF and cytokine dosage were collected before the beginning of the aerobic training and 24 hours after the last session of the 12th week.

Statistical analysis

The sample size used was calculated considering the similarity in methodology and clinical outcomes evaluated in a previous study [28]. The sample size was calculated using the Gpower software (version 3.1.9.2, North Rhine-Westphalia, Germany), adopting a 5% significance level, 80% power, and a 0.8 correlation coefficient for all variables. The calculations were based on the mean SBP values obtained in the aforementioned study, which demonstrated the need for at least 76 participants, of which, when considering a possible average loss of 8%, according to the formula described by Whitley & Ball et al. [29], we arrive at a total of 82 participants, 41 in each group.

Patient characteristics were expressed as absolute and relative values. Data distribution was analysed using the Shapiro-Wilk test. Parametric data were expressed as mean and standard in addition to promoting a gain in physical conditioning, as found in the present study, in which the IG participants walked more metres in the 6MWT, while non-parametric data were presented as the median. Baseline and post-intervention data (clinical, cytokines, and quality of life) were compared using the paired t-test or Wilcoxon test for intragroup comparisons, and the independent t-test or Mann-Whitney test for intergroup comparisons. One-way ANOVA followed by the Bonferroni post hoc test was used to compare PAF levels. Variables potentially associated with PAFC16:0 and PAFC18:1 plasma levels were explored by multivariate linear regression. The "backward" method was used to adjust the regression model. The linear relationship between independent and dependent variables, absence of multicollinearity, presence of homoscedasticity, independent distribution of errors, and normality of residuals were assumptions observed and respected in the final regression model. All analyses were performed using SPSS Statistics for Windows (version 23.0, IBM, Armonk, USA). The significance level for all statistical tests was set at 5%, and a statistician blinded to the groups included in the study analysed all data. The effect size was calculated by using Cohen's d, and the results were interpreted following Cohen as follows: small (0.21–0.49), medium (0.50–0.79), or large (≥ 0.80) [30].

RESULTS

The flowchart indicating patients' progression through the study is shown in Figure 1. Fifty-seven participants were initially allocated to the intervention group (IG) and forty-three to the untrained control group (CG). Thirty-six trained and forty-one untrained participants completed the study and were evaluated at the end of treatment.

The investigated sample was mainly composed of women aged 66.51 ± 7.53 years. The average of participants classifies them as overweight (BMI = 29.50 ± 4.51 kg/m^2) (Table 1). Regarding alcohol and tobacco consumption, 23.4% of the sample were alcohol consumers and 20.8 were ex-alcohol consumers; 18.2% were smokers and 53.2% were ex-smokers. Both alcohol consumers and ex-alcohol consumers were classified as low-consumption, evaluated by the AUDIT (Alcohol Use Disorders Identification Test) [31]. Angiotensin-receptor blockers (ARB) (68.83%) and diuretics (45.45%) were the main drugs used. Diabetes mellitus was the most frequent comorbidity (61.03%). Most participants (67.5%) were classified

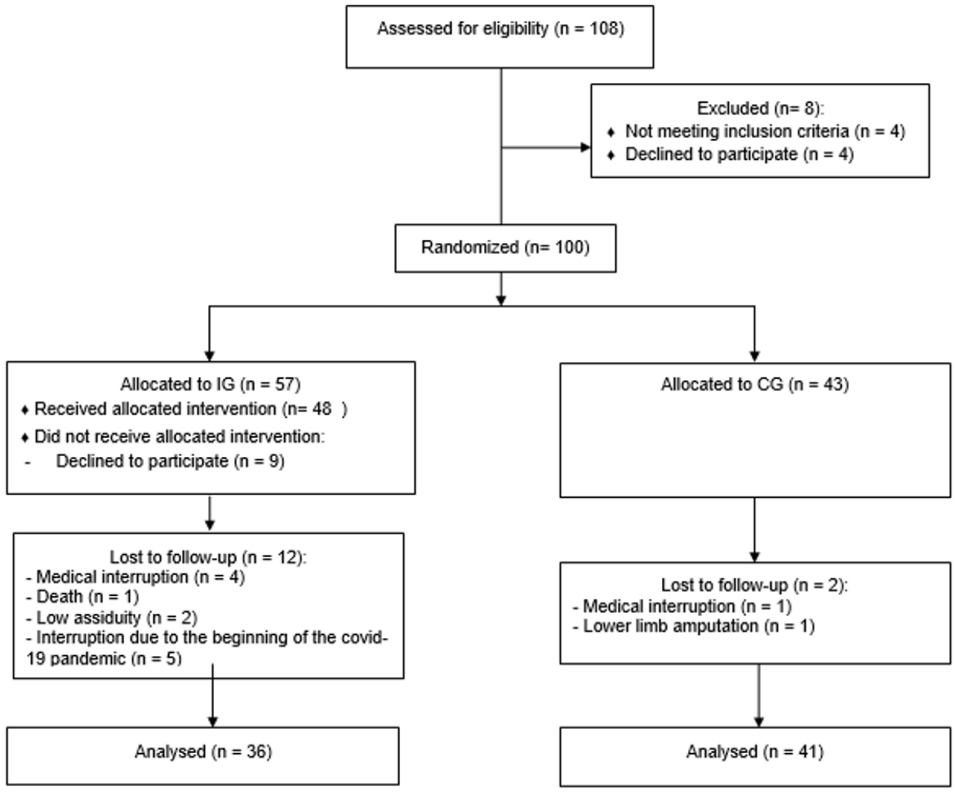


FIG. 1. CONSORT diagram showing the participants' progress through the trial.

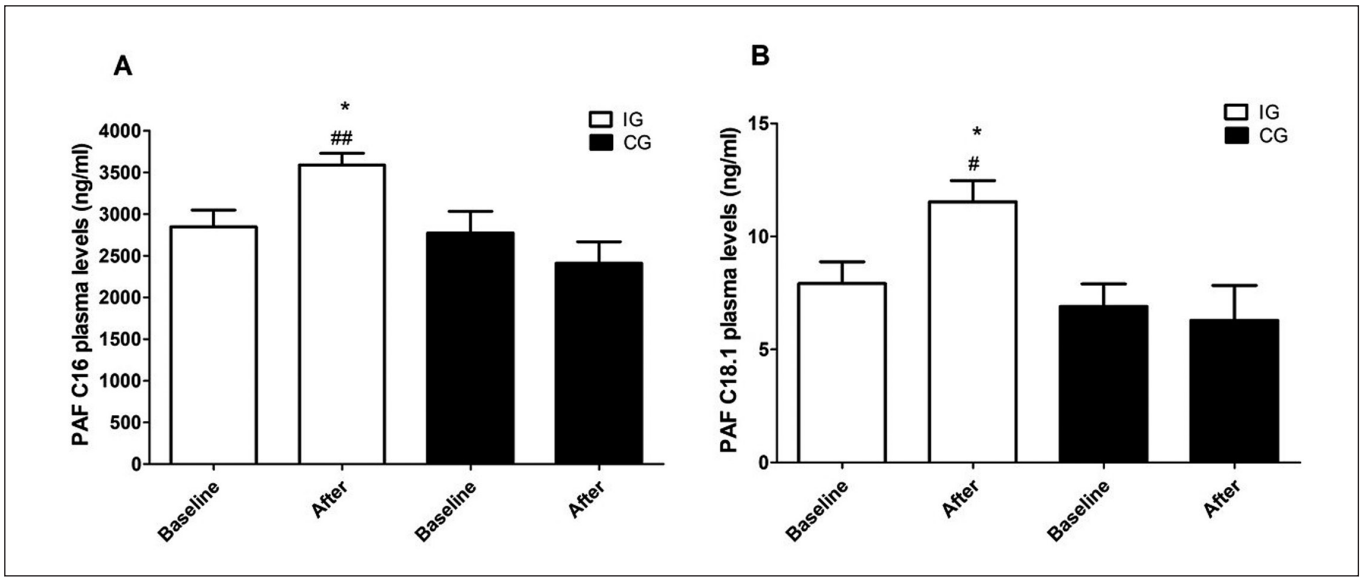


FIG. 2. Effect of aerobic training on plasma levels of PAFC16 and PAFC18.1. Data are presented as mean+SEM. $^{*}p < 0.05$ indicates statistically significant differences compared to baseline. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ indicate statistically significant differences between the intervention group (IG) and the control group (CG). One-way ANOVA followed by Bonferroni post-test.

as moderately active according to the physical activity profile assessed by the HPA questionnaire (Table 1).

As indicated in Table 2, post-intervention arterial blood pressure levels were significantly reduced [124.72 ± 1.66 vs. 133.05 ± 2.38 (95% CI 2.93–13.72, p = 0.048) for SBP and 76.94 ± 1.48 vs. 82.22 ± 1.44 (95% CI 4.82–5.73, p = 0.034) for DBP] in trained subjects compared to baseline. The effect size, as measured by Cohen's d, was d = 4.12 and 3.60, respectively, indicating a large effect. Intergroup comparison indicated that trained participants exhibited reduced delta levels after 12 weeks [-8.33 ± 2.65 vs. 1.43 ± 2.47 (95% CI -17.00–-2.53, p = 0.049) for SBP and -5.27 ± 2.3 vs. 4.36 ± 1.56 (95% CI 4.01–4.70, p = 0.02) for

TABLE 1. Baseline characteristics of the participants.

Variables	IG (n = 36)	CG (n = 41)	Total (n = 77)
Age (years)	66.97 ± 6.99	66.12 ± 8.03	66.51 ± 7.53
Men, n (%)	15 (41.7%)	16 (39.0%)	31 (40.3%)
Women, n (%)	21 (58.3%)	25 (61.0%)	46 (59.7%)
BM (Kg)	76.15 ± 13.89	76.18 ± 14.62	76.17 ± 14.19
BMI (Kg/m^2)	29.65 ± 3.47	29.37 ± 5.30	29.50 ± 4.51
AC (cm)	100.63 ± 1.71	98.78 ± 1.82	99.70 ± 1.52
SBP (mmHg)	133.05 ± 2.38	133.41 ± 2.48	133.23 ± 2.41
DBP (mmHg)	82.22 ± 1.44	81.70 ± 1.43	81.96 ± 1.39
HAP scoring	59.11 ± 3.22	59.39 ± 2.49	59.26 ± 1.99
Life habits, n (%)			
Alcohol consumers	8 (22.2)	10 (24.4)	18 (23.4)
Ex-alcohol consumers	11 (30.6)	5 (12.2)	16 (20.8)
Non-alcohol consumers	17 (47.2)	26 (63.4)	43 (55.8)
Smoker	6 (16.7)	8 (19.5)	14 (18.2)
Ex-Smoker	19 (52.8)	22 (53.7)	41 (53.2)
Non-Smoker	11 (30.6)	11 (26.8)	22 (28.6)
Medications, n (%)			
ARBs	22 (61.11)	31 (75.60)	53 (68.83)
β-Blockers	15 (41.66)	16 (39.02)	31 (40.25)
Diuretics	21 (58.33)	14 (34.14)	35 (45.45)
ACEi	7 (19.44)	7 (17.07)	14 (18.18)
CCBs	8 (22.22)	14 (34.14)	22 (28.57)
Associated pathologies, n (%)			
Diabetes Mellitus	21 (58.33%)	26 (63.41%)	47 (61.03%)
Dyslipidemia	5 (13.88%)	9 (21.95%)	14 (18.18%)
CABG	2 (5.55%)	0	2 (2.59%)
MI	8 (22.22%)	1 (2.43%)	9 (11.68%)
Hypothyroidism	6 (16.66%)	8 (19.51%)	14 (18.18%)
Stable angina	1 (2.77%)	0	1 (1.29%)

BM = body mass; BMI = body mass index; AC = abdominal circumference; SBP = systolic blood pressure; DBP = diatolic blood presure; HAP = human activity profile; ARBs = angiotensin-II receptor blockers; ACEi = angiotensin-converting enzyme; CCBs = calcium-channel blockers; CABG = coronary artery bypass graft surgery; MI = miocardial infaction. Data are presented as frequency or mean ± SEM.

DBP] (Table 2). The effect size for intergroup comparisons of blood pressure values also presented a large effect (d = 5.18 for SBP and d = 6.21 for DBP). Thus, this result suggests that the aerobic training protocol was effective in reducing blood pressure in hypertensive subjects. Furthermore, PAF plasma levels were significantly increased in trained subjects compared to baseline [11.51 ± 4.51 vs. 7.91 ± 4.6 (95% CI -7.15–-0.07, p < 0.004, d = 0.90 for PAFC18:1 and 3587.09 ± 677.69 vs. 2848.61 ± 957.63 (95% CI -139.4–-82.49, p < 0.004, d = 0.89 for PAFC16:0)] and to untrained participants [11.51 ± 4.51 vs. 6.28 ± 4.6 (95% CI (95% CI 0.52 to 9.95, p < 0.05, d = 0.74 for PAFC18:1 and 3587.09 ± 677.69 vs. 2410.5 ± 770.83 (95% CI 301.9 to 2051, p < 0.001, d = 1.68 for PAFC16:0)] after 12 weeks (Figure 2). According to Cohen's d, we considered the effect size of the results found with plasma PAF levels to be large, except for the comparison of PAFC18 levels between groups after 12 weeks, which was medium. Decreased blood pressure values and increased PAF levels after the intervention are also shown in Figure 3. The distance walked in the 6MWT [406.26 ± 17.95 vs. 491.82 ± 18.71 (95% CI -118.70–-52.41, p < 0.05, d = 4.70)] and MEP [92.61 ± 4.57

TABLE 2. Clinical variables, quality of life and cytokines levels of participants at baseline and post 12 weeks.

	IG (n = 36)			CG (n = 41)			IG vs CG		
	Baseline	Post	Sig	Baseline	Post	Sig	IG delta	CG delta	Sig
Clinical variables									
BM (Kg)	76.15 ± 2.31	76.05 ± 2.32	-1.01–1.21[a]	76.18 ± 2.28	75.75 ± 2.40	-0.58–1.46[a]	-0.10 ± 0.55	-0.43 ± 0.50	-1.15–1.82[c]
BMI (Kg/m^2)	29.65 ± 0.57	29.37 ± 0.62	-0.40–0.94[a]	29.37 ± 0.82	29.29 ± 0.89	-0.31–0.46[a]	-0.27 ± 0.33	-0.07 ± 0.19	-0.94–0.54[c]
AC (cm)	100.63 ± 1.71	100.06 ± 2.24	-2.42–3.56[a]	98.78 ± 1.82	99.80 ± 1.95	-0.28–2.33[a]	-0.56 ± 1.47	-1.02 ± 0.64	-2.62–3.53[c]
SBP (mmHg)	133.05 ± 2.38	124.72 ± 1.66	2.93–13.72[a,*]	133.41 ± 2.48	134.85 ± 2.56	-6.44–3.57[a]	-8.33 ± 2.65	1.43 ± 2.47	-17.00–-2.53[c,*]
DBP (mmHg)	82.22 ± 1.44	76.94 ± 1.48	4.82–5.73[b,**]	81.70 ± 1.43	86.07 ± 1.47	-6.24–-4.29[b,**]	-5.27 ± 2.3	4.36 ± 1.56	4.01–4.70[d,**]
HR (bpm)	70.86 ± 1.97	68.83 ± 1.90	-1.48–5.53[a]	72.24 ± 1.74	71.95 ± 2.07	-2.97–3.56[a]	-2.20 ± 1.72	-0.29 ± 1.61	-6.45–2.98[c]
MIP (cmH_2O)	-91.33 ± 4.74	-99.11 ± 3.88	-16.86–1.31[a]	-94.46 ± 4.21	-91.24 ± 4.28	6.40–9.13[b]	7.77 ± 4.47	-3.22 ± 3.61	-4.02–-2.41[d]
MEP (cmH_2O)	92.61 ± 4.57	107.69 ± 2.78	-23.95–-6.21[a,*]	94.31 ± 2.96	94.61 ± 3.54	-6.26–5.67[a]	15.08 ± 4.36	0.29 ± 2.95	4.50–25.07[c,*]
6-MWT (m)	406.26 ± 17.95	491.82 ± 18.71	-118.70–-52.41[a,*]	424.02 ± 9.86	416.92 ± 9.69	-5.00–19.20[a]	85.56 ± 16.32	-7.0 ± 38.35	59.65–126.66[c,*]
Quality of life (score 0 to 100)									
FC	67.22 ± 3.63	75.02 ± 3.98	-15.77–0.16[a]	75.97 ± 3.23	76.58 ± 3.56	-7.38–6.16[a]	7.80 ± 3.92	0.61 ± 3.35	-0.13–1.35[d]
LPA	50.02 ± 6.05	72.19 ± 5.53	3.16–6.43[b,**]	56.78 ± 5.73	63.29 ± 6.0	-19.94–24.37[b]	22.16 ± 7.24	6.51 ± 6.69	-3.97–35.28[c]
Pain	54.69 ± 3.95	68.38 ± 4.36	-24.27–-3.11[a,*]	54.29 ± 4.33	57.58 ± 4.09	-10.49–3.91[a]	13.69 ± 5.21	3.29 ± 3.56	-1.92–22.73[c]
GH	66.02 ± 3.32	76.91 ± 2.94	-18.32–-3.45[a,*]	63.22 ± 2.82	56.51 ± 3.32	0.59–12.82[a,*]	10.88 ± 3.66	-6.70 ± 3.02	8.20–26.98[c,*]
VIT	59.75 ± 3.89	75.13 ± 2.74	-23.09–-7.68[a,*]	59.63 ± 3.92	59.92 ± 3.81	-6.09–5.50[a]	15.38 ± 3.79	0.29 ± 2.86	5.75–24.44[c,*]
AS	67.69 ± 5.09	83.02 ± 3.77	-25.00–-5.65[a,*]	77.58 ± 3.28	80.90 ± 4.24	-13.87–16.78[b]	15.33 ± 4.76	3.31 ± 4.39	-0.88–24.91[c]
LAE	48.88 ± 7.18	65.72 ± 6.30	-14.87–18.80[b]	40.65 ± 6.21	60.48 ± 6.75	14.10–19.55[b,**]	16.83 ± 8.34	19.82 ± 6.85	-18.28–21.35[d]
MH	68.19 ± 3.87	77.94 ± 3.18	-16.30–-3.19[a,*]	68.12 ± 3.43	68.58 ± 3.08	-5.19–4.26[a]	9.75 ± 3.23	0.46 ± 2.34	1.47–17.10[c,*]
Cytokines levels									
INF-y (pg/mL)	0.00 (2.48)	0.00 (2.63)	-0.85–0.85[b]	0.00 (0.00)	0.00 (0.00)	-0.00–0.00[b]	-0.00–0.0	0.00 (0.00)	-0.00–0.00[d]
TNF-α (pg/mL)	0.16 (0.31)	0.16 (0.36)	-0.09–0.09[b]	0.00 (0.16)	0.16 (0.20)	-0.02–0.02[b]	0.00 (0.08)	0.16 (0.20)	-0.11–0.20[d]
IL-10 (pg/mL)	0.23 (0.49)	0.10 (0.32)	-0.05–0.21[b]	0.10 (0.23)	0.10 (0.23)	-0.05–0.05[b]	0.00 (-0.16)	0.00 (-0.13)	-0.02–0.02[d]
IL-6 (pg/mL)	1.66 (2.01)	1.53 (1.19)	-0.49–0.75[b]	1.53 (1.29)	0.81 (1.59)	-0.05–0.21[b]	0.08 (-0.41)	0.00 (-0.27)	-0.06–0.06[d]
IL-4 (pg/mL)	0.12 (0.62)	0.06 (0.62)	-0.10–0.22[b]	0.12 (0.12)	0.00 (0.12)	-0.04–0.04[b]	0.00 (-0.14)	0.00 (-0.12)	-0.02–0.02[d]
IL-2 (pg/mL)	0.00 (0.32)	0.32 (0.32)	-0.40–0.23[b]	0.00 (0.32)	0.32 (0.32)	-0.10–0.10[b]	0.00 (0.32)	0.00 (0.32)	-0.07–0.07[d]

BM = body mass; BMI = body mass index; AC = abdominal circumference; SBP = systolic blood pressure; DBP = diatolic blood pressure; MIP, maximal inspiratory pressure; MEP = maximal expiratory pressure; 6-MWT = six-minute walking test; FC = functional capacity; LPA = Limitation of physical aspects; GH = general health; VIT = vitality; AS = social aspects; LEA = limitation of emotional aspects; MH = mental health; INF-$_\gamma$ = Interferon-gamma; TNF-α = Tumor Necrosis Factor-alpha; IL-10 = Interleukin 10; IL-6 = Interleukin 6; IL-4 = Interleukin 4; IL-2 = Interleukin 2. Data indicated as the mean and standard error of the mean, or median and interquartile range. [a]Paired T test (IC95%); [b]Wilcoxon test (valor p); [c]Independent T test (IC95%); [d]Mann–Whitney test (p value). * and ** indicate statistical difference for IC95%.

vs. 107.69 ± 2.78 (95% CI -23.95–-6.21; d = 4.10) were also increased comparing the post-training results with baseline and with the delta values registered in the untrained group [85.56 ± 16.32 vs. -7.0 ± 38.35 (95% CI 59.65–126.66, p < 0.04, d = 5.27) for 6MWT and 15.08 ± 4.36 vs. 0.26 ± 2.25 (95% CI 4.50–25.07, p < 0.01, d = 0.99) for MEP] (Table 2). These previous results with MEP and 6MWT show a large effect size. The intervention proved to be effective in increasing circulating PAF levels in addition to controlling blood pressure, exerting a beneficial effect by improving functional capacity and expiratory muscle strength in trained participants. Conversely, cytokine levels were similar in both groups (Table 2).

Multiple linear regression analysis indicated that MEP was the strongest predictor (F [3.19] = 6.322; p = 0.001; R^2 adjusted = 0.499) for PAFC16:0 plasma levels in subjects undergoing aerobic training. SBP (p = 0.436) and DBP (p = 0.085) showed no significant association with PAFC16:0. SBP (p = 0.742), DBP (p = 0.269) and MEP (p = 0.935) also did not show a significant association with PAFC18.1. It is important to highlight that the number of plasma samples collected for both cytokine and PAF measurements was reduced (29 samples in the control group and 27 in the intervention group). 12 participants refused to collect blood in the CG and 9 in the IG.

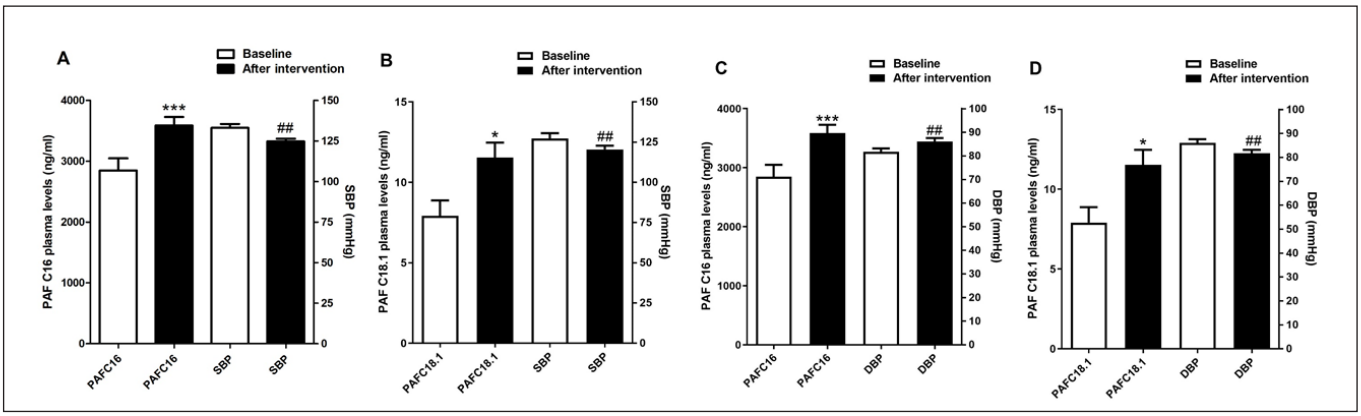


FIG. 3. Reduction of blood pressure levels and increase of PAF levels after aerobic training. Data are presented as mean+SEM. $^{*}p < 0.05$ and $^{***}p < 0.01$ indicate statistically significant differences compared to baseline. $^{\#\#}p < 0.01$ indicates statistically significant differences between the intervention group (IG) and the control group (CG). One-way ANOVA followed by Bonferroni post-test.

The trained subjects also showed a significant improvement in quality of life when the domains limitation of physical aspects [72.19 ± 5.53 vs. 50.02 ± 6.05 (95% CI 3.16–6.43, p = 0.006, d = 2.50)], pain [68.38 ± 4.36 vs. 54.69 ± 3.95 (95% CI -24.27–-3.11, p = 0.03, d = 3.29)], general health [76.91 ± 2.94 vs. 66.02 ± 3.32 (95% CI -18.32–-3.45, p = 0.001, d = 3.47)], vitality [75.13 ± 2.74 vs. 59.75 ± 3.89 (95% CI -23.09–-7.68, p = 0.002, d = 4.64)], social aspects [83.02 ± 3.77 vs. 67.69 ± 5.09 (95% CI -25.00–-5.65, p = 0.004, d = 3.45)] and mental health [77.94 ± 3.18 vs. 68.19 ± 3.87 (95% CI -16.30–-3.19, p = 0.02, d = 1.78)] were compared to baseline (Table 2). In addition, untrained subjects presented a worsening in the domain general health status [63.22 ± 2.82 vs. 56.51 ± 3.32 (95% CI 0.59–12.82, p = 0.03, d = 2.18)] and a better score in the domain limitation of emotional aspects [60.48 ± 6.75 vs. 40.65 ± 6.21 (95% CI 3.16–6.43, p = 0.01, d = 3.06)] compared to baseline. From the intergroup comparison, trained subjects obtained better quality of life delta scores in the domains of general health [10.88 ± 3.66 vs. -6.70 ± 3.02 (95% CI 8.20–26.98, p = 0.005, d = 3.25)], vitality [15.38 ± 3.79 vs. 0.29 ± 2.86 (95% CI 5.75–24.44, p = 0.002, d = 4.68)], and mental health [9.75 ± 3.23 vs. 0.46 ± 2.34 (95% CI 1.47–17.10, p = 0.004, d = 2.99)] (Table 2). A large effect size was found in the previously presented results of the quality of life domains.

DISCUSSION

The present study demonstrated that aerobic training was efficient in reducing blood pressure while increasing circulating PAF levels in hypertensive individuals. In addition, increased functional capacity, expiratory muscle strength, and quality of life were verified by subjects undergoing aerobic training.

The study was composed mainly of overweight elderly subjects, most of them women. Corroborating the current evidence, SAH is more prevalent in the elderly population [32], and this disease is closely correlated to vascular stiffness caused by calcium accumulation, smooth-muscle hyperplasia within the tunica media, as well as quantitative and qualitative changes in vascular collagen [33]. In addition, overweight is directly related to SAH development [34]. In general, SAH rates are higher in women than men over 65 years old, and the hormonal imbalance associated with menopause seems to be related to this higher prevalence [35]. Smoking and alcohol intake are also directly related to SAH development [36]; these were frequent habits among the participants in this study. Although the HAP questionnaire indicated that most study participants were moderately active, the data previously presented showed that they had several risk factors for the development of SAH and other cardiovascular diseases.

Interestingly, the aerobic training protocol developed in this study proved to be a relevant treatment strategy, exerting a marked impact on the physical, functional, and psychological aspects evaluated. In addition to controlling blood pressure, aerobic training improved physical performance, expiratory muscle strength, and quality of life in hypertensive participants. There is consistent evidence of exercise-induced blood pressure control [37]. However, the mechanisms involved in this effect are not completely understood. Thus, the involvement of PAF in mechanisms linked to SAH pathophysiology suggests a potential effect of this molecule on aerobic training-associated blood pressure control [38, 39]. Accordingly, there is evidence that PAF exerts a hypotensive effect [7, 9, 10].

During exercise, there is an increase in the activation of the sympathetic autonomic nervous system, mainly to maintain cardiac output under the demand imposed by the intensity [40]. Furthermore, previous evidence has shown that PAF is released in response to sympathetic activity, primarily as negative feedback to initial vasoconstriction [9]. Thus, by a probable mechanism of increasing the energy supply to the tissues under the demand for exercise, the PAF

induces a decrease in peripheral vascular resistance. Few mechanisms have been elucidated with PAF-induced hypotension. In addition, studies have ruled out the involvement of the renin-angiotensin system, muscarinic, β-adrenergic, dopaminergic, eicosanoids, calcium influx, thyrotropin-releasing hormone, steroids, or histaminergic mechanisms [7]. Thus, the mechanisms demonstrated in the hypotensive effect of this substance have been by modulation of vascular smooth muscle tone via negative feedback mechanism triggered by central activation of the sympathetic nervous system or peripherally promoting renal vasodilation [7, 9, 10], which may justify their release during the exercise.

In resting conditions, experimental studies have shown the hypotensive effect of PAF. A study found a dose-dependent decrease in mean arterial blood pressure in a model of spontaneously hypertensive rats after intravenous PAF infusion [38]. A similar effect was demonstrated in dogs by another study in the same year [39]. In addition, administration of the PAF receptor antagonist CV-3988 increased blood pressure in rats [41]. These findings suggest that PAF is involved in blood pressure control under normal conditions and during SAH.

We found a significant increase in PAFC16:0 and PAFC18:1 plasma levels in trained subjects. Thus, considering the increased plasma PAF levels after aerobic exercise, to the best of our knowledge this was the first clinical study to investigate and find an increase in this substance after aerobic training in humans. Haynes et al. [42] documented increased post-exercise PAF levels. However, they used eccentric exercise and evaluated this effect on platelet-mediated thrombotic risk. Platelet-activating factor (PAF) is a family of lkyl- cetyl glycerol- hosphorylcholines that has a fatty acid linked by an ether bond at the sn-1 position (fatty acids containing C16 or C18 carbon chains are the most abundant), an acetyl group at the sn-2 position, and a polar head of phosphorylcholine at the sn-3 position [43]. The modulating effect of PAF on blood pressure was also demonstrated by intra-arterial infusion of PAFC16:0 and PAFC18:1, which was able to induce renal vasodilatation and systemic hypotension in rats [42]. PAF is synthesized by two markedly different pathways known as the "*de novo*" and "remodelling" pathways. The remodelling pathway is responsible for the proinflammatory PAF production in acute and chronic inflammations [44]. Conversely, the *de novo* pathway was initially thought to be responsible for constitutive PAF production, maintaining basal PAF levels [45]. To produce its effects, PAF binds to specific receptors on smooth muscle cells, cardiomyocytes, neutrophils, monocytes-macrophages, eosinophils, Kupffer cells, and endothelial cells [7].

Although PAF is related to the continuous activation of inflammatory cascades during the development of inflammation-related chronic disorders [44], changes in anti- (IL-4, IL-10) and proinflammatory (IFN-γ, TNF-α, IL-2, IL-6) cytokine levels were not identified in the present study. Supporting our results, there is evidence that PAF can be produced in the non-inflammatory remodelling pathway via lysophosphatidylcholine acyltransferase 1 (LPCAT1), a member of the lysophospholipid acyltransferase family that is involved in PAF biosynthesis, which is neither activated nor up-regulated by inflammatory stimuli [45]. Furthermore, a study demonstrated that the PAF-induced hypotensive effect was prevented by Nω-nitro-L-arginine, an inhibitor of nitric oxide (NO) biosynthesis in endothelial cells, suggesting that NO, the "endothelium-derived relaxing factor", may be involved in PAF-mediated SAH control [46]. In addition, evidence has shown the contribution of NO to the regulation of blood pressure after exercise training [47]. NO was found to be an endothelial-derived relaxing factor and may play an important role not only in endothelial relaxation but also in the prevention of endothelial cell dysfunction [47]. Exercise-induced shear stress stimulates NO release, upregulating NO synthesis [48]. Thus, in addition to the non-inflammatory pathway, this previous evidence also indicates that PAF may also reduce blood pressure via NO; however, more studies are needed to evaluate this process after aerobic training, since an association between increased levels of PAF and reduced arterial blood pressure was not found in the present study.

Multiple regression analysis did not find an association between plasma PAF levels and post-intervention arterial blood pressure. Linear regression assumes that relationships between variables are linear and that errors are normally distributed and have constant variance. If these assumptions are not met, the regression results may be inaccurate [48]. Thus, the difference between the number of PAF samples and blood pressure may have influenced the linear regression results. The reduced PAF sample may also have been a limitation of the present study.

Furthermore, the increase in PAF levels after aerobic training may not be involved in the control of SAH, but with other beneficial effects that both exercise and PAF participate in, such as activation of the immune system, neuronal plasticity, or body weight control [49–52]; however, further studies are needed to evaluate this process.

A moderate determination coefficient ($R^2 = 0.499$) was found between increased plasma PAF C16:0 levels and expiratory muscle strength. A study conducted by our research group also demonstrated increased expiratory muscle strength and reduced SBP levels in hypertensive subjects after eek aerobic exercise training [53]. In addition, another study using aerobic exercises for upper and lower limbs through virtual cardiac rehabilitation for 15 weeks found increased expiratory muscle strength and reduced DBP in hypertensive patients [23].

Despite the gain in expiratory muscle strength having been associated with the reduction in SAH, as demonstrated in previous studies, and with the increase in PAF levels in the present study, there are few data in the literature that support this effect, especially after exercise. A study found a significant increase of PAF in the bronchoalveolar lavage fluid of race-trained horses [54]. In addition, PAF is responsible for potentiating the phagocytosis activity exerted by alveolar macrophages and neutrophils, which is crucial for lung clearance [55, 56]. Although further studies are needed, based on the previous evidence, we can suggest that PAF contributes to lung

ventilation, and consequently to respiratory muscle strength. Furthermore, as during exercise there is an increase in the release of this substance, its contribution to alveolar clearance can facilitate gas exchange and lung efficiency, improving the ventilatory machinery and consequently respiratory muscle strength.

Limitations and future directions

Although the study demonstrated several benefits of aerobic training, some limitations must be considered. Accordingly, a group of normotensive subjects would be relevant to compare pre-and post-exercise PAF levels with the results recorded in hypertensive subjects. However, it was difficult to obtain the number of hypertensive participants according to the calculated sample size, so it would be even more difficult to include normotensive individuals. Although our sample complied with the sample size calculation, the number of participants was too small to expand the use of independent variables in the multiple regression model. Furthermore, several factors may have limited the observed association of increased PAF levels with the reduction in blood pressure, such as I) the intensity of the exercise, which was of low intensity; II) the duration of the aerobic training programme, which can be considered short, and the discrepancy between the BMI of the participants, since some were overweight and others normal weight, and III) a reduced sample for PAF dosage. Future research, excluding these previous biases, will be necessary to evaluate the involvement of PAF in the control of hypertension induced by exercise, in which resistance exercise can also be an alternative.

CONCLUSIONS

Taken together, our results indicated that the aerobic training protocol developed in this study had a beneficial impact on blood pressure, physical performance, respiratory muscle strength, and quality of life in hypertensive patients. In addition, increased circulating PAF levels in trained subjects were associated with increased expiratory muscle strength, requiring more studies in future to assess the participation of this substance in blood pressure control.

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Disclosure of interest

The authors report no conflict of interest.

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Serum myokines as potential biomarkers of myostatin inhibition in sport doping: a preliminary study on their baseline levels in elite athletes

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ABSTRACT: We considered in this study the possibility of developing an indirect procedure for detecting myostatin inhibition/suppression, a practice that is prohibited as doping in sport. We have specifically considered the potential diagnostic utility of human serum myokines as indirect markers of myostatin inhibition. Myostatin, its main antagonist follistatin, and other myokines (follistatin-like 1, musclin, oncostatin, osteonectin, irisin, brain derived neurotrophic factor, and insulin-like growth factor-1) were selected as a panel of potential biomarkers whose levels may be altered following myostatine suppression. The serum levels of myostatin and of the nine myokines were measured in elite athletes of different age, sex, and sport discipline, and their cross correlation assessed by multivariate analysis. All myokines resulted to be measurable in human serum, except for musclin and irisine, whose levels were below the limits of quantitation in a reduced number of samples. Serum concentrations varied of different orders in magnitude (musclin and osteonectin < 1 ng/mL; follistatin, myostatine and irisine 1–5 ng/mL; brain-derived neurotrophic factor, follistatin-like 1 and iinsulin-like growth factor-1 > 10 ng/mL), while no significant differences were found between female and male subjects, with the exceptions of follistatin-like 1 and musclin, showing a higher concentrations in females ($p < 0.05$). Levels of insulin-like growth factor 1 and brain derived neurotrophic factor were significantly higher in power athletes than in endurance ones. Multivariate statistics showed that musclin, follistatin-like 1 and oncostatin are more clustered and correlated to myostatin than other myokines, suggesting they could be considered as potential biomarkers of doping by myostatin inhibitors.

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INTRODUCTION

Myokines are a class of small proteins mainly produced and released from skeletal muscle cells and strictly involved in myogenesis, muscle contraction, and exercise-associated metabolic changes. In general, myokines play a significant role in various physiological functions, including muscle growth and metabolic homeostasis (reviewed in [1]). Myostatin (MYO), also known as Growth Differentiation Factor-8 (GDF-8), is a 25 kDa myokine belonging to the Transforming Growth Factor beta (TGF-β) family. MYO is expressed in skeletal muscles, acting as the key negative regulator of skeletal muscle cell growth and differentiation [2]. MYO specifically acts on muscle cells with the dual action of inhibiting the proliferation and differentiation of the myoblasts. Recent experimental evidence has revealed that muscles of knock-out mice for the MYO gene resulted to be two to three times larger than the wild-type ones. Transgenic mice expressed high levels of the MYO propeptide, and/or high levels of follistatin (FOL), which is the main natural antagonist of MYO, were expressed. The results of these experiments showed an increase in muscle similar to what had been observed in knockout mice for the MYO gene [3].

In recent years, great relevance has been devoted to these proteins in the processes of communication with other organs, such as liver and adipose tissue. However, since the main role of MYO is the inhibition of muscle growth, it is possible to obtain a condition of hypertrophy by antagonizing MYO in several ways. Substances or factors that can inhibit or suppress MYO could be therefore appealing for doping purposes, allowing to increase muscle mass, with an enhancement in terms of strength and, consequently, of the athletic performance.

Enhancement of muscle growth is one of the most desirable effects in sport doping. In this context, MYO inhibition/suppression sounds as a very effective strategy for the non-physiological, illicit improvement of sport performance, especially in power sports/sport disciplines. For the above reasons, anti-MYO agents are included in the list of doping substances and methods of the World Anti-Doping

Agency (WADA): section S4 ("Hormone and metabolic modulators") specifically mentions as a doping strategy the use of "MYO inhibitors such as i) Agents reducing or ablating MYO expression, ii) MYO binding proteins (such as FOL, and MYO propeptide) and iii) MYO-neutralizing antibodies" [4]. No direct methods are currently available for the detection of all the many possible substances and methods triggering MYO inhibition.

Due to the wide variety of substances and methods that can be used to inhibit/suppress MYO, the detection of doping by MYO inhibition represents one of the most demanding challenges for the anti-doping scientific community. At present, there are no known official methods for the detection in serum/urine of antibodies neutralizing MYO, but, even though these methods were available, they would be useful only for detecting a small part of the different possible inhibition/suppression systems. For instance, a method specifically developed for the detection of anti-MYO antibodies would have no efficacy in the case the inhibition would be performed using FOL and/or gene therapy with siRNAs.

Nowadays, skeletal muscle is considered as an endocrine organ secreting hormone-like factors that can influence the metabolism of other organs and tissues. During muscle contraction, the production and secretion of specific myokines are stimulated, and myokines can act with endocrine, autocrine and/or paracrine action. In addition to MYO and to its primary antagonist FOL [5], other myokines of interest are the following: the adipomyokine Follistatin-like 1 [6] (FSTL-1), Musclin (MUS) [7], Oncostatin (ONC) [8], Osteonectin (OST) [9], Irisin (IRI) [10], Brain Derived Neurotrophic Factor (BDNF) [11], Insulin-like Growth Factor 1 (IGF1) [12], some interleukins such as IL-6, IL-13, IL-15 [13] and some other growth factors such as Fibroblast Growth Factor-21 (FGF-21) [14] and also Erythropoietin (EPO) [15]. The activity of all these myokines is related to the trophic state and to the activity of the skeletal muscle; moreover, their action is often synergistic.

The primary way to inhibit MYO is by the use of FOL – a ubiquitous glycoprotein produced by the anterior pituitary gland [16], that, consequently, cannot be classified as a myokine – and/or of antibodies binding to MYO itself or to the ActRIIB receptor. Recently, a spliced form of FOL was produced and successfully delivered into muscles by using adeno-associated viruses (AAV) with no appearance of adverse effects [17]. Other methods that can be illicitly used to inhibit MYO are based on the silencing of the MYO gene by gene therapy (GT), via the transfer of genetic material (RNA, DNA, or genetically modified cells) to human cells, normally with the purpose to treat specific diseases [18]. It was also shown that in vivo injections of MYO binding antibodies can be effective in neutralizing MYO as a therapeutic strategy to treat dystrophies [19]. In recent years RNA interference (RNAi) has also been proven to achieve MYO inhibition. More specifically, RNAi uses small RNA molecules, called short interfering RNA (siRNA), that bind to mRNA and promote post-transcriptional gene silencing [20]. A recent study also reported that the use of antisense oligonucleotides can be effective in MYO blockade and that the inhibition can be achieved also by exon skipping [21]. Finally, apart from siRNA, other possibilities to inhibit MYO gene expression are represented by microRNAs (miRs), that are small endogenous non-coding single strand RNAs whose function is to regulate gene expression in an epigenetic way [22]. In particular, miRs induce gene silencing by overlapping with complementary sequences present on the mRNA target molecule; this link entails a repression of the translation or the degradation of the target molecule at a post-transcriptional level. Several miRs, called MyomiRs, are expressed in muscle cells where they act by repressing several genes responsible for the growth and development of the muscle cells themselves. Their expression is cell- and tissue-specific, and their role as cellular biomarkers in the diagnosis of pathologies is progressively being explored. The majority of miRs are present within the cell, but they have also been found in significant levels also extracellularly, in many biological fluids, such as plasma, serum, saliva, and urine.

Such a broad variety of possible ways to reduce the levels of MYO make the development of traditional, "direct" antidoping analytical methods, aimed to detect all the potential MYO inhibitors/suppressors, virtually impossible. In cases like this, the only possible detection strategy is based on the monitoring of a panel of diagnostic biomarkers, whose levels are significantly altered following MYO inhibition/suppression. The basic idea of the present work is therefore to identify possible biomarkers that can be perturbed by the MYO suppression. We focused on protein targets (not only MYO itself and FOL, but also some other myokines closely related to MYO) and on their possible correlation. Since the main aim of the study was to design a procedure that could be applied in the framework of doping control, our first step was to assess the normal physiological levels of the selected biomarker(s) and the possible correlation among the targeted biomarkers themselves in elite athletes testing negative to the antidoping tests. The analysis of these data would indeed represent the first step in the aim to detect a "signature" of the abuse of the inhibition/suppression of MYO, whenever the normal values are perturbed by non-physiological causes. If proved sufficiently robust, multi-parametric assays of this kind can be taken into consideration as effective initial testing methods.

On the grounds stated above, we have therefore considered the following:

1) To explore the potential application of human serum myokines as indirect markers of doping and principally to define ranges of baseline serum levels of the principal myokines in a database of elite athletes.
2) To evaluate the correlation among the myokines tested and their variability considering sex, age and sport/discipline.

MATERIALS AND METHODS

Participants

Serum samples from elite athletes (see Table 1 for details on the composition of the selected athlete population) collected for routine doping control tests and previously reported as negative have been

assayed for the selected panel of following, potential biomarkers: Myostatin (MYO); Follistatin-like 1 (FSTL-1); Musclin/Osteocrin (MUS); Oncostatin (ONC); Osteonectin (OST); Irisin (IRI); Brain-derived Neurotrophic Factor (BDNF); Follistatin (FOL); Insulin-like growth factor 1 (IGF1), in compliance with the World Anti-Doping Agency ethical code for the accredited laboratories [23]. All samples considered in the present study were from athletes who explicitly expressed their consent for the samples to be used for research purposes on the doping control form, after anonymization and after all anti-doping analyses were completed, with no adverse analytical finding reported for any other doping substance/method. The information reported on the doping control form, indicating sex and age of the subject, allowed to exclude that more than one sample among those considered in the study was collected from the same subject. Athletes were divided into sub-groups according to their sex and to the type of sport to which they belong. Athletes practicing POW sports were selected among disciplines such as weightlifting, body building, short-distance athletics (less than 200 meters). Athletes practicing endurance sports (END) were selected among disciplines such as cycling, football, tennis, triathlon, long-distance athletics (more than 1000 meters).

Immunological Assays

Different immunological methods with antibodies specific for the proteins of interest were used. More specifically, MYO and FOL levels were measured by enzyme-linked immunosorbent assay (ELISA, R&D Systems, article # DGDF80 and DFN00 respectively) on a Victor3 plate reader (Perkin Elmer) using quality controls prepared by the recombinant reference standards included in the respective kits; IRI and IGF1 serum levels were measured by radioimmunoassays (RIA, Phoenix Pharmaceuticals) on a Wizard2 Gamma Counter (Perkin Elmer); the analysis of all other analytes was performed via multiplex cytometric beads array (CBA, Merck Millipore) on xMAP Luminex 200 Flow Cytometer (Merck Millipore). Samples were assayed in duplicate and results were considered valid when intra-day precision was estimated to be less than 10% of coefficient of variation.

Table 1 summarizes the characteristics of the athletes and the immunological assays applied in this research.

Data Analysis

Descriptive statistics was performed by using Statistica 12.0 (Statsoft) and SPSS 17.0 (IBM) software packages. Kolmogorov-Smirnov test was used to assess the normality of data distribution. Correlations among biomarkers were calculated according to Kendall's tau beta index. Differences between groups have been determined by Mann-Whitney U test except where indicated. Statistical significance was set at $p < 0.05$, high significance was considered when $p < 0.01$. A Principal Component Analysis (PCA) was run for a reduced and paired subset of the database ($N = 41$) in which all athletes resulted assayed for all the analytes.

Ethics

The authors declare that the experiments reported in the manuscript were performed in accordance with the ethical standards of the Helsinki Declaration and that the participants signed an informed consent form. Specifically, anonymized samples were selected among those belonging to athletes who had given written consent for their samples to be used for research purposes at the moment of sample collection for the doping control test, in agreement with the WADA Ethical rules as detailed in the WADA International Standards for Laboratories.

RESULTS

Table 2 summarizes the results of the assays of myokines in elite athletes' serum.

TABLE 1. Details on the elite athletes' population selected for the construction of the database and immunological assays applied. Pow = Power sport, End = Endurance sport, FCBA = Flow cytometry bead array, IRMA = Immunoradiometric assay, ELISA = Enzyme-Linked Immunosorbent Assay, I-125 = Iodine isotope 125; LOD = limit of detection.

	All	Age mean	Males	Females	Pow	End	Assay	Detection	LOD	Precision	
		(min-max)					Test			Intra-assay	Inter-Assay
BDNF	56	26.6 (17–46)	43	13	18	38	FCBA	Fluorescence	6 pg/mL	< 10%	< 15%
FSTL-1	58	26.7 (17–46)	45	13	19	39	FCBA	Fluorescence	296 pg/mL	< 10%	< 15%
IRI	41	26.9 (17–46)	35	6	16	25	FCBA	Fluorescence	150 pg/mL	< 10%	< 15%
MUS	51	26.8 (17–46)	39	12	15	36	FCBA	Fluorescence	37 pg/mL	< 10%	< 15%
MYO	53	26.6 (17–46)	41	12	18	35	ELISA	Absorbance	5.3 pg/mL	< 5%	< 10%
ONC	58	26.8 (17–46)	45	13	19	39	FCBA	Fluorescence	0,4 pg/mL	< 10%	< 15%
OST	55	26.8 (17–46)	44	11	18	37	FCBA	Fluorescence	2.6 ng/mL	< 10%	< 15%
IGF1	57	26.7 (17.46)	44	13	19	38	IRMA	I-125 decay count	10 ng/mL	< 10%	< 10%
FOL	54	26.8 (17–46)	42	12	19	35	ELISA	Absorbance	83 pg/mL	< 5%	< 10%

As it can be seen, serum levels varied by different orders of magnitude among the different target compounds: ONC and MUS showed concentrations lower than 1 ng/mL; levels of FST, MYO and IRI ranged between 1 and 5 ng/mL, while BDNF, FSTL-1, IGF1 and were highly expressed in serum (BDNF and FSTL-1 approximately between 10 and 30 ng/mL and IGF1 approximately 200 ng/mL). A statistically significant difference was found between female and male subjects regarding FSTL-1 and MUS, where female athletes showed higher values of these analytes. Moreover, another statistically significant difference was found in the levels of IGF1 depending on the type of sport practiced by the athletes: subjects belonging to the POW group presented significantly higher serum values of IGF1. The serum concentration of MYO resulted 4 to 5 times higher than FOL. Differently, FSTL-1 presented higher average serum values than MYO and the ratio in all samples resulted greater than 1. Figure 1 shows the correlation plots of the analytes versus the athletes' age. As it can be seen, negative and statistically significant correlation was found only for IGF1 (Kendall's tau beta index of correlation = -0.55, $p < 0.01$).

A weak, but significant, positive correlation was found for FOL (Kendall's tau beta index of correlation = 0.31, $p < 0.01$). PCA analysis (figure 2) showed that three myokines, MUS, FSTL-1 and ONC, are more related to MYO as they cluster very closely together in the first component of the plot.

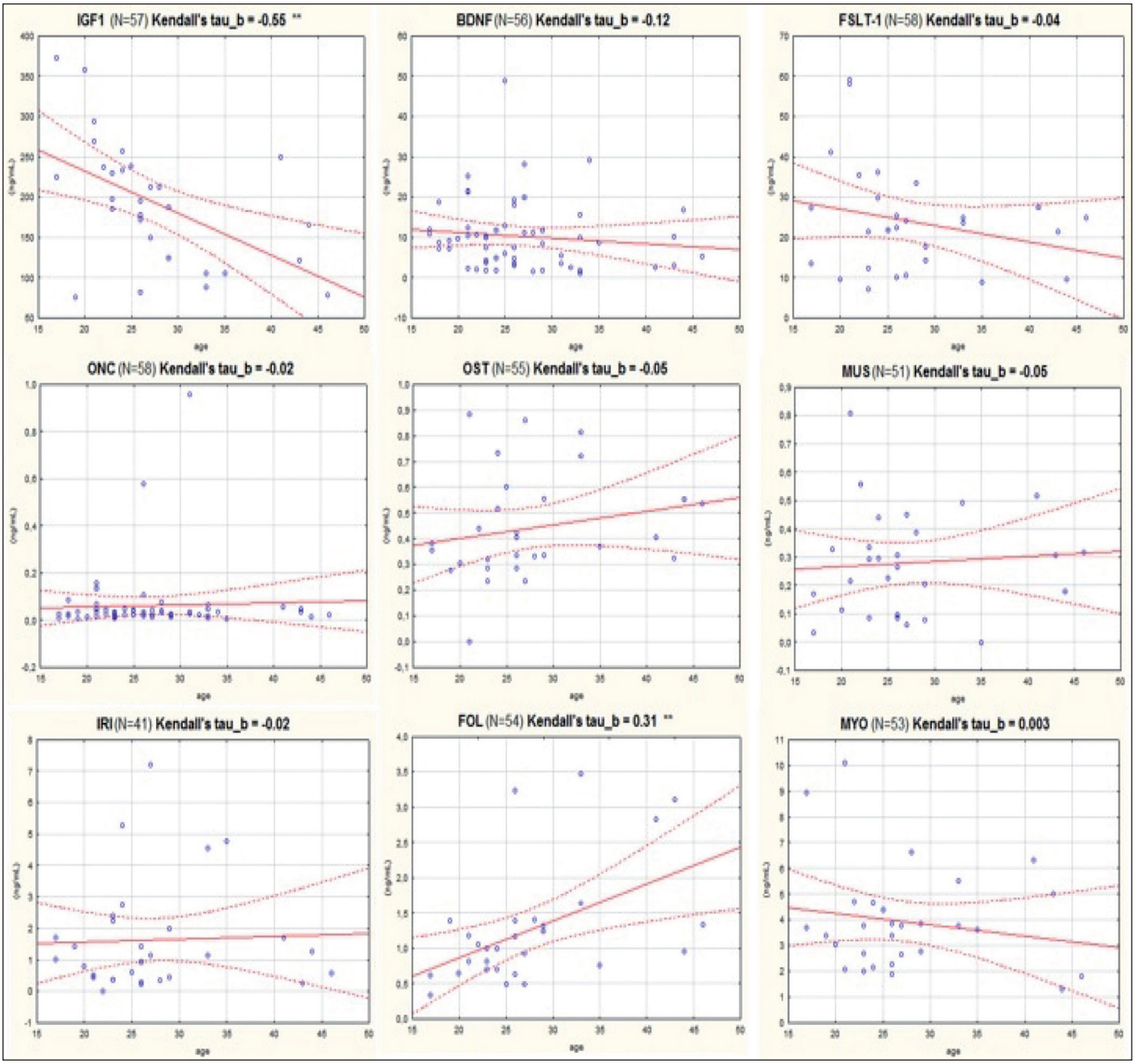


FIG. 1. Correlation with age of the levels of each of nine biomarkers considered in this study.
IGF1: Insulin-like growth factor 1; BDNF: Brain-derived Neurotrophic Factor; FSTL-1: Follistatin-like 1; ONC: Oncostatin; OST: Osteonectin; MUS: Musclin/Osteocrin; IRI: Irisin; FOL: Follistatin; MYO: Myostatin.

TABLE 2. Serum level of Myostatin (MYO), Follistatin (FOL), Insulin-like Growth Factor 1(IGF1), Oncostatin (ONC), Osteonecton (OST), Follistatin-like-1 (FSTL-1), Musclin (MUS), Irisin (IRI), Brain Derived Growth Factor (BDNF) in elite athletes and values comparison among groups of interest. M = males, F = females, POW = power typically anaerobic sports, END = endurance typically aerobic sports. * = significance ($p < 0.05$), ** = highly significant ($p < 0.01$).

		N	mean (ng/mL)	std.dev	std.err.mean	*p*-value
BDNF	ALL	56	10.32	8.71	1.16	
	M	43	10.14	8.66	1.32	0.99
	F	13	10.92	9.22	2.56	
	POW	18	15.75	1.10	2.59	0.001**
	END	38	7.75	6.03	0.98	
FSLT-1	ALL	58	27.35	18.26	2.40	
	M	45	24.26	15.44	2.301	0.014*
	F	13	38.01	23.46	6.506	
	POW	19	28.45	1.582	3.629	0.58
	END	39	26.81	1.951	3.123	
IRI	ALL	41	1.62	1.56	0.24	
	M	35	1.424	1.191	0.201	0.43
	F	6	2.736	2.83	1.155	
	POW	16	1.831	1.971	0.493	0.86
	END	25	1.479	1.251	0.25	
MUS	ALL	51	0.320	0.19	0.03	
	M	39	0.293	0.199	0.032	0.03*
	F	12	0.395	0.117	0.034	
	POW	15	0.333	0.185	0.047	0.76
	END	36	0.309	0.19	0.032	
MYO	ALL	53	3.85	1.89	0.26	
	M	41	3.871	1.883	0.294	0.39
	F	12	4.345	1.912	0.552	
	POW	18	4.332	2.683	0.632	0.69
	END	35	3.796	1.309	0.221	
ONC	ALL	58	0.061	0.14	0.02	
	M	45	0.068	0.16	0.024	0.20
	F	13	0.036	0.013	0.004	
	POW	19	0.045	0.04	0.019	0.47
	END	39	0.069	0.171	0.027	
OST	ALL	55	0.42	0.24	0.03	
	M	44	0.397	0.233	0.035	0.25
	F	11	0.534	0.262	0.079	
	POW	18	0.441	0.215	0.051	0.49
	END	37	0.417	0.258	0.042	
IGF1	ALL	57	197.3	73.6	9.744	
	M	44	193.9	76.7	11.568	0.30
	F	13	208.5	63.1	17.491	
	POW	19	236.7	67.608	15.51	0.006**
	END	38	177.6	69.058	11.203	
FOL	ALL	54	1.37	1.21	0.09	
	M	42	1.127	0.587	0.09	0.33
	F	12	1.165	0.918	0.265	
	POW	19	1.022	0.319	0.073	0.80
	END	35	1.196	0.789	0.133	

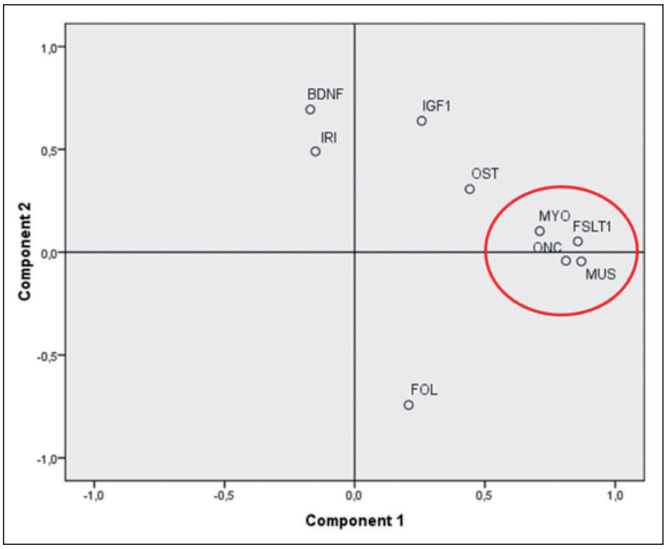


FIG. 2. Principal component analysis of a paired subset of athletes (N=41). Component #1 explain 33% of the total variance and can be related to the development and trophy of muscles and bones. Component #2 explain another 17% of the variance and can be interpreted as the component mainly related to the energetic metabolism of the skeletal muscle. IGF1: Insulin-like growth factor 1; BDNF: Brain-derived Neurotrophic Factor; FSTL-1: Follistatin-like 1; ONC: Oncostatin; OST: Osteonectin; MUS: Musclin/ Osteocrin; IRI: Irisin; FOL: Follistatin; MYO: Myostatin.

DISCUSSION

Myokines in Athletes Serum: Descriptive Statistics

All myokines analyzed resulted to be measurable in all samples of human serum, except for MUS and IRI, whose concentrations were in some samples below the limits of quantification (0.037 and 0.15 ng/mL respectively). Myokines showed levels of serum concentration of different orders in magnitude: OST and MUS resulted to have a concentration lower than 1 ng/mL; FOL, MYO and IRI between 1 and 5 ng/mL, while BDNF, FSTL-1 and IGF1 were highly expressed in serum with concentration higher than 10 ng/mL.

For FSTL-1 and MUS, females exhibited higher serum values than males ($p < 0.05$). However, the overall limited number of female individuals in the database may be a confounder of this result.

A statistically significant difference was also found considering the type of sport practiced by the athletes (POW vs. END) with regard to IGF1. POW group presented significantly higher serum values of IGF1. The reasons for this result can be found in the peculiar characteristics of strength power sport types. POW athletes perform high intensity performances in a very short time range, and which exploit an anaerobic alactacid energy metabolism by using phosphocreatine as the main substrate for ATP regeneration. IGF1 is a growth factor that is secreted in response to the stimulation of growth hormone which in turn is under the control of the hypothalamus. IGF1 is a growth factor essential for development and growth of tissues and bones. Despite skeletal muscle is not the main secretory organ, IGF1 has been recognized recently as a myokine to all extents [24].

Acute high intensity exercise is a very powerful stimulus for GH secretion, which in turn can therefore lead to increased IGF1 production hence the higher serum values found in the power group. In any case, the true relationship between IGF-1 and physical exercise is still to be fully understood. Other studies report contradictory results where certainly the IGF1 increases following a high intensity exercise but while a low intensity exercise would lead to the maintenance or even the decrease of the serum values of IGF1 [25].

FOL/MYO and MYO/FSTL-1 Ratios

On average, the serum concentration of MYO resulted to be 4 to 5 times higher than FOL. The ratio between the two analytes has a wide, non-normal distribution and resulted lower than 1 with a slightly asymmetric distribution (mean value = 0.34). A reversal of the FOL/MYO ratio could indicate an inhibition of MYO through the fraudulent use of its natural antagonist(s). However, the high variability we found for the ratio (CV = 64%), suggests that it may be difficult to fix a population threshold of abuse in order to use the ratio as an index of MYO inhibition. For antidoping purposes, it may be more appropriate to follow the variation of individual MYO/FOL ratio longitudinally. A similar consideration can be made with respect to the MYO/FSTL-1 ratio. In this case, FSTL-1 exhibits higher average serum values than MYO and the ratio in all samples is greater than 1. The alteration of the ratio to values close to 1 or even below 1 could be caused by the inhibition of MYO through an over-expression of its inhibitor FSTL-1. However, also in this case, we found a very wide distribution of the ratio values (mean ratio = 7.8, CV = 85.4%) making it difficult to establish population-based thresholds.

Correlation with Age

The decrease in serum IGF-1 with age is well a known phenomenon [26–27]. With respect to sport doping, IGF-1 is not only prohibited as such, but it is also one of the two biomarkers to detect GH intake: the age of the subject is taken into account in the GH-Score formula applied in doping control to determine the decision limit of the abuse [28].

Our data are in agreement with the above: a negative and statistically significant correlation with age was found for IGF1 (Kendall's tau beta index of correlation = -0.55, $p < 0.01$, see Figure 1).

On the other hand, another statistically significant correlation with age was found for FOL (Kendall's tau beta index of correlation = 0.31, $p < 0.01$), although this correlation seems to be weak as indicated by the index of correlation that is < of 0.5. A more detailed analysis was performed by dividing the athletes into two groups of equal numbers (up to 25 years of age N = 29, and from 25 years upwards N = 28). Athletes over 25 years of age have higher FOL (1.42 ng / mL vs. 0.85 ng / mL) and also in this case the result obtained is statistically significant ($p << 0.01$). FOL actually increases with age and this result is confirmed in observational studies from literature: mean FOL levels do not change during puberty, but are higher in adult and postmenopausal women [29]. On the other hand, we found no

correlation between age and MYO in our database. From the literature, it is known that MYO slightly increases until 57 years of age and then it starts to decrease [30]. Moreover, some studies found that serum MYO levels follow a circannual cycle, showing a peak during the spring season, as they are directly dependent on the 25-hydroxy-Vitamin D levels [31]. It was also found that females have slightly lower serum levels of MYO than men [32]. However, in our analysis we did not find such differences: we found MYO values to be moderately higher in females, but without being statistically significant and this result may also be due to the small number of female athletes in our database. Moreover, the fact that FOL shows a positive correlation with the age of athletes while on the contrary MYO does not (at least in the age range considered in this study), confirms that any monitoring of the MYO / FOL ratio for anti-doping purposes would perform better if executed at the longitudinal level by applying indi-vidual thresholds of abuse.

Correlation among Myokines

Correlation among myokines was assessed by calculating Kendall's Tau beta index of and by performing a Principal Component analysis (PCA) (see again Figure 2). PCA analysis shows that three myokines, MUS, FSTL-1 and ONC, are closely related to MYO. These myokines cluster very closely together in the first component of the plot. This component can be interpreted as the one associated to the development of skeletal muscle mass and its state of trophy. The second component (explaining another 20% of the total variance) can be interpreted as linked to the trophic and energetic/anabolic state of muscle tissue, where analytes such as BDNF, IRI and IGF1 show the highest values. PCA results are corroborated by the significance that emerged from the calculation of the correlation indices as described in Table 3.The pairwise correlations between ONC, MUS and FSTL-1 are high and highly significant ($p << 0.01$). MYO correlates moderately but significantly with FSTL-1 and ONC ($p < 0.05$) and more weakly with MUS. The close proximity of FSTL-1, MUS and ONC to MYO can be explained the physiological features of these three myokines. FSTL-1 is, like myostatin, a myokine that accentuates hypertrophy [33], specifically of the cardiac muscle and improves regeneration after heart attacks. It belongs to the Osteonectin protein family also known as SPARC (Secreted Protein Acidic and Rich in Cysteine). It is known that FSTL-1 is principally secreted by skeletal muscle cells, it is induced in skeletal and cardiac muscle by hypertrophic response and it has a protective effect on muscle vasculature [34]. Most important of all, FSTL-1 it is a MYO inhibitor as well as FOL [35].

ONC promotes bone formation [36]. Levels of ONC, which was initially identified in the bone, increase during muscle development and regeneration but also during en-durance exercise and muscle hypertrophy. The close proximity between ONC and MYO is not surprising when we consider that MYO itself is involved in the formation of osteoclasts and bone resorption [37]. MUS is an exercise responsive myokine that is known to improve exercise capacity and physical endurance [7]. Apart from the properties and functions that each of these myokines possesses, their correlation with myostatin suggests that inhibition of myostatin (evidenced by a reduction in its serum levels) may also affect the serum values of these three related-myokines. This circumstance is of considerable importance in the view to consider the myokines as effect biomarkers for anti-doping purposes.

CONCLUSIONS

In this pilot study, we examined a pool of human myokines to assess their variability in serum samples of professional athletes and their potential feasibility as biomarkers to reveal the manipulation of muscle cells regulators for doping purposes.Results can be summarized as follows:

– All myokines were assayable, although their levels in human serum varied by different magnitude in concentrations. Due to the limited availability of studies concerning specific myokines in sports, the concentration values of the myokines reported here can serve as a starting point to assess reference ranges by further studies, conducted on a broader population of athletes.

TABLE 3. Correlations among myokines and the other analytes calculated by Kendall's tau beta index. BDNF: Brain-derived Neurotrophic Factor; FSTL-1: Follistatin-like 1; ONC: Oncostatin; OST: Osteonectin; MUS: Musclin/Osteocrin; IRI: Irisin; FOL: Follistatin; MYO: Myostatin.

	BDNF	FSLT-1	ONC	OST	MUS	IRI	FOL	MYO
BDNF	-							
FSLT-1	0,05	-						
ONC	0,09	**0,46****	-					
OST	-0,05	0,11	0,03	-				
MUS	0,09	**0,50****	**0,56****	0,19	-			
IRI	0,20	-0,15	-0,23	0,21	-0,14	-		
FOL	-0,17	0,05	0,13	-0,02	0,10	-0,21	-	
MYO	-0,08	0,17	0,23*	0,04	0,13	-0,10	-0,05	-

* = significance ($p < 0.05$), ** = highly significant ($p < 0.01$).

– MYO serum concentration was found to be higher than FOL concentration. The MYO/FOL ratio showed a wide distribution that does not suggest its application to doping control analysis as a diagnostic paremeter for Myostatin inhibition. Individual and longitudinal-based follow up of the MYO/FOL ratio, as in the framework of the Athlete Biological Passport, also needs to be explored.
– A group of three myokines (MUS, FSTL-1 and ONC) clusters very close together with myostatin. This correlation makes these three myokines to be good candidates as potential biomarkers of inhibition practices operated on myostatin. Further studies, made also on a greater number of athletes and treated with myostatin inhibitors will further clarify the real extent of this correlation.

Results here obtained suggest that, although muscle damage due to very intense training could lead to a leakage of myokines into serum, the identification of a range of baseline levels, especially for MYO and for four of the target analytes here considered (FOL, FSTL-1, MUS and ONC), with the possibility of monitoring their variation longitudinally – as in the framework of the "Athlete biological Passport" – also following additional studies on their intra-individual variability, may be the starting point to develop a novel strategy to reveal the different forms of doping based on MYO inhibition/suppression.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Conflict of interest declaration

The authors declare no conflict of interest.

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Contemporary practices of Portuguese and Brazilian soccer coaches in designing and applying small-sided games

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ABSTRACT: This descriptive study aimed to investigate the current practices of Portuguese and Brazilian soccer coaches in the design and implementation of small-sided games (SSGs) in soccer. A total of 187 male coaches participated in the online survey, consisting of 82 Portuguese and 105 Brazilian individuals. These coaches held various positions within the technical staff, with 63 serving as head coaches, 38 as assistant coaches, 38 as physical trainers, and 48 in other roles related to the technical staff. Additionally, the participants represented both youth (n = 102) and adult competitive levels (n = 59), along with some who were not currently associated with a specific group. The survey consisted of 32 questions divided into three main sections: (i) the timing of SSG application, (ii) the methods used for applying SSGs, and (iii) the reasons for applying SSGs. The Chi-square test revealed a statistically significant association between nationality and the frequency of SSGs used in training sessions during the pre-season ($p = 0.039$) and in-season ($p < 0.001$). Moreover, significant association between nationality and the time allocated to employing SSGs for targeting aerobic training ($p < 0.001$) was found. There was a significant association between nationality and the weekly frequency of SSGs use for targeting sprint training ($p = 0.019$). The Chi-square test identified significant associations between nationality and the use of SSGs for targeting technical training ($p = 0.002$), as well as for tactical training ($p = 0.002$). In summary, this study underscores that SSGs are primarily employed to enhance aerobic fitness, change of direction, technical skills, and tactical behaviors. Coaches generally favor employing SSGs two to three times a week, with typical sessions lasting between 16 to 30 minutes. Notably, the major discrepancies between nationalities lie in the importance assigned to the use of SSGs. However, in practice, the formats and objectives for implementing SSGs remain relatively similar.

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INTRODUCTION

Small-sided games (SSGs) are drill-based exercises that aim to simplify the complexity of the formal game while utilizing task constraints to enhance players' perception of specific behaviors and objectives [1]. While often referred to as SSGs, it is more accurate to describe them as "small-sided and conditioned games," or simply "conditioned games." This terminology better encapsulates the coach's capacity to employ task constraints for shaping player behavior by adjusting rules and task objectives [1]. For instance, a seemingly conventional 11 vs. 11 game can undergo transformation into a conditioned game when the coach modifies the task objective (e.g., scoring in two goals instead of the regular one) or task rules (e.g., limiting players to two touches, reducing the pitch dimensions to half-field) [1,2]. However, due to the prevailing usage of SSGs in the literature as a general term, we will use "SSG" interchangeably with the more precise term "conditioned games."

These types of games have gained popularity in soccer training environments, mainly due to their ability to exaggerate the occurrence of desired behaviors while providing an intense form of exercise [3]. As a coach, there is the potential to manipulate task objectives, such as employing small goals or focusing on ball possession as the target. Additionally, rules of play can be adjusted, encompassing variations in the number of players involved, pitch size and configuration, limitations on intra- and inter-personal coordination (e.g., restricting actions like touches on the ball or prohibiting actions like dribbling), and time constraints (e.g., setting time limits for specific actions like shooting). These adaptable features provide a versatile framework for creating dynamic and purposeful games tailored to specific training objectives, harnessing task constraints to maximize the desired outcomes. Consequently, SSGs offer a comprehensive integration of various performance dimensions, encompassing tactical-technical [2,4,5], physical/physiological [6,7], and psychological/sociological aspects [8].

Due to their flexibility, SSGs offer numerous opportunities for researchers to explore the acute and chronic adaptations resulting from manipulating different constraints [9–11]. Over the past two decades, researchers have increasingly focused on investigating the effects of these modified constraints, as SSGs provide a rich training environment that offers diverse opportunities while ensuring that players are engaged in the specific dynamics of soccer [12–14]. This emphasis on specificity enhances the effectiveness of training and optimizes the chances of improving players' performance [6].

For instance, it is well-established that smaller formats, characterized by fewer players involved in the game, typically induce a significantly greater internal load compared to larger formats involving more players [15]. Conversely, expanding the pitch size to over 100 m^2 substantially increases the external load demands, including parameters such as total distance covered and distance covered at higher speeds, when compared to smaller pitches [16]. Furthermore, playing with a smaller number of participants significantly increases the frequency of technical actions, such as passes, ball receptions, and dribbles [5], as well as individual tactical behaviors like penetration and delaying tactics [17]. On the other hand, larger formats typically reduce the frequency of individual technical actions while enhancing collective behaviors such as mobility and the exploration of wider areas [18].

The acute responses mentioned above can ultimately influence chronic adaptations. For example, it is well-established that small-sided games, especially those with fewer participants like 1v1 to 4v4, are particularly effective for enhancing endurance performance, making them similarly effective to running-based high-intensity interval training [19] or conventional endurance training [6]. Conversely, research indicates that when using larger formats, SSGs may not be as effective as running-based high-intensity interval training for improving sprint performance or change-of-direction, as shown in various studies [7].

Interestingly, despite the extensive research and established nature of SSGs in scientific literature, there is limited literature [20] that specifically examines how frequently sports coaches incorporate these drills into their training regimens and the factors that influence their usage. Consequently, there is a lack of systematic analysis regarding how coaches have been integrating SSGs into their daily practice. Much of the existing research has primarily focused on experimental or descriptive studies, with a specific emphasis on assessing the acute or chronic effects of SSGs on players, rather than providing insights into the actual coaching practices surrounding these games.

In terms of the frequency of SSG usage in training scenarios, a descriptive quantitative study tracked the types of exercises employed by a coach over a period of five months [21]. The results showed that, on average, coaches dedicated approximately 19.8 ± 13.4 minutes of training sessions to small-sided formats (ranging from 1v1 to 5v5), and another 19.2 ± 7.4 minutes to large-sided formats (ranging from 6v6 to 11v11) of SSGs [21].

In order to gain deeper insights into the perspectives of coaches regarding SSGs, a qualitative study published in 2017 [22] conducted interviews with two experienced soccer coaches. The findings from these interviews indicated that coaches considered SSGs important due to their ability to effectively combine tactical principles and physical fitness components within the same exercise, resulting in positive performance transfer during matches. For instance, in a qualitative report, one coach expressed, "Through the implementation of small-sided games, I can effectively implement my game model with high intensity, addressing and refining the specific situations I have in mind, all while enhancing the players' skills." [22] Another coach mentioned, "These games enable me to assess whether my tactical ideas are being well-executed by the players. In these drills, the athletes have the opportunity to focus on aspects that are directly applicable to the game, doing so with intensity and at a pace that closely resembles actual match conditions." [22]

Furthermore, another qualitative study [20] focused on exploring how coaches design SSGs and align their design with their objectives in real practice scenarios. This study revealed that experienced coaches demonstrated a high level of skill in implementing task constraints that allowed for the observation of desired physiological stimuli during practice sessions [20]. Notably, there was a strong correlation ($r = 0.827$) between the estimated maximal heart rate of the coach and the actual maximal heart rate recorded in the players [20].

These studies [20–22] shed light on the usage and importance of SSGs in coaching practices. However, it is evident that further research is necessary to gain a comprehensive understanding of the various factors influencing the frequency and utilization of SSGs by coaches across different sports contexts. This includes considering factors related to different countries, which often have unique cultural aspects that impact training methods and approaches. Similar to studies conducted in the field of strength and conditioning [23], where practices related to physical fitness development and training load monitoring have been extensively described [24–26], a survey focusing on how coaches utilize SSGs is also required to enhance our understanding of who uses SSGs, why they are used, where they are implemented, and when they are incorporated into training sessions. This type of research has the potential to broaden our knowledge about how coaches and sports scientists perceive and utilize SSGs in their daily activities, providing valuable insights that can optimize their coaching practices.

Among various nations, the utilization of SSGs has notably thrived in Portugal and Brazil. In Portugal, the development of tactical periodization [27], alongside influential coaches like José Mourinho, has spurred the adoption of ecologically-based training exercises, leading to a significant rise in the interest and incorporation of SSGs into coaching practices. Consequently, there has been a concurrent expansion in the SSG-related literature. Brazil has closely followed this trend, as SSGs gained popularity as a foundational training approach for both youth and adult soccer players, aligning with the ecological exercise paradigm [28,29]. Given the established use of SSGs in both countries, it becomes particularly intriguing to explore and compare their practical applications within daily training sessions.

Consequently, the purpose of this study was to conduct a survey and examine the usage of SSGs in the context of soccer training. A quantitative approach was employed, utilizing questionnaires that were disseminated among coaches at various competitive levels in both Portuguese and Brazilian contexts. By collecting and analyzing this data, the study aimed to provide valuable insights into the utilization of SSGs, shed light on current practices, and offer recommendations to enhance the effectiveness of SSG implementation in soccer training programs. Additionally, the study will perform comparisons between the Portuguese and Brazilian contexts to assess how SSGs are implemented in these distinct realities.

This study offers a survey and characterization of the utilization of SSGs in daily coaching practices, employing a descriptive research design. This can prove invaluable for coaching federations and training institutions, as it provides insights into how coaches have been integrating these games into their training routines. It can also facilitate the evaluation and potential reformulation of coaching approaches, particularly if improper games have been employed in specific contexts or for certain objectives. Given its descriptive nature, the primary objective is to provide a comprehensive overview of how coaches are utilizing SSGs and to ascertain whether their use aligns with the latest scientific literature. Any identified gaps could serve as valuable feedback for coaching training centers, enabling them to enhance their educational content on this topic.

MATERIALS AND METHODS

Study design

This study utilized a cross-sectional study design to investigate and describe the current practices of Portuguese and Brazilian soccer coaches concerning the design and implementation of SSGs in soccer. The a priori sample size calculation utilized an effect size threshold of 0.3, which corresponds to a small effect size. This choice was made due to the absence of similar studies that could provide a mean difference between populations as a reference point. Additionally, a p-value of 0.05 and a power of 0.95 were established for the analysis. In terms of degrees of freedom, since the majority of studies involved a dichotomous questions comparing two populations (Portuguese and Brazilian), a value of 2 was assigned. Utilizing these specified parameters in the G*power software (version 3.1) for a chi-squared goodness-of-fit test (option of contingency tables), the recommended a priori sample size was determined to be 172.

A diverse group of coaches, including head coaches, assistant coaches, and physical trainers, were invited to participate in the survey and share their practices and perspectives regarding the utilization of SSGs in soccer training. The primary objective was to gain a comprehensive understanding of how SSGs are currently being designed and integrated into soccer training programs, aiming to identify prevailing trends and approaches employed by coaches in both Portugal and Brazil. The study adhered to ethical standards for research involving human subjects, in accordance with the principles outlined in the Declaration of Helsinki. Prior to participating in the survey, informed consent was obtained from all participants. It is important to note that participation was entirely voluntary, and coaches had the option to withdraw from the study at any point without facing any adverse consequences or penalties.

Setting

The online survey was administered using Google Forms and was available for participation from July 9, 2022, to July 18, 2022. The recruitment process for participants involved reaching out to coaches who expressed an interest in SSGs during a webinar held on July 9, 2022. Participants in this webinar were initially introduced to the fundamental concepts and significance of SSGs in a broad manner. This introductory session aimed to provide a comprehensive understanding of the terminologies and concepts associated with SSGs.

Subsequently, the coaches received a detailed clarification regarding these games and their significance before proceeding with the survey. Additionally, the survey link was shared through the researchers' networks, which included contacts in various soccer clubs and former students actively engaged in the sport.

To prioritize confidentiality and encourage candid responses, the survey was designed to be completely anonymous. This approach ensured that coaches could freely provide their feedback and insights in a secure and confidential manner, fostering an environment of openness and trust.

Participants

The survey successfully gathered responses from 187 participants, all from Portugal and Brazil, and there were no missing data points. The participant breakdown included 82 Portuguese men, and 105 Brazilian men. These coaches occupied various roles within the technical staff, with 63 individuals serving as head coaches, 38 as assistant coaches, 38 as physical trainers, and 48 in other capacities related to the technical staff, such as match analysts and physiologists. Furthermore, the participants encompassed both youth (n = 102) and adult competitive levels (n = 59), with some not currently affiliated with a specific team or coaching position.

Table 1 presents an insightful overview of the participants' characteristics and backgrounds. It includes information on their academic qualifications, certificate levels in soccer, years of experience in soccer, and their current involvement in competitive levels of the sport. This data provides valuable context for understanding the perspectives and experiences of the participants in relation to SSGs.

Small-sided games survey

For this study, a comprehensive survey was developed to investigate the use and value of SSGs. The survey's main domains, covering "when," "how," and "why" SSGs are used, were initially drafted by a team of three researchers, with questions based on existing literature.

To ensure the survey's quality and validity, three independent academic experts in SSGs, recognized soccer researchers according to ExpertScape, were invited to review the draft. Their valuable feedback led to revisions and improvements in the wording and structure of the questions.

Next, the revised survey was sent to three additional experts, consisting of four soccer coaches with a minimum UEFA B license. Among them, two had no academic background in sports sciences, while the other two did. Their perspectives were considered to further enhance the survey's content.

After incorporating feedback from both the academic experts and soccer coaches, the final version of the survey was sent back to the initial three academic experts for their final evaluation. Once their approval was obtained, the survey was presented as a supplementary file and launched online using Google Forms.

Participants were provided with a clear explanation of the survey's purpose, research design, and assurances of anonymity, confidentiality, and data protection before proceeding. Their voluntary participation and consent were confirmed before they completed the questionnaire.

The survey encompassed a demographic questionnaire comprising 18 closed questions to gather data on the participants' academic and professional backgrounds. In the "when" domain, 14 questions were presented to explore the frequency of Small-Sided Games (SSGs) implementation during the season, within a training week, and within individual training sessions. The "how" domain consisted of 43 questions aimed at identifying the primary objectives of using SSGs and how coaches select specific task constraints to align with these objectives. Lastly, the "why" domain featured 15 questions focused on understanding the perceived importance of utilizing SSGs for different training objectives. For the original version of the questionnaire, please refer to the supplementary file 1.

It is crucial to provide clarity regarding the terminology used in this survey. SSGs were broadly defined and explained to the participating coaches as drill-based games that maintain the dynamics of the actual game while incorporating task constraints and simplifying the format of the official game. However, it is worth noting that the more accurate term for these activities would be "conditioned games," as it comprehensively describes their nature [1]. Nevertheless, due to the widespread use and popularity of the term "SSGs," it was used interchangeably with the term "conditioned games" in this survey. Hence, irrespective of the particular format of play employed (e.g., 1v1 without goals, 5v5 with regular goals, 10v10 on half the pitch), all these activities were collectively categorized under the umbrella term of SSGs. This categorization was based on their shared design, which incorporates task constraints aimed at emphasizing specific behaviors and achieving desired player outcomes while preserving the fundamental dynamics of the game.

Data acquisition and statistical procedures

The data gathered from the Google Forms survey was exported and compiled into an Excel file for analysis. For fixed response questions, a frequency analysis was conducted, which involved examining the distribution and frequency of different response options. This allowed for a quantitative assessment of the participants' choices and preferences within the predefined response categories.

In the inferential analysis, the Chi-square test was employed to investigate potential relationships between two categorical variables, such as nationality and the survey question. When dealing with ordinal scales, the Mann-Whitney U test was utilized to assess differences between nationalities. These statistical tests were conducted using SPSS software (version 28.0.0.0, IBM, Chicago, USA), with statistical significance set at a p-value < 0.05.

RESULTS

Table 2 presents information regarding the usage of SSGs in Portugal and Brazil, specifically focusing on the pre-season and in-season periods. No significant differences were found between the number

TABLE 1. Demographic characterization of survey respondents.

	Portuguese	Brazilian
	Men (N = 82)	**Men (N = 105)**
Relationship with soccer		
Soccer player or former soccer player	72 (87.8%)	57 (54.3%)
Age		
18–25 years old	27 (32.9%)	34 (32.4%)
26–30 years old	21 (25.6%)	16 (15.2%)
31–35 years old	13 (15.9%)	11 (10.5%)
36–40 years old	9 (11.0%)	16 (15.2%)
41–45 years old	4 (4.9%)	15 (14.3%)
46–50 years old	6 (7.3%)	4 (3.8%)
51–55 years old	1 (1.2%)	2 (1.9%)
> 55 years old	1 (1.2%)	7 (6.7%)
Education		
Basic education (1st to 9th school years)	0 (0%)	0 (0%)
Secondary school (10th to 12th school years)	23 (28.0%)	9 (8.6%)
Graduation	29 (35.4%)	46 (43.8%)
Post-graduation/specialization	6 (7.3%)	27 (25.7%)
Master degree	21 (25.6%)	15 (14.3%)
Ph.D. degree	3 (3.7%)	8 (7.6%)
Graduation is related with sports sciences and/or physical education	67 (81.7%)	102 (97.1%)
Role in soccer		
Head coach	27 (32.9%)	36 (34.3%)
Assistant coach	23 (28.0%)	15 (14.3%)
Physical trainer	16 (19.5%)	22 (21%)
Physiologist	0 (0%)	5 (4.8%)
Match analyst	3 (3.7%)	5 (4.8%)
Other	13 (15.9%)	22 (21%)
Soccer License level		
UEFA “C” level	26 (31.7%)	1 (1.0%)
UEFA “B” level	18 (22.0%)	0 (0%)
UEFA “A” level	5 (6.1%)	4 (3.8%)
UEFA “Pro” level	1 (1.2%)	1 (1.0%)
CBF “C” level	0 (0%)	3 (2.9%)
CBF “B” level	0 (0%)	4 (3.8%)
CBF “A” level	0 (0%)	2 (1.9%)
CBF “Pro” level	0 (0%)	0 (0%)
Experience in soccer		
0–5 years of experience	34 (41.5%)	52 (49.5%)
6–10 years of experience	23 (28.0%)	12 (11.4%)
11–15 years of experience	4 (4.9%)	10 (9.5%)
16–20 years of experience	4 (4.9%)	4 (3.8%)
21–25 years of experience	1 (1.2%)	3 (2.9%)
> 25 years of experience	1 (1.2%)	6 (5.7%)
Current practice (competitive level)		
5 to 10 years old	12 (14.6%)	12 (11.4%)
11 to 13 years old	11 (13.4%)	21 (20.0%)
14 to 16 years old	13 (15.9%)	15 (14.3%)
17 to 19 years old	8 (9.8%)	10 (9.5%)
Adults (amateurs)	15 (18.3%)	18 (17.1%)
Adults (professionals)	12 (14.6%)	14 (13.3%)

TABLE 2. Characterization of the application timing ("when") of small-sided games in soccer training during the pre-season.

	Pre-season	Pre-season	In-season	In-season
	Brazilian (n = 105)	**Portuguese (n = 82)**	**Brazilian (n = 105)**	**Portuguese (n = 82)**
How many field training sessions per week does the team you manage have?				
1	2 (2%)	0 (0%)	3 (3%)	0 (0%)
2	12 (11%)	11 (13%)	30 (29%)	10 (12%)
3	15 (14%)	19 (23%)	15 (14%)	36 (44%)
4	14 (13%)	17 (21%)	15 (14%)	16 (20%)
5	13 (12%)	18 (22%)	22 (21%)	9 (11%)
6	13 (12%)	5 (6%)	9 (9%)	4(5%)
7	4 (4%)	1 (1%)	3 (3%)	0 (0%)
> 7	9 (9%)	4 (5%)	4 (4%)	0 (0%)
Not applicable	14 (13%)	7 (9%)	14 (13%)	7 (9%)
In how many of your weekly training sessions do you use SSGs?				
1	5 (5%)	3 (4%)	6 (6%)	2 (2%)
2	23 (22%)	14 (17%)	24 (23%)	21 (26%)
3	14 (13%)	24 (29%)	15 (14%)	34 (41%)
4	11 (10%)	15 (18%)	19 (18%)	14 (17%)
5	14 (13%)	3 (4%)	18 (17%)	4 (5%)
6	2 (2%)	0 (0%)	5 (5%)	0 (0%)
7	1 (1%)	0 (0%)	0 (0%)	0 (0%)
> 7	0 (0%)	0 (0%)	4 (4%)	0 (0%)
All the sessions	22 (21%)	7 (9%)	0 (0%)	0 (0%)
Not applicable	13 (12%)	16 (20%)	14 (13%)	7 (9%)
On average, how long does your training session last?				
0–30 min	7 (7%)	1 (1%)	9 (9%)	5 (6%)
31–60 min	27 (26%)	17 (21%)	43 (41%)	22 (27%)
61–90 min	50 (48%)	53 (65%)	35 (33%)	45 (55%)
> 90 min	8 (8%)	4 (5%)	4 (4%)	3 (4%)
Not applicable	13 (12%)	7 (9%)	14 (13%)	7 (9%)
How much time do you dedicate to the use of SSGs in your training session?				
0–15 min	4 (4%)	3 (4%)	7 (7%)	4 (5%)
16–30 min	36 (34%)	38 (46%)	35 (33%)	35 (43%)
31–45 min	24 (23%)	20 (24%)	27 (26%)	22 (27%)
46–60 min	22 (21%)	9 (11%)	18 (17%)	10 (12%)
61–75 min	3 (3%)	4 (5%)	2 (2%)	4 (5%)
> 75 min	3 (3%)	1 (1%)	2 (2%)	0 (0%)
Not applicable	13 (12%)	7 (9%)	14 (13%)	7 (9%)
How often do you use SSGs in your warm-up routine on a weekly basis?				
Never	4 (4%)	5 (6%)	4 (4%)	6 (7%)
< half of the number of sessions	29 (28%)	29 (35%)	33 (31%)	35 (35%)
> half of the number of sessions	36 (34%)	33 (40%)	39 (37%)	27 (33%)
All the sessions	24 (23%)	8 (10%)	15 (14%)	7 (9%)
How often do you integrate SSGs in the main part of your training sessions (from the end of the warm-up until the start of the cool-down)?				
Never	2 (2%)	1 (1%)	4 (4%)	1 (1%)
< half of the number of sessions	22 (21%)	23 (28%)	25 (24%)	24 (29%)
> half of the number of sessions	48 (46%)	39 (48%)	44 (42%)	39 (48%)
All the sessions	21 (20%)	12 (15%)	17 (16%)	11 (13%)
How often do you use SSGs during the cool-down phase on a weekly basis?				
Never	37 (35%)	32 (39%)	35 (33%)	31 (38%)
< half of the number of sessions	26 (25%)	30 (37%)	27 (26%)	35 (43%)
> half of the number of sessions	19 (18%)	11 (13%)	23 (22%)	6 (7%)
All the sessions	9 (9%)	2 (2%)	3 (3%)	2 (2%)

TABLE 3. Characterization of the application methods ("how") of small-sided games in soccer training – improving physical fitness

	To develop aerobic fitness	To develop aerobic fitness	To develop sprint performance	To develop sprint performance	To develop change of direction	To develop change of direction
	Brazilian (n = 105)	**Portuguese (n = 82)**	**Brazilian (n = 105)**	**Portuguese (n = 82)**	**Brazilian (n = 105)**	**Portuguese (n = 82)**
Do you employ SSGs as a training method focused on the development of abilities?						
Yes	83 (79%)	59 (72%)	59 (56%)	32 (39%)	80 (76%)	54 (66%)
No	11 (10%)	17 (21%)	32 (30%)	43 (52%)	14 (13%)	23 (28%)
Not applicable	11 (10%)	6 (7%)	12 (11%)	7 (9%)	11 (10%)	5 (6%)
If yes, what types of game formats do you most frequently use for the development of aerobic fitness?						
1v1 to 4v4	29 (28%)	28 (34%)	41 (39%)	23 (28%)	48 (46%)	32 (39%)
5v5 to 8v8	17 (16%)	6 (7%)	9 (9%)	4 (5%)	8 (8%)	5 (6%)
8v8 to 11v11	1 (1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1%)
Combining more than one group of formats	36 (34%)	25 (30%)	9 (9%)	5 (6%)	24 (23%)	16 (20%)
Regarding the previous formats, do you use them most frequently?						
Formats with numerical balance (e.g., 2v2)	21 (20%)	16 (20%)	21 (20%)	9 (11%)	20 (19%)	23 (28%)
Formats with numerical imbalance (e.g., 3v2; 2v2+2) using jokers/wildcards for whom attacking	47 (45%)	35 (43%)	32 (30%)	23 (28%)	48 (46%)	22 (27%)
Formats with numerical imbalance (e.g., 3v2; 2v2+2) using jokers/wildcards for whom defending	15 (14%)	8 (10%)	6 (6%)	0 (0%)	12 (11%)	9 (11%)
When do you intend to use SSGs for the development of the ability how much training session time do you allocate to this objective?						
0–10 min	6 (6%)	10 (12%)	16 (15%)	16 (20%)	18 (17%)	12 (15%)
11–20 min	27 (26%)	34 (41%)	29 (28%)	14 (17%)	35 (33%)	30 (37%)
21–30 min	41 (39%)	10 (12%)	13 (12%)	2 (2%)	25 (24%)	11 (13%)
> 35 min	9 (9%)	5 (6%)	1 (1%)	0 (0%)	2 (2%)	1 (1%)
How many times per week (number of sessions per week) do you intend to use SSG for the development of the ability?						
1–2	48 (46%)	43 (52%)	40 (38%)	30 (37%)	54 (51%)	42 (51%)
3–4	33 (31%)	15 (18%)	17 (16%)	2 (2%)	25 (24%)	12 (15%)
5–7	2 (2%)	1 (1%)	2 (2%)	0 (0%)	1 (1%)	0 (0%)

of training sessions per week during pre-season (U = 2849.5; Z = -0.229; p = 0.819) and in-season (U = 2850.0; Z = -1.423; p = 0.155) among nationalities while using the Mann-Whitney U test.

The Chi-square test revealed a statistically significant association between nationality and the frequency of SSGs used in training sessions during the pre-season ($X_{(8)}$ = 16.218; p = 0.039) and in-season ($X_{(7)}$ = 28.165; p < 0.001). Regarding the duration of training sessions, no statistically significant association with nationality was found in the pre-season ($X_{(4)}$ = 7.275; p = 0.122) and in-season ($X_{(4)}$ = 8.960; p = 0.062). The Chi-square test showed no statistically significant association between nationality and the duration of SSGs per session during the pre-season ($X_{(6)}$ = 6.220; p = 0.399) and in-season ($X_{(6)}$ = 5.874; p = 0.437). The Chi-square test also revealed no statistically significant association between nationality and the frequency of SSG use during the warm-up phase in the pre-season ($X_{(4)}$ = 6.832; p = 0.145) and in-season ($X_{(4)}$ = 5.132; p = 0.274). Similarly, the Chi-square test indicated no statistically significant association between nationality and the frequency of SSG use during the main part of training in the pre-season ($X_{(4)}$ = 2.262; p = 0.688) and in-season ($X_{(4)}$ = 3.541; p = 0.472).

Although there were no statistically significant associations between nationality and the frequency of SSG use during the cool-down phase of training in the pre-season ($X_{(4)} = 6.844$; $p = 0.144$), significant interactions were observed in the in-season data ($X_{(4)} = 12.033$; $p = 0.017$).

Table 3 presents the frequency of answers regarding how SSGs are used for developing physical fitness. The Chi-square test did not identify any statistically significant association between nationality and the use of SSGs for targeting aerobic training ($X_{(1)} = 4.475$; $p = 0.062$). However, significant interactions were observed for sprint ($X_{(1)} = 7.217$; $p = 0.007$) and change-of-direction ($X_{(1)} = 5.599$; $p = 0.018$).

Similarly, the Chi-square test did not reveal any statistically significant association between nationality and the choice of SSGs formats for targeting aerobic training ($X_{(3)} = 4.329$; $p = 0.228$), sprint training ($X_{(2)} = 0.129$; $p = 0.938$), or change-of-direction ($X_{(3)} = 1.504$; $p = 0.681$).

Furthermore, there was no statistically significant association between nationality and the numerical relationships used in SSGs for targeting aerobic training ($X_{(2)} = 0.521$; $p = 0.771$), sprint training ($X_{(2)} = 4.673$; $p = 0.097$), or change-of-direction ($X_{(2)} = 5.456$; $p = 0.065$).

However, the Chi-square test did reveal a statistically significant association between nationality and the time allocated to employing SSGs for targeting aerobic training ($X_{(3)} = 18.254$; $p < 0.001$). No significant interactions were found for sprint ($X_{(3)} = 6.895$; $p = 0.075$) and change-of-direction ($X_{(3)} = 2.408$; $p = 0.492$).

Lastly, there was no statistically significant association between nationality and the weekly frequency of SSGs use for targeting aerobic training ($X_{(2)} = 3.399$; $p = 0.183$) and change-of-direction ($X_{(2)} = 2.102$; $p = 0.350$). However, significant associations were observed for sprint training ($X_{(2)} = 7.960$; $p = 0.019$).

Table 4 provides insight into how Portuguese and Brazilian respondents use SSGs for developing technical skills and tactical behaviors. The Chi-square test identified statistically significant associations between nationality and the use of SSGs for targeting technical training ($X_{(1)} = 9.748$; $p = 0.002$), as well as for tactical training ($X_{(1)} = 9.644$; $p = 0.002$).

Similarly, a statistically significant association was observed between nationality and the choice of SSGs formats for targeting technical training ($X_{(3)} = 8.663$; $p = 0.034$). However, no statistical interactions were found for tactical training ($X_{(3)} = 3.596$; $p = 0.308$).

Moreover, there was a statistically significant association between nationality and the numerical relationships employed in SSGs for targeting technical training ($X_{(2)} = 9.363$; $p = 0.009$). Nevertheless, no significant interactions were detected for tactical training ($X_{(2)} = 1.840$; $p = 0.398$).

Furthermore, a statistically significant association was found between nationality and the time allocated for employing SSGs in technical training ($X_{(3)} = 13.791$; $p = 0.003$). However, no significant association was observed for tactical training ($X_{(3)} = 4.887$; $p = 0.180$).

Lastly, a statistically significant association existed between nationality and the weekly frequency of SSGs use for technical training ($X_{(2)} = 6.412$; $p = 0.041$), whereas no significant association was found for tactical training ($X_{(2)} = 0.814$; $p = 0.666$).

Table 5 provides an overview of how SSGs are developed with different types of fields and task objectives, aiming to target various capacities in players. Important to disclose that penetration games involve objectives such as overcoming a line through dribbling, effectively implementing the penetration principle of play. This principle entails players facing their direct opponents, attempting to bypass them through dribbling, and advancing towards the target. The Chi-square test did not indicate any statistically significant association between nationality and pitch size for the purpose of increasing physiological stimulus ($X_{(1)} = 0.906$; $p = 0.341$), acceleration and deceleration ($X_{(1)} = 0.180$; $p = 0.672$), and developing technical actions ($X_{(1)} = 0.171$; $p = 0.679$). However, it did reveal significant associations when it came to increasing sprint ($X_{(1)} = 5.136$; $p = 0.023$) and developing tactical behaviors ($X_{(1)} = 9.056$; $p = 0.003$).

Similarly, the Chi-square test showed no statistically significant association between nationality and pitch size for increasing physiological stimulus ($X_{(4)} = 4.485$; $p = 0.344$), sprint ($X_{(4)} = 2.630$; $p = 0.622$), acceleration and deceleration ($X_{(4)} = 1.698$; $p = 0.791$), and developing tactical behaviors ($X_{(4)} = 2.598$; $p = 0.627$). However, it did reveal a significant association concerning technical development ($X_{(4)} = 13.554$; $p = 0.009$).

Table 6 illustrates the perceived relevance of using SSGs for developing different capacities. The Chi-square test indicate statistically significant association between nationality and the importance of SSGs for developing aerobic fitness ($X_{(4)} = 9.689$; $p = 0.046$), muscular power ($X_{(4)} = 11.907$; $p = 0.018$), sprint ($X_{(4)} = 27.318$; $p < 0.001$), change-of-direction ($X_{(4)} = 22.642$; $p < 0.001$), agility ($X_{(4)} = 22.055$; $p < 0.001$), offensive technical skills ($X_{(4)} = 15.891$; $p < 0.001$), defensive technical skills ($X_{(4)} = 19.013$; $p < 0.001$), offensive tactical behaviors ($X_{(4)} = 12.289$; $p = 0.015$), defensive tactical behaviors ($X_{(4)} = 11.185$; $p = 0.025$) and game model ($X_{(4)} = 16.818$; $p = 0.002$). No significant associations were found regarding SSG's importance to maximum strength development ($X_{(4)} = 2.618$; $p = 0.624$).

DISCUSSION

The present research aimed to characterize the usage and application of small-sided games (SSGs) in soccer by coaches from Portugal and Brazil. Out of a total sample of 201 coaches, it was found that the majority of them implement SSGs two to three times a week in both pre-season and in-season training periods. These SSG drills typically last between 16 to 30 minutes, as reported by most respondents.

Most coaches stated that they primarily incorporate SSGs during warm-up and the main part of their training sessions. Additionally, the study revealed that coaches often use SSGs to target aerobic

fitness and change-of-direction, while not as frequently for sprinting. However, a significant majority reported using SSGs for stimulating technical and tactical training.

When examining the formats of SSGs, it was evident that coaches preferred smaller formats (1v1 to 4v4) for targeting aerobic fitness, change of direction, and technical skills. Conversely, medium formats (5v5 to 8v8) were more favored for developing tactical behaviors. Furthermore, coaches tended to opt for larger fields when seeking to stimulate sprinting actions and tactical training, while smaller fields were chosen for enhancing aerobic fitness, change of direction, and technical abilities.

In terms of task objectives, ball possession was the most preferred for increasing physiological stimulus, accelerations, decelerations, and technical skills. On the other hand, penetration games, where players must overcome lines for scoring, were favored for stimulating sprinting actions. Additionally, objectives using regular goals and a goalkeeper were the most frequently chosen for promoting tactical behaviors during SSGs.

When are small-sided games employed?

The popularity of SSGs in these countries can be attributed to various factors, including the influence of tactical periodization [27,30], a well-known training approach in Portugal, which emphasizes the use of drill-based games like SSGs to enhance player development across multiple levels. As a result, it is understandable that coaches frequently integrate SSGs into their training routines, although not necessarily in every session.

The substantial use of SSGs in training sessions aligns with the findings of a previous study that examined the training process of a professional soccer team over half of a season [21]. In that

TABLE 4. Characterization of the application methods ("how") of small-sided games in soccer training – improving technical skills and tactical behaviors

	To develop technical skills	To develop technical skills	To develop tactical behaviors	To develop tactical behaviors
	Brazilian (n = 105)	**Portuguese (n = 82)**	**Brazilian (n = 105)**	**Portuguese (n = 82)**
Do you employ SSGs as a training method focused on the development of abilities?				
Yes	90 (86%)	58 (71%)	88 (84%)	57 (70%)
No	6 (6%)	17 (21%)	6 (6%)	17 (21%)
Not applicable	9 (9%)	7 (9%)	11 (10%)	8 (10%)
If yes, what types of game formats do you most frequently use for the development of aerobic fitness?				
1v1 to 4v4	41 (39%)	40 (49%)	11 (10%)	8 (10%)
5v5 to 8v8	6 (6%)	1 (1%)	11 (10%)	6 (7%)
8v8 to 11v11	1 (1%)	0 (0%)	5 (5%)	0 (0%)
Combining more than one group of formats	42 (40%)	17 (21%)	61 (58%)	43 (52%)
Regarding the previous formats, do you use them most frequently?				
Formats with numerical balance (e.g., 2v2)	30 (29%)	34 (41%)	32 (30%)	25 (30%)
Formats with numerical imbalance (e.g., 3v2; 2v2+2) using jokers/wildcards for whom attacking	50 (48%)	19 (23%)	42 (40%)	27 (33%)
Formats with numerical imbalance (e.g., 3v2; 2v2+2) using jokers/wildcards for whom defending	10 (10%)	5 (6%)	14 (13%)	5 (6%)
When do you intend to use SSGs for the development of the ability how much training session time do you allocate to this objective?				
0–10 min	13 (12%)	15 (18%)	9 (9%)	7 (9%)
11–20 min	35 (33%)	33 (40%)	20 (19%)	19 (23%)
21–30 min	35 (33%)	9 (11%)	38 (36%)	25 (30%)
> 35 min	7 (7%)	1 (1%)	21 (20%)	6 (7%)
How many times per week (number of sessions per week) do you intend to use SSG for the development of the ability?				
1–2	50 (48%)	40 (49%)	44 (42%)	29 (35%)
3–4	32 (30%)	18 (22%)	40 (38%)	27 (33%)
5–7	8 (8%)	0 (0%)	4 (4%)	1 (1%)

TABLE 5. Characterization of the task objectives used ("how") for physiological/physical and technical/tactical stimulus

	To increase physiological stimulus	To increase physiological stimulus	To increase the running speed	To increase the running speed	To increase accelerations and decelerations
	Brazilian (n = 105)	**Portuguese (n = 82)**	**Brazilian (n = 105)**	**Portuguese (n = 82)**	**Brazilian (n = 105)**
Use bigger fields (> 1/8 of the field)	43 (41%)	28 (34%)	75 (71%)	70 (85%)	39 (37%)
Use smaller fields (< 1/8 of the field)	62 (59%)	54 (66%)	30 (29%)	12 (15%)	66 (63%)
Which task objective do you often use?					
Ball possession	50 (48%)	32 (39%)	13 (12%)	11 (13%)	29 (28%)
Penetration zones	29 (28%)	18 (22%)	63 (60%)	52 (63%)	26 (25%)
Small goals	15 (14%)	19 (23%)	13 (12%)	7 (9%)	28 (27%)
Youth size goals+GK	7 (7%)	9 (11%)	4 (4%)	6 (7%)	13 (12%)
Regular goals+GK	4 (4%)	4 (5%)	12 (11%)	6 (7%)	9 (9%)

	To increase accelerations and decelerations	To develop technical skills	To develop technical skills	To develop tactical behaviors	To develop tactical behaviors
	Portuguese (n = 82)	**Brazilian (n = 105)**	**Portuguese (n = 82)**	**Brazilian (n = 105)**	**Portuguese (n = 82)**
Use bigger fields (> 1/8 of the field)	28 (34%)	35 (33%)	25 (30%)	80 (76%)	76 (93%)
Use smaller fields (< 1/8 of the field)	54 (66%)	70 (67%)	57 (70%)	25 (24%)	6 (7%)
Which task objective do you often use?					
Ball possession	23 (28%)	55 (52%)	36 (44%)	34 (32%)	20 (24%)
Penetration zones	19 (23%)	11 (10%)	16 (20%)	15 (14%)	9 (11%)
Small goals	22 (27%)	13 (12%)	20 (24%)	6 (6%)	5 (6%)
Youth size goals+GK	7 (9%)	12 (11%)	8 (10%)	9 (9%)	10 (12%)
Regular goals+GK	11 (13%)	14 (13%)	2 (2%)	41 (39%)	38 (46%)

GK: goalkeeper

study [21], it was revealed that SSGs, in various formats, accounted for up to 39 minutes of the total training session time. This parallel supports the idea that SSGs have become an integral part of modern soccer training programs, playing a crucial role in developing players' skills.

Considering the use of SSGs during warm-up routines can be attributed to their positive effects on athletic performance, particularly in terms of reactive agility, lower limb power, and sprint performance. Previous studies [31,32] have shown that incorporating SSGs into warm-up strategies can lead to improved acute athletic responses, which can be highly beneficial for preparing players for the demands of the subsequent training session or match.

By introducing technical and tactical challenges during the warm-up phase, SSGs not only enhance the physical preparedness of players but also help to stimulate decision-making abilities early on in the training session [33]. This can be especially advantageous for players as they transition from warm-up to the main part of the training session, as they are already mentally and physically primed to engage in game-like scenarios.

During the main part of the training session, which occurs after the warm-up and before the cool down, it was evident from the survey that SSGs are widely implemented by coaches from both Brazil and Portugal, regardless of the phase of the season. In fact, in more than half of the training sessions, SSGs are utilized, indicating their versatility and potential to address various training objectives.

The flexibility of SSGs allows coaches to design training sessions with specific aims in mind. These games can be employed to develop technical skills [5], hone tactical behaviors [4], or enhance physical fitness [6]. The existing literature supports the positive adaptations that can be achieved through the use of SSGs, providing a sound rationale for coaches' inclination to incorporate them into their training routines.

The popularity of SSGs in the main part of training sessions can be attributed to their effectiveness in creating game-like scenarios [1], fostering decision-making abilities, and promoting team cohesion. The dynamic and competitive nature of these games provides an engaging and challenging environment for players, contributing to their overall development.

The widespread use of SSGs in the main part of training sessions reflects the growing recognition of their efficacy among coaches. As the body of evidence supporting the benefits of SSGs continues to grow [3,6], it is likely that coaches will continue to integrate these games into their training methodologies.

Why are small-sided games chosen?

While Brazilian coaches largely emphasize that SSGs are crucial for developing attacking and defensive tactical principles, the majority of Portuguese coaches consider them important but not very important. Similarly, most Brazilian coaches view SSGs as very important for improving aerobic fitness and agility, whereas Portuguese coaches consider them important but not of the same high importance. These disparities in perceived importance may reflect variations in coaching philosophies, training methods, or tactical approaches in each country's soccer culture.

Indeed, the observed differences between countries in the perceived importance of SSGs for specific components may be attributed to various factors, including differences in coaching education, available literature, or access to alternative training methods. However, it is noteworthy that SSGs are consistently considered important to very important for developing aerobic fitness, agility, technical skills, and tactical behaviors. This alignment with existing literature [6,34,35] highlights that coaches are well-informed about the advantages of using SSGs and are in line with recent evidence supporting their effectiveness.

Conversely, for improving strength, power, or sprint performance, coaches express less confidence in the utility of SSGs. This stance is also in line with existing evidence, which often suggests the superiority of other training methods for these specific physical qualities [7]. Coaches seem to recognize the need for more specialized and time-efficient approaches to target these capacities effectively, which may not be optimally addressed through SSGs alone.

How are small-sided games implemented?

The existing literature on SSGs' ability to improve aerobic fitness and endurance performance has been steadily growing [6], with studies suggesting that SSGs can be as effective as continuous or intermittent running-based exercises in enhancing aerobic fitness. This finding has led to the popularity of SSGs, as they enable coaches to combine technical and tactical stimulus with an appropriate aerobic workout [15]. Particularly, the smaller formats of SSGs tend to induce heart rate responses exceeding 85% of maximal heart rate [36], making them effective in providing a significant aerobic stimulus. These findings offer valuable insights into the reasons provided by coaches, with 71.3% of Portuguese and 77.2% of Brazilian coaches expressing their positive use of SSGs for developing specific physical components. The high percentage of coaches employing SSGs for this purpose aligns with the increasing body of evidence supporting the effectiveness of these drills in enhancing aerobic fitness and other physical capacities.

The coaches' preference for using small formats of play (1v1 to 4v4) for 11 to 20 minutes with imbalance formats to stimulate aerobic fitness aligns with the existing literature on the subject. The adoption of these specific training approaches by coaches indicates their awareness of the optimal conditions required to effectively target and enhance aerobic power in their players.

Smaller formats of play, such as 1v1 to 4v4, offer several advantages for improving aerobic fitness [15]. The reduced playing area and the increased number of high-intensity actions, including sprints, accelerations, and decelerations, create a more intense and demanding physical workload for the players [37].

Additionally, the use of imbalance formats, where one team has numerical superiority over the other, further intensifies the aerobic demands on the players [38]. The team with fewer players is required to cover more ground and engage in more frequent bursts of high-intensity movements, increasing the overall metabolic demand. Moreover, the coaches' selection of an 11 to 20-minute duration for SSGs is well-founded. Research suggests [6] that this timeframe is adequate for eliciting physiological adaptations and promoting positive changes in aerobic fitness.

It is noteworthy that coaches frequently employ smaller formats (1v1 to 4v4) of SSGs to stimulate change of direction in soccer players. The rationale behind this choice is rooted in the potential benefits of exposing players to the challenges of frequent changes of direction that occur in these game scenarios. While the heterogeneity of the number of accelerations, decelerations, and changes of direction in soccer is considerable [39], and some improvements in agility may be attributed to enhanced reaction time rather than a direct physical ability to change direction [40], studies have suggested that small-sided games can be effective forms of training to develop this skill [33].

Despite the lack of consistent research evidence specifically comparing changes of direction in small-sided games to multidirectional accelerations or sprints [35], coaches may believe that the specific demands of small formats can still contribute to the development of change-of-direction abilities in players. Indeed, agility is a multifaceted skill that encompasses perceptual-cognitive abilities, decision-making, and physical attributes. While small-sided games may not specifically target all aspects of agility, the repetitive exposure to rapid changes of direction in these game scenarios can still be beneficial for players. Coaches' preference for using SSGs for change-of-direction training is likely based on the belief that regular exposure to such dynamic movements can lead to adaptations and improved efficiency in executing agile actions on the field.

Among the various physical qualities analyzed, coaches expressed less frequency in SSGs to improve linear sprint performance. This

TABLE 6. Characterization of the reasons ("why") for using small-sided games in soccer training

	Brazilian (n = 105)	**Brazilian (n = 105)**	**Brazilian (n = 105)**	**Brazilian (n = 105)**	**Brazilian (n = 105)**
	Not Important	**Slightly Important**	**Moderately Important**	**Important**	**Very Important**
Importance of SSGs for the Development of Aerobic Fitness	2 (2%)	13 (12%)	6 (6%)	35 (33%)	49 (47%)
Importance of SSGs for the Development of Maximum Strength	9 (9%)	19 (18%)	19 (18%)	30 (29%)	28 (27%)
Importance of SSGs for the Development of Muscular Power	8 (8%)	12 (11%)	21 (20%)	32 (30%)	32 (30%)
Importance of SSGs for the Development of Maximum Running Speed (Sprint)	3 (3%)	20 (19%)	11 (10%)	30 (29%)	41 (39%)
Importance of SSGs for the Development of Change of Direction Abilities	3 (3%)	11 (10%)	9 (9%)	20 (19%)	62 (59%)
Importance of SSGs for the Development of Agility	2 (2%)	14 (13%)	4 (4%)	29 (28%)	56 (53%)
Importance of SSGs for the Development of Offensive Technical Skills (e.g., passing, receiving, shooting)	0 (0%)	10 (10%)	6 (6%)	21 (20%)	68 (65%)
Importance of SSGs for the Development of Defensive Technical Skills (e.g., tackling, intercepting)	0 (0%)	12 (11%)	3 (3%)	24 (23%)	66 (63%)
Importance of SSGs for the Development of Offensive Tactical Behaviors (e.g., penetration, offensive coverage, mobility, width)	2 (2%)	11 (10%)	5 (5%)	25 (24%)	62 (59%)
Importance of SSGs for the Development of Defensive Tactical Behaviors (e.g., defensive containment, defensive coverage, balance, concentration)	1 (1%)	10 (10%)	5 (5%)	29 (28%)	60 (57%)
Importance of SSGs for the Development of the Game Model	1 (1%)	14 (13%)	6 (6%)	29 (28%)	55 (52%)
	Portuguese (n = 82)	**Portuguese (n = 82)**	**Portuguese (n = 82)**	**Portuguese (n = 82)**	**Portuguese (n = 82)**
	Not Important	**Slightly Important**	**Moderately Important**	**Important**	**Very Important**
Importance of SSGs for the Development of Aerobic Fitness	3 (4%)	8 (10%)	7 (9%)	42 (51%)	22 (27%)
Importance of SSGs for the Development of Maximum Strength	10 (12%)	14 (17%)	19 (23%)	24 (29%)	15 (18%)
Importance of SSGs for the Development of Muscular Power	1 (1%)	11 (13%)	11 (13%)	42 (51%)	17 (21%)
Importance of SSGs for the Development of Maximum Running Speed (Sprint)	5 (6%)	12 (15%)	27 (33%)	29 (35%)	9 (11%)
Importance of SSGs for the Development of Change of Direction Abilities	0 (0%)	3 (4%)	16 (20%)	34 (41%)	29 (35%)
Importance of SSGs for the Development of Agility	0 (0%)	8 (10%)	9 (11%)	44 (54%)	21 (26%)
Importance of SSGs for the Development of Offensive Technical Skills (e.g., passing, receiving, shooting)	0 (0%)	3 (4%)	11 (13%)	33 (40%)	35 (43%)
Importance of SSGs for the Development of Defensive Technical Skills (e.g., tackling, intercepting)	1 (1%)	5 (6%)	8 (10%)	37 (45%)	31 (38%)
Importance of SSGs for the Development of Offensive Tactical Behaviors (e.g., penetration, offensive coverage, mobility, width)	0 (0%)	5 (6%)	9 (11%)	34 (41%)	34 (41%)
Importance of SSGs for the Development of Defensive Tactical Behaviors (e.g., defensive containment, defensive coverage, balance, concentration)	0 (0%)	6 (7%)	6 (7%)	40 (49%)	30 (37%)
Importance of SSGs for the Development of the Game Model	1 (1%)	6 (7%)	16 (20%)	34 (41%)	25 (30%)

preference aligns with existing evidence suggesting that SSGs might not be the most efficient method for enhancing sprinting abilities in soccer players [7]. There are several reasons behind this trend observed among coaches.

Firstly, SSGs may not adequately expose players to high-intensity sprint actions, especially in smaller field formats [41]. In typical SSGs scenarios, the playing area is restricted, which may limit the distance players can cover at maximum speed [39]. As a result, players may not reach peak sprinting velocities during these games, leading to suboptimal training stimuli for enhancing linear sprint performance.

Secondly, the contextual demands of soccer matches do not always prioritize frequent sprints. Soccer involves frequent changes of pace, direction, and tactical decisions, requiring players to adopt various movement patterns. As such, players are not consistently exposed to sustained linear sprinting throughout a match, making SSGs less conducive to replicating this specific aspect of game performance [14].

The majority of coaches expressing their use of SSGs for technical skills development which is not surprising. SSGs offer a unique advantage of providing a high-intensity stimulus while seamlessly integrating technical and tactical aspects of the game. This characteristic makes them particularly attractive for coaches aiming to enhance players' technical abilities within the context of realistic match scenarios.

Recent systematic reviews have supported this notion [34], showing that SSGs can be highly effective in improving individual technical skills, such as passing and dribbling, when compared to more analytical exercises. The dynamic and game-like nature of SSGs allows players to practice their technical skills in situations that closely resemble real-game scenarios, enhancing transferability to actual match performance.

Coaches predominantly expressed a preference for using smaller formats (1v1 to 4v4), which is consistent with the existing literature [5]. Smaller formats have been shown to increase the number of technical actions performed by each player within a given time frame [5]. This heightened frequency of actions in SSGs is likely to result in more opportunities for skill development and improvement following training interventions.

In the context of tactical behaviors, coaches also showed a significant preference for using SSGs as a means of improvement. Interestingly, in contrast to the preference for smaller formats in developing technical skills, coaches tended to opt for medium formats (5v5 to 8v8) for enhancing tactical behaviors. This choice is likely due to the larger formats allowing players to better emulate the model of play, enabling them to explore and practice important principles of the game, such as playing in unity and creating space in attack or fostering defensive concentration [4].

The analysis of task constraints and objectives employed by coaches revealed interesting trends in their preferences. Coaches tend to favor smaller fields for stimulating accelerations, decelerations, and technical skills, likely due to the increased diversity in movements and the proximity required for technical actions. On the other hand, coaches expressed a preference for using bigger fields to elicit physiological responses, promote more sprint actions, and facilitate the development of tactical behaviors.

These choices appear to align with existing evidence on the use of SSGs. Bigger fields have been shown to significantly increase players' heart rate responses [16], making them suitable for targeting physiological fitness. Moreover, the larger playing area encourages more sprint actions, which can be crucial for specific training objectives [41]. Conversely, smaller fields may reduce the number of sprints but create a higher frequency of accelerations and decelerations [42], allowing players to practice precise technical skills.

The task objectives chosen by coaches offer valuable insights into their preferences for using specific SSG formats. Coaches primarily favored ball possession games for enhancing physiological stimulus, accelerations, decelerations, and technical actions, which aligns with the literature [43]. The emphasis on ball possession encourages players to constantly create space, leading to more frequent changes of direction and bursts of speed, as well as favoring players to circulate the ball more often, which increases the number of receives, passes, and dribbles.

For stimulating sprints, coaches mostly opted for penetration zones, which involve players overcoming a line or covering a specific distance with the ball as the primary objective of the task. This format provides opportunities for explosive bursts of speed and acceleration, contributing to the development of sprinting abilities.

In terms of tactical behaviors, the regular game format with a goalkeeper was the most favored option. This format closely replicates the conditions of a real match, encouraging players to apply tactical principles and strategies in a realistic context. The presence of a goalkeeper adds complexity to the task, requiring players to make decisions based on defensive and offensive scenarios.

Study limitations and future research

This study has several limitations that should be acknowledged. Firstly, as a survey-based study, the responses provided by coaches are subjective in nature, and caution should be exercised while generalizing the findings. Future research could consider complementing the survey data with direct observations of coaching practices in clubs to triangulate the information.

Additionally, while the survey had a considerable number of participants, it focused solely on coaches from Portugal and Brazil. The training philosophies and coaching education programs in these countries might differ from those in other regions, limiting the generalizability of the findings to a broader international context. Therefore, caution should be exercised when applying these results to other countries or regions.

To overcome these limitations, future research endeavors should endeavor to broaden the survey's scope to encompass coaches from diverse cultural backgrounds and geographic regions. Indeed,

training approaches, models, and styles can vary significantly from one country to another. Therefore, it is essential for future research to extend the survey to encompass various regions that may employ distinct training methodologies. This will enable a more comprehensive and comparative analysis of coaching practices worldwide. The completed survey is available in supplementary file 1, facilitating its replication in different contexts and offering researchers the opportunity to compare results with our findings.

CONCLUSIONS

This survey successfully characterized the implementation of SSGs by Portuguese and Brazilian soccer coaches, shedding light on when, how, and why these training drills are utilized. The findings indicate that coaches generally prefer to use SSGs two to three times a week, with a typical duration of 16 to 30 minutes per session. These games are predominantly employed to stimulate aerobic fitness, change of direction, as well as technical skills and tactical behaviors. Smaller formats (1v1 to 4v4) were favored for targeting various fitness, technical, and tactical capacities, while coaches adjusted task objectives and field dimensions based on the specific components they aimed to develop. Overall, the coaches considered SSGs as very important for stimulating aerobic fitness, technical skills, and tactical behaviors.

This study adds valuable insights to the existing knowledge of how coaches incorporate SSGs into their training routines. The findings may lead to future recommendations for optimizing training instructions, offer novel ideas for diversifying and modifying training practices, and provide a fresh perspective on the implementation of small-sided games in soccer training.

Availability of Data and Materials

All data generated or analyzed during this study are available at the request of the corresponding author.

Competing interests

The authors declare that they have no competing interests

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Circulating total 25(OH)D and calculated free 25(OH)D in professional academy footballers at a northerly latitude in the UK

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ABSTRACT: There is limited data on the vitamin D status of UK-based professional academy footballers. Therefore, the objective of this study was to report total 25(OH)D, free 25(OH)D and free $1,25(OH)_2D$ at the end of the winter (March) and summer periods (October) in a cohort (n = 27) of professional academy footballers in northern England. Blood samples were collected to measure total 25(OH)D, parathyroid hormone, vitamin D binding protein, albumin and calcium. Free 25(OH)D and $1,25(OH)_2D$ were calculated. Dietary vitamin D intake and retrospective summer sunlight exposure were also collected. At the end of winter, 2/27 (7.4%) players were vitamin D deficient (25(OH)D < 30 nmol/l) and 11/27 (40.7%) were insufficient (25(OH)D > 30 nmol/l < 50 nmol/l). By the end of summer, none were deficient but 3/14 (21.4%) were still insufficient. Median total 25(OH)D (82.2 nmol/l [IQR: 50.3–90.2] vs. 54.2 nmol/l [IQR: 36.8–71.9]; P = .02), free 25(OH)D (25.8 pmol/l [IQR: 15.1–33.1] vs. 13.2 pmol/l [IQR: 9.0–14.9]; P = .005) and free $1,25(OH)_2D$ (389 fmol/l [IQR: 209–594] vs. 212 fmol/l [IQR: 108–278]; P = .034) were significantly higher at the end of summer than the end of winter. At the end of winter, free 25(OH)D was lower (P = .003) in those vitamin D insufficient (8.8 pmol/l [IQR: 5.5–11.8]) vs. sufficient (13.7 pmol/l [IQR: 12.0–17.0]). There was a high prevalence of vitamin D insufficiency at the end of the winter. Free 25(OH)D was also lower at the winter timepoint and in players that were insufficient vs. sufficient.

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INTRODUCTION

In addition to its involvement in calcium and phosphate homeostasis, vitamin D has been associated with important roles that are relevant to athletes [1–5]. Vitamin D status in footballers has been shown to correlate with measures of physical performance, including VO_{2max}, sprint times and vertical jump height [1]. Vitamin D deficiency and insufficiency may also impair immune function in athletes [4] and influence injury incidence [5].

A meta-analysis of 23 studies involving 2, 313 athletes showed that 56% of athletes across different geographical locations were vitamin D insufficient. The prevalence of vitamin D insufficiency was highest in the winter and spring seasons and was significantly higher in the UK than other geographical locations, including Spain, France, Australia, and the USA [6]. Vitamin D status is therefore of particular interest to UK sportspersons, but currently there is limited information in this area in UK-based footballers. The two existing studies from the UK to date have been carried out on professional players [2, 7]. One of these studies found that 55% of footballers had a circulating 25(OH)D < 50 nmol/l [2] during the winter (November-January) months, but this data was based on a sample of 11. A brief communication also reported 65% of 20 Premier League footballers were vitamin D insufficient in the winter [7]. To our knowledge no studies have reported vitamin D status in UK-based professional academy footballers.

Vitamin D status is currently assessed by measuring circulating total 25-hydroxyvitamin D (25(OH)D) because it reflects contributions from cutaneous synthesis and from the diet and has a relatively long circulating half-life of 13–18 days [8]. The gold-standard method for measuring total 25(OH)D concentration is liquid chromatography mass-spectrometry (LC-MS/MS) [9]. So far, no study has reported vitamin D status in UK footballers using this method and only a few studies have used this method in other European countries [10]. Whilst one previous study in the UK has reported seasonal variations in total 25(OH)D level in UK footballers (in August and December) [7], as far as we are aware, no studies have been conducted at the optimal time point to capture the winter vitamin D nadir (March).

Existing studies in UK footballers have also only reported total 25(OH)D to assess vitamin D status. There has been debate as to whether this is the most appropriate biomarker to use [11]. More specifically, around 85% – 90% of total 25(OH)D and $1,25(OH)_2D$ in circulation is bound to vitamin D binding protein (VDBP) and around

10% – 15% is bound to albumin. Unbound or free 25(OH)D accounts for < 1% of total circulating vitamin D [11] but the free hormone hypothesis suggests that only hormones that are liberated from their carrier proteins exert a physiological effect [12]. In healthy adults, a stronger association has been shown between free 25(OH)D and bone mineral density [13] and free 25(OH)D and parathyroid hormone (PTH) [14] than total concentrations. Therefore, free 25(OH)D concentrations may be an important metabolite to consider in athletes.

To our knowledge, no previous studies have assessed total 25(OH)D in UK-based professional academy football players at the optimal seasonal timepoints using the gold-standard approach and reported free vitamin D metabolites. Therefore, the purpose of this study was to examine these in a cohort of UK-based academy players at a professional football club in northern England.

MATERIALS AND METHODS

Participants

A convenience sample of 27 male professional footballers (aged 16–21 years) from a football academy situated at a northerly latitude in the UK (53° North) were recruited to the study. The study was approved by the Institutional Ethics Committee (SBS16/17136). Exclusion criteria were injury or illness within 6 weeks prior to measurement of blood biochemistry and/or unwillingness to complete study measurements (e.g., phobia of venipuncture). All data collection was performed in accordance with the ethical standards of the Helsinki Declaration and all participants provided written informed consent.

Study design

This is a cross-sectional, observational study to determine the vitamin D status of this cohort of footballers at the end of winter and the end of summer. Measurements were taken in March, when vitamin D status is expected to be at its annual nadir, and the beginning of October when there has been opportunity for endogenous synthesis of vitamin D over the summer months and, therefore, vitamin D levels are likely to be at their peak [15].

At the end of winter timepoint, blood samples were collected to measure serum total 25(OH)D, $1,25(OH)_2D$, parathyroid hormone (PTH), vitamin D binding protein (VDBP), albumin, calcium, creatinine and phosphate. Free 25(OH)D and free $1,25(OH)_2D$ were calculated. Dietary vitamin D intake was collected by an estimated four-day dietary record and retrospective summer sunlight exposure was collected by questionnaire. At the end of summer timepoint, a further blood sample was collected from each participant for repeated determination of vitamin D status and related biochemistry.

Study measurements

Anthropometric measurements

Height was measured in metres to the nearest 0.1 centimetre using a stadiometer and body mass to the nearest 0.1 kilogram using an electronic balance scale. Body mass index (BMI) was calculated using Quetelet's index.

Sample handling

Samples were collected into serum separating tubes (SST). Blood in SST tubes was left to clot for 30 minutes at room temperature and centrifuged at 3000 g for 10 minutes. The serum was aliquoted and stored at -80°C until analysis.

Total serum 25(OH)D

Total $25(OH)D_2$ and $25(OH)D_3$ were measured by liquid chromatography with tandem mass spectrometry (LC-MS/MS) which is the method used by the National Institute of Standards and Technology [16]. Measurements were carried out at the laboratory of the Institute of Human Development (University of Manchester, UK). This laboratory participates in the Vitamin D External Quality Assessment Scheme, and the assay was calibrated against the National Institute of Standards and Technology standard. Samples (200 μl) and a deuterated internal standard (d_6-25(OH)D were prepared using 100 μl methanol:isopropanol (80:20) and then extracted with 1 ml of hexane. This extracted 25(OH)D was blown down, reconstituted in 150 μl of 66% methanol and injected onto a waters phenyl column attached to the mass spectrometer. The extract was eluted with an isocratic gradient over 5 minutes. Analysis was carried out in positive ion mode using the transitions m/z 401 > 159 for 25(OH)D and Mm/z 407 > 159 for d_6-25(OH)D.

In this study, vitamin D deficiency (total 25(OH)D < 30nmol/l) and insufficiency (total 25(OH)D between 30 nmol/l and 50 nmol/l) was defined using the Institute of Medicine classifications [8] and these thresholds are widely used by UK healthcare practitioners.

Other biochemistry

Vitamin $1,25(OH)_2D$ was measured by chemiluminescence immuno assay (CLIA) after an extraction step on the IDS-iSYS (Immunodiagnostic Systems, Boldon, UK) at the Bone Biochemistry Laboratory (University of Sheffield, UK). The inter-assay CV was 6.0%. Intact PTH was measured by an automated sandwich CLIA on the IDS-iSYS (Immunodiagnostic Systems, Boldon, UK) at the Bone Biochemistry Laboratory (University of Sheffield, UK). The manufacturers inter-assay CV is < 2.0%. Serum albumin, calcium, creatinine, and phosphate were measured using an automated colorimetric assay on the Cobas c701 (Roche Diagnostics, Mannheim, Germany) in the Chemical Chemistry laboratory (Sheffield Teaching Hospitals, UK). The inter assay precision was < 2.0% for all tests. Vitamin D binding protein (VDBP) was measured using a polyclonal non-competitive two-site enzyme-linked Sandwich immunoassay (Genways, San Diego, USA). The inter- and intra- assay CV were 3.9% and 3.3%, respectively.

Calculated free 25(OH)D and free $1,25(OH)_2D$

Free 25(OH)D and $1,25(OH)_2D$ was calculated using the concentrations of albumin and VDBP and their respective binding affinities for 25(OH)D.

The formula used for free 25(OH)D was [17]:

$$\text{Free } 25(\text{OH})\text{D} = \frac{\text{Total } 25(\text{OH})\text{D}}{1 + (6 \text{ x } 10^5 \text{ x albumin}) + 7 \text{ x } 10^8 \text{ x VDBP}}$$

The formula used for $1,25(\text{OH})_2\text{D}$ was [18]:

$$\text{Free } 1,25(\text{OH})_2\text{D} =$$

$$= \frac{\text{Total } 1,25(\text{OH})_2\text{D}}{1 + (5.4 \text{ x } 10^4 \text{ M} - 1 \text{ x albumin}) + (3.7 \text{ x } 10^7 \text{M} - 1 \text{ x VDBP})}$$

Dietary vitamin D and sunlight exposure assessment

Participants were asked to complete an estimated 4-day diet diary to assess dietary intake of vitamin D at the end of winter timepoint. Data was collected on two training days, one match day and one rest day. After completion, a trained nutritionist interviewed the participants to establish the accuracy of the completed diaries. Dietary vitamin D intake was calculated using Nutritics (Dublin, Ireland).

Habitual sunlight exposure was estimated at baseline using a retrospective sunlight exposure questionnaire that was adapted from a previous study [19]. Despite known difficulties in quantifying sun exposure due to many influencing factors [20], data derived from questionnaires has been shown to be correlated with vitamin D status [21].

For each month of the year participants were asked to score how often they were usually outside and exposed to the sun. A score of 3 for 'often', '2' for occasionally, and '1' for seldom. The score given was multiplied by the total body areas exposed in each month by using the percentages below from the 'rule of nine' to estimate the surface area of the skin exposed to sunlight. The 'rule of nines' is a tool used by medical practitioners to assess the total body surface area involved in burns patients. It has previously been used in vitamin D research to estimate the surface area of the skin exposed to sunlight [22]:

- Head = 9% (front and back together)
- Both arms = 9% (L) + 9% (R)
- Both legs = 18% (L) + 18% (R)
- Torso = 18% (front) + 18% (back)
- Groin = 1%

For example, a participant ticking 'occasionally', and 'head' and 'arms' would get a score for that month of (2*(0. 09 + 0.09 + 0.09)) = 0.54. By using this scoring system, a score for 'summer sunlight exposure' was derived by calculating the sum of the scores for months where endogenous vitamin D synthesis can occur in the UK (April to September).

Statistical analysis

Statistical analyses were performed using the Statistical Analysis Package for Social Sciences (SPSS V.26 for Windows, Chicago, Illinois, USA).

Each variable is presented as median and inter-quartile range (IQR) due to the non-normal distributions. Each variable was tested for normality with a Shapiro-Wilk test. Normality was assumed if Shapiro-Wilk test was not statistically significant ($P > .05$) but no paired- or between-participant variables met the assumptions of

TABLE 1. Participant characteristics (n = 27) from the end of winter (March) timepoint.

Variables	Median	IQR
Age (years)	18	17–18
Height (cm)	179.4	174.1–185.3
Body mass (kg)	76.2	67.5–81.6
BMI (kg/m^2)	23.2	22.3–24.0
Total 25(OH)D (nmol/l)	50.2	40.4–61.8
Total $1,25(\text{OH})_2\text{D}$ (pmol/l)	25.5	15.1–33.4
Calculated free 25(OH)D (pmol/l)	12.8	8.8–14.9
Calculated free $1,25(\text{OH})_2\text{D}$ (fmol/l)	242	174–349
Parathyroid Hormone (pg/ml)	31.7	21.8–42.0
Serum Phosphate (mmol/l)	1.19	1.14–1.28
Serum Creatinine (μmol/l)	94	87–103
eGFR (ml/min/1.73m^2)	88	79–90
Serum Calcium (mmol/l)	2.42	2.37–2.48
Adjusted Serum Calcium (mmol/l)	2.19	2.15–2.23
Albumin (g/l)	52	51–54
Vitamin D Binding Protein (μg/ml)	322	273–391
Total Alkaline Phosphatase (IU/l)	101	78–124

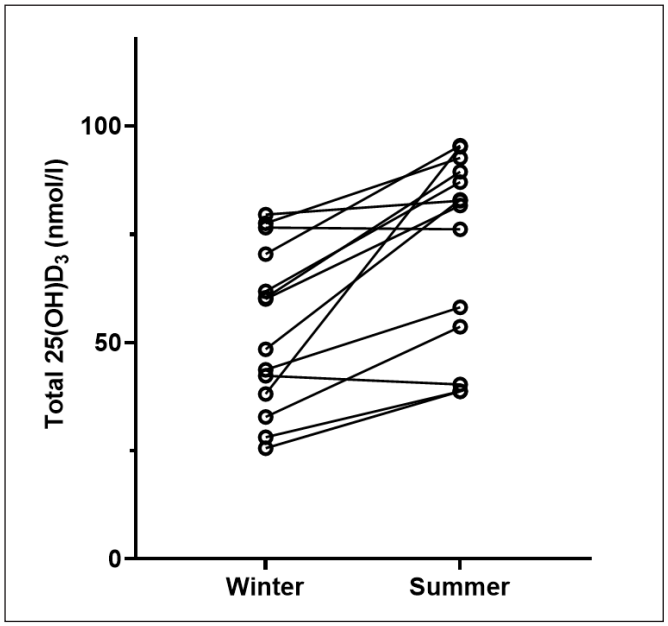


FIG. 1. Change in median total 25(OH)D status from the end of winter (n=14) the to end of summer (n = 14). Circles represent individual participant values

parametric testing. Therefore, non-parametric Mann-Whitney U test was used to assess for differences between the groups. For paired comparisons of variables, a non-parametric Wilcoxon test was used. A Pearson's correlation coefficient (where Shapiro-Wilk was $P > .05$) or Spearman's Rho (where Shapiro-Wilk was $P < .05$) were used to determine significant relationships between variables. For all tests a $P < 0.05$ was set as the critical level of significance.

RESULTS

Blood samples were obtained in March from all 27 participants. Thirteen of the participants had left the Academy by October, so samples were captured from the remaining 14 players. Completed diet diaries and sunlight exposure questionnaires were obtained from twenty participants. In all blood samples, vitamin $25(OH)D_2$ was undetectable and so we have not reported concentrations of this metabolite. The participant characteristics at the start of the study at the end of winter timepoint are shown in Table 1.

Vitamin D metabolites

At the end of winter, 2/27 (7.4%) players were vitamin D deficient (25(OH)D < 30nmol/l), 11/27 (40.7%) were insufficient (25(OH)D > 30nmol/l < 50 nmol/l) and 14/27 (51.9%) were sufficient. By the end of summer, none were deficient, 3/14 (21.4%) were insufficient and 11/14 (78.6%) were sufficient. Analysis of a subset where we had March and October samples was completed (n = 14). Total 25(OH)D concentration was lower at the end of winter (median: 54.2 nmol/l [IQR: 36.8–71.9]) compared to the end of summer (median: 82.2 nmol/l [IQR: 50.3–90.2]) and this difference was significant (Z = -3.107, P = .02) (Figure 1).

Calculated free 25(OH)D and calculated free $1,25(OH)_2D$ were significantly lower at the end of winter than the end of summer. PTH and VDBP were significantly higher at the end of winter than the end of summer but there was no significant difference in adjusted serum calcium or albumin between the two timepoints (Table 2).

At the end of winter timepoint, there were no statistically significant differences in total $1,25(OH)_2D$, calculated free $1,25(OH)_2D$, PTH, adjusted serum calcium, VDBP or albumin between players who were insufficient (total 25(OH)D < 50nmol/l) and sufficient (total 25(OH)D > 50nmol/l). However, calculated free 25(OH)D was significantly higher in those who were sufficient compared to those who were insufficient (Table 3).

Correlations between total 25(OH)D, free 25(OH)D and PTH.

There was no relationship between total 25(OH)D and PTH at the end of winter (r = -.033, P = .870) or end of summer timepoints (r = -.415, P = .158). There was also no relationship between free 25(OH)D and PTH at the end of winter (r = -.033, P = .870) or end of summer timepoints (r = -.033, P = .870).

TABLE 2. End of winter (March) and end of summer (October) values for PTH, adjusted serum calcium, VDBP, albumin and other vitamin D metabolites.

Variables	March (n = 14)		October (n = 14)		P-value
	Median	IQR	Median	IQR	
PTH (pg/ml)	33.2	25.1–47.0	21.7	17.1–24.5	.004
Adjusted Serum Calcium (mmol/l)	2.20	2.13–2.26	2.16	2.10–2.19	.345
Total $1,25(OH)_2D$ (pmol/l)	15.6	12.9–32.8	25.1	15.7–36.5	.140
Calculated free 25(OH)D (pmol/l)	13.2	9.0–14.9	25.8	15.1–33.1	.005
Calculated free $1,25(OH)_2D$ (fmol)	212	108–278	389	209–594	.034
VDBP (μg/ml)	338	278–407	204	179–232	.005
Albumin (g/l)	52	50–54	51	49–53	.555

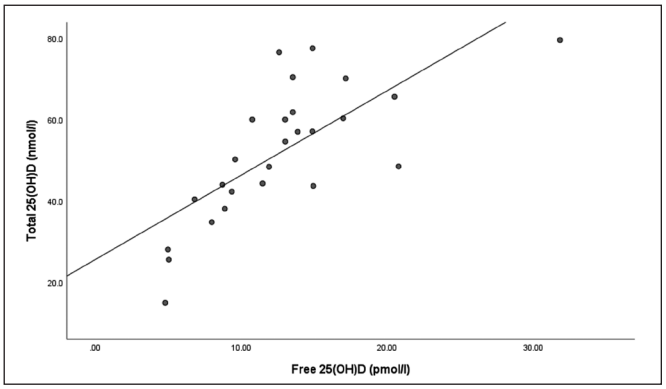


FIG. 2. The relationship between total 25(OH)D and free 25(OH)D at the end of winter timepoint.

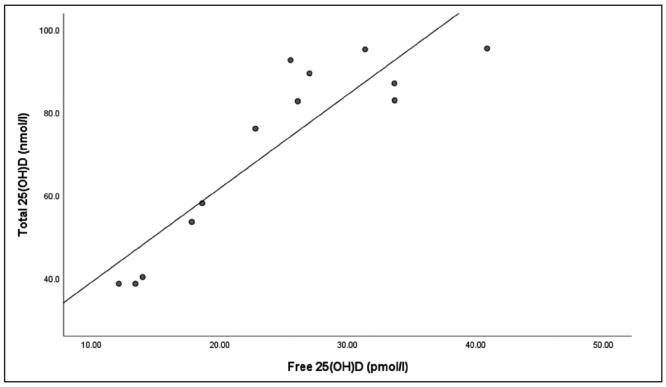


FIG. 3. The relationship between Total 25(OH)D and free 25(OH)D at the end of summer timepoint.

TABLE 3. PTH, adjusted serum calcium, VDBP, albumin and other vitamin D metabolites in players who were insufficient (total 25(OH)D < 50 nmol/l) vs. players who were sufficient (total 25(OH)D >50 nmol/l) at the end of winter (March).

Variables	Deficient/Insufficient (n = 13)		Sufficient (n = 14)		P-value
	Median	IQR	Median	IQR	
PTH (pg/ml)	29.2	22.2–39.7	33.2	17.3–48.5	.756
Adjusted Serum Calcium (mmol/l)	2.2	2.16–2.22	2.19	2.15–2.24	.805
$1,25(OH)_2D$ (pmol/l)	24.3	13.2–29.8	27.6	17.3–27.6	.225
Calculated free 25(OH)D (pmol/l)	8.8	5.5-11.8	13.7	12.9-17.0	.003
Calculated free 1, 25(OH)2D (fmol/l)	212	154-334	242	212-357	.432
VDBP (μg/ml)	321	236-384	323	273-393	.667
Albumin (g/l)	51	49-54	53	51-54	.128

There were strong relationships between total 25(OH)D and free 25(OH)D at the end of winter (r = .766, P = < .001) (Figure 2) and end of summer timepoints (r = .883, P < .001) (Figure 3).

Dietary vitamin D & sunlight exposure

Overall median dietary vitamin D intake was 2.8 μg/day (IQR: 1.6–6.3). Median dietary vitamin D intake in players with sufficient 25(OH)D concentrations was 3.7 μg/day (IQR: 2.8–8.8), whereas it was 1.6 μg/day (IQR: 1.3–3.3) in players with insufficient 25(OH)D concentrations. This difference was statistically significant (U = 15.0, n_1 = 11, n_2 = 9, P = .007).

The median summer sunlight exposure score in players that were sufficient in 25(OH)D was 10.18 (IQR: 8.74–11.62) and was 9.93 (IQR: 8.12–11.42) in those that were insufficient, but this difference was not significant (U = 46.5, n_1 = 11, n_2 = 9, P = .824).

DISCUSSION

Our study has identified a high prevalence of total $25(OH)D_3$ insufficiency in professional academy football players at the end of winter (7.4% vitamin D deficient and 40.7% insufficient). To our knowledge, no other study has reported total 25(OH)D concentrations in professional academy footballers in the UK, but our data are congruent with findings from other studies of academy and professional level football players across Europe, which have demonstrated winter insufficiency rates of up to 75% [2, 7, 23–25].

The fall in total $25(OH)D_3$ concentration from the end of summer to the end of winter in professional football players has previously been established [7] and this reflects that of the general UK population [15]. Findings from our study also show that total 25(OH)D was higher at the end of summer (median: 82.2 nmol/l) compared to the end of winter (median: 54.2 nmol/l). However, even after the summer period around one fifth of players tested were still insufficient (21.4%). This prevalence of insufficiency is much higher in comparison to other studies of elite football players in Europe that have reported summer period total 25(OH)D concentrations [7, 23, 26] but these studies were carried out on professional first team, rather than academy level players. This is interesting and, taken with the vitamin D insufficiency rates from the winter period, may highlight a lack of nutrition related advice provided to academy compared to professional players and/or poor compliance with nutrition-related advice in academy level players. These findings are also important when we consider that adequate vitamin D status may be important for optimal performance [1–3, 10, 27] and injury prevention [5, 28], particularly in professional academy players who typically have greater training loads in comparison to the first team [29].

Total $1,25(OH)_2D$ was not different between the two timepoints. This is not surprising due to tight homeostatic control of this metabolite [30]. However, our results show that PTH concentration was lower in academy players at the end of summer compared to the end of winter, although it was within the normal range (10–65 ng/ml) at both time points. A study in professional Polish football players has also shown lower concentrations of total 25(OH)D (62.4 ± 24.8 nmol/l vs. 77.1 ± 22.6 nmol/l, P = 0.0003) and higher PTH (25.37 ± 7.95 pg/ml vs. 21.37 ± 6.88 pg/ml, P = 0.0062) at the end of winter compared to the end of summer [31]. PTH influences bone turnover and therefore future studies should consider any detrimental effects of low 25(OH)D on bone health outcomes in young professional footballers. However, despite the difference in PTH in our study, there were no differences in serum calcium concentration between the two timepoints. There was also no relationship between PTH and total 25(OH)D or free 25(OH)D at either timepoint. Other studies in footballers have also failed to detect a significant association between total 25(OH)D and PTH [26, 32]. However, this is likely due to the small sample sizes in these studies and the relatively weak inverse association previously reported between total 25(OH)D and PTH at a population level [33]. To our knowledge no study has previously reported the association between free 25(OH)D and PTH in footballers but a significant inverse association has been demonstrated in non-athlete populations [34].

Due to recent interest in free vitamin D metabolites, we also report free 25(OH)D and free $1,25(OH)_2D$. Free 25(OH)D was similar to concentrations reported elsewhere in healthy people [12, 35]. Like total 25(OH)D, we found a lower free 25(OH)D at the end of

winter compared to the end of summer. We also found a strong correlation between total 25(OH)D and free 25(OH)D at both study timepoints. This is congruent with literature from other population subgroups where free 25(OH)D has been shown to be highly correlated with total 25(OH)D [14, 34, 36, 37]. Some studies have shown that free 25(OH)D might be better correlated with health and biochemical outcomes than total 25(OH)D, including BMD and markers of calcium homeostasis [13, 14]. Although, recent studies have indicated that the measurement of free 25(OH)D may only be useful in certain health conditions that perturb VDBP concentration, such as pregnancy and cirrhosis [35]. A group has recently reported no significant relationship between calculated free 25(OH)D and physical performance (handgrip strength, vertical jump, and aerobic fitness) in 24 Polish football players [27], but this is the only study to date. Therefore, the relevance of reporting free vitamin D metabolites in footballers, particularly in relation to performance outcomes, still requires investigation in studies with much larger sample sizes.

VDBP concentrations were higher at the end of winter than the end of summer and this would, in part, explain the lower calculated free 25(OH)D and free $1,25(OH)_2D$ concentrations at the end of winter. Seasonal variations in VDBP have been reported elsewhere, with increased VDBP in response to low vitamin D status and this may be to maximize uptake of vitamin D from skin [38].

At the end of winter timepoint, there were no differences in PTH, adjusted serum calcium, total $1,25(OH)_2D$ or free $1,25(OH)_2D$ between players who were vitamin D insufficient compared to those who were sufficient. This might be expected as variations in calciotropic hormones in otherwise healthy people may only be seen at extreme total 25(OH)D concentrations. However, calculated free 25(OH)D was higher in those who were sufficient compared to those who were insufficient, as expected due to the strong correlation between the two metabolites [36, 37].

There was no difference in sunlight exposure scores between the two groups but due to the many factors influencing cutaneous synthesis and difficulties in assessing sunlight exposure [20], these findings should be treated with caution. One possible contributor to insufficiency in these players is dietary vitamin D intake. Median intake was significantly lower in the insufficient group (1.6 μg/day) compared to the sufficient group (3.7 μg/day). For both groups, this is lower than the UK Reference Nutrient Intake (10 μg/day) and for the insufficient group this is less than the intake reported by adolescents and younger adults (2.4 μg/day from all sources) in the UK [15]. This may highlight the importance of dietary intake in academy players, especially during the winter period when opportunity for subcutaneous synthesis in the UK is minimal. There was no vitamin D supplementation programme for these players within the academy at the time of study, but it is possible that this might not reflect practices elsewhere.

Our study has several strengths. First, we have used a gold-standard method to assess vitamin D status (first study in UK to report total 25(OH)D using LC-MS/MS in young footballers) and in optimising the timing of assessment to capture the annual peak and nadir for vitamin D status in the UK (end of winter and summer periods). Second, we have also reported calculated free 25(OH)D and $1,25(OH)_2D$ in footballers using a polyclonal assay to measure VDBP. There are limitations of the calculated method for assessing free 25(OH)D and free $1,25(OH)_2D$, including the assumption of invariant binding affinity of different VDBP alleles with 25(OH)D [39], despite evidence that this is not the case [40]. Future studies in footballers may therefore look to use direct measurement methods for free 25(OH)D. The study has other limitations, including the absence of data corresponding to health and performance outcomes and a relatively small sample size including a loss-to-follow-up of 13 players due to players leaving the football club. There are also known difficulties in quantifying sun exposure due to many influencing factors, including time of day, season, skin type, cloud cover, clothing, and sunscreen use [20]. There are also known difficulties in dietary assessment of vitamin D intakes because it is found in very few food sources. Therefore, consumption/lack of consumption of vitamin D containing foods during the recording period could impact on estimates of habitual intakes.

Future studies need a larger sample size, perhaps across multiple football clubs, and should also consider the relevance of reporting free vitamin D metabolites in footballers, particularly in relation to performance outcomes.

CONCLUSIONS

We have demonstrated a high prevalence of vitamin D insufficiency at the end of the winter in academy level footballers in the UK, with a fifth still insufficient by the end of the summer. We also observed lower free 25(OH)D at the end of the winter and in those who were insufficient compared to sufficient at the winter timepoint. Vitamin D intake was very low, which suggests a need for targeted dietary advice and appropriate use of supplementation in this cohort of academy level footballers. The high prevalence of deficiency could have implications for performance and injury outcomes (including bone health) in academy players and this should be the focus of further study with consideration given to the relevance of reporting free vitamin D metabolites.

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Conflict of interest declaration

The authors declare no conflict of interest.

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Artificial intelligence in sport: Exploring the potential of using ChatGPT in resistance training prescription

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ABSTRACT: OpenAI's Chat Generative Pre-trained Transformer (ChatGPT) technology enables conversational interactions with applications across various fields, including sport. Here, ChatGPT's proficiency in designing a 12-week resistance training programme, following specific prompts, was investigated. GPT3.5 and GPT4.0 versions were requested to design 12-week resistance training programmes for male and female hypothetical subjects (20-years-old, no injury, and 'intermediate' resistance training experience). Subsequently, GPT4.0 was requested to design an 'advanced' training programme for the same profiles. The proposed training programmes were compared with established guidelines and literature (e.g., National Strength and Conditioning Association textbook), and discussed. ChatGPT suggested 12-week training programmes comprising three, 4-week phases, each with different objectives (e.g., hypertrophy/strength). GPT3.5 proposed a weekly frequency of ~3 sessions, load intensity of 70-85% of one repetition-maximum, repetition range of 4-8 (2-4 sets), and tempo of 2/0/2 (eccentric/pause/concentric/'pause'). GPT4.0 proposed intermediate- and advanced programme, with a frequency of 5 or 4 sessions, 60-90% or 70-95% intensity, 3-5 sets or 3-6 sets, 5-12 or 3-12 repetitions, respectively. GPT3.5 proposed rest intervals of 90-120 s, and exercise tempo of 2/0/2. GPT4.0 proposed 60-180 (intermediate) or 60-300 s (advanced), with exercise tempo of 2/1/2 for intermediates, and 3/0/1/0, 2/0/1/0, and 1/0/1/0 for advanced programmes. All derived programmes were objectively similar regardless of sex. ChatGPT generated training programmes which likely require additional fine-tuning before application. GPT4.0 synthesised more information than GPT3.5 in response to the prompt, and demonstrated recognition awareness of training experience (intermediate vs advanced). ChatGPT may serve as a complementary tool for writing 'draft' programme, but likely requires human expertise to maximise training programme effectiveness.

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INTRODUCTION

Generative Pre-training Transformer (GPT) from OpenAI is a language model that attracted 1 million users within 5 days of launching the free model 3.5 (GPT3.5) on November 30, 2022 (https://openai.com/). Its paid successor, GPT4.0, launched on March 14, 2023 (https://openai.com/), enhanced its suitability for tasks requiring advanced reasoning. Based on Natural Language Processing, GPT generates human-like conversations through applications (chatbots or ChatGPT), providing contextually accurate responses to users' inputs [1]. As such, ChatGPT has the potential to offer support in various fields, including academia and sport [2].

In academia, chatbots may serve as a "research assistant" to generate ideas, receive feedback, and summarise literature [2, 3, 4]. In sports, ChatGPT can produce training prescriptions, including plans, suggestions, and performance feedback based on specified information [2]. This is particularly useful in situations like Coronavirus disease 2019, where training support may be scarce [5]. In preparing training programmes, coaches utilise books, scholarly articles, and online resources [6]. Yet, the wealth of information, coupled with biases inherent in diverse data sources (e.g., erroneous conclusions or perceptions), remains challenging and time consuming to navigate. Globally, GPT technologies appear to offer an increasingly popular approach to streamlining such information gathering and synthesis. However, reported examples of its use in sport and exercise are scarce [2, 3].

Pre-trained on a large corpus of online data, the accuracy of ChatGPT in exercise prescription is not fully understood [7]. Such limitations must be recognised before the replacement of human intelligence can be countenanced in this area, through widespread adoption across the general population. Thus, this 'short communication' aimed to (i) explore and inform readers about ChatGPT technology in sports and exercise, (ii) highlight the potential use of ChatGPT for exercise prescription in resistance training, and (iii) compare the resistance training programmes designed by GPT3.5 and GPT4.0 in a hypothetical male and female participant.

MATERIALS AND METHODS

In this study, we assessed ChatGPT's ability to prescribe training for hypothetical individuals characterised as either intermediate (moderately resistance-trained) or advanced (well resistance-trained) via a series of prompts. The training programmes proposed by ChatGPT were carefully compared with authoritative literature, including the textbook of the National Strength and Conditioning Association (NSCA) and various review papers. The authors had expertise in sports science and exercise prescription; possessed strength and conditioning qualifications; and had 10–20 years of experience in designing and assessing resistance training programmes for athletes of varying experience levels, ranging from adolescent to Olympic. These backgrounds may add a valuable perspective to the appraisal (Table 1).

Exercise prescription using ChatGPT

Separately, we requested GPT3.5 and GPT4.0 (July 20, 2023 versions; OpenAI, L.L.C., San Francisco, CA, USA) to provide a 12-week resistance training programme to develop muscular strength, i.e., intermediate-level training programmes (abbreviated as GPT3.5 and $GPT4.0_{INT}$ for each version, respectively). Technologies auto-generated texts based on three prompts (Figure 1). The texts (training programmes) generated by ChatGPT were transformed or condensed (manually) into table format to facilitate review and appraisal (supplementary file 1 [S1], and supplementary file 2 [S2]). Our preliminary prompts yielded comparable recommendations for both the male and female; therefore, we only report male responses in this brief report. Subsequently, we asked GPT4.0 to create an advanced training programme ($GPT4.0_{ADV}$) for the same participants, using the same prompts except for the training level (Figure 1).

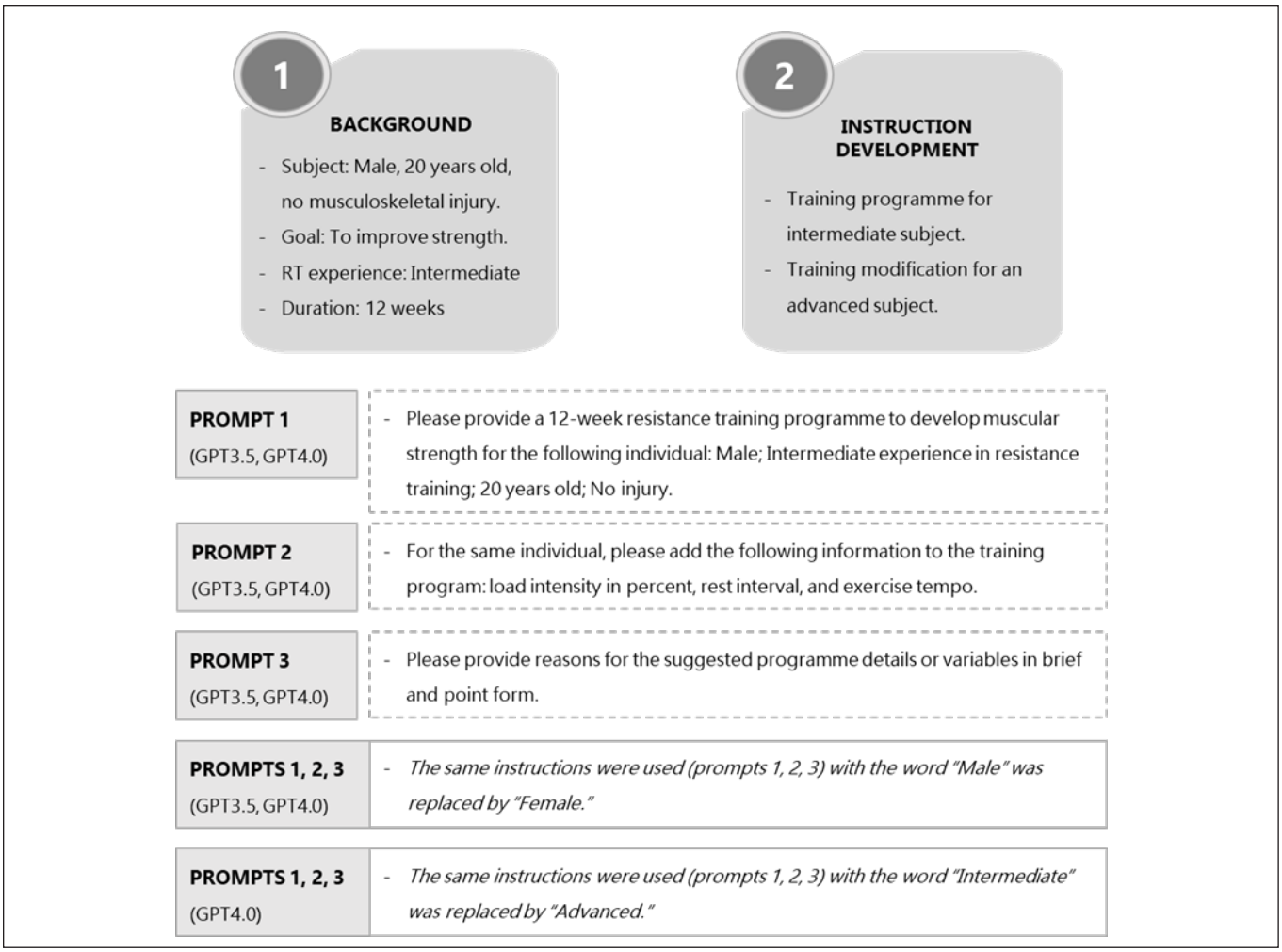


FIG. 1. Prompts or instructions used to generate training programmes for intermediate (GPT3.5 and $GPT4.0_{INT}$) and advanced ($GPT4.0_{ADV}$) resistance training in male and female subjects.

TABLE 1. Summary of the appraisal between and ChatGPT generated training programmes and scientific literature

	GPT 3.5 INTERMEDIATE	GPT 4.0 INTERMEDIATE	GPT 4.0 ADVANCED
Programming			
Exercise selection	Moderate	Moderate	Moderate
Weekly frequency	Strong	Strong	Strong
Load intensity	Strong	Strong	Strong
Set and repetition	Moderate	Strong	Strong
Tempo	Moderate	Moderate	Moderate
Rest interval	Weak	Strong	Strong
Principles			
Progressive overload	Strong	Strong	Strong
Variation	Strong	Strong	Strong
Specificity	Moderate	Moderate	Moderate
Recovery	Moderate	Moderate	Strong
Strategies			
Weekly routines	Strong	Strong	Strong
Contemporary practices	Weak	Weak	Weak

Note: Ratings are classified as 'strong', 'moderate', or 'weak', which are assigned by considering the appropriateness and completeness of how particular variables are integrated within the overall programme, including the degree to which the variables align with established scientific standards and practices.

RESULTS

A summary of ChatGPT's responses (Table 1), the requested 12-week resistance training programmes (S1) and reasoning (S2), are provided. Briefly, both GPT3.5 and GPT4.0 proposed three periodised phases of training (foundation *and* high volume; strength building *and* moderate volume/high intensity; and intensification *and* low volume/very high intensity) lasting 4 weeks. The advanced programme also utilised 4-week phases, but proposed block periodisation (accumulation, intensification, and realisation). Preliminary analysis specific to "female prompts" revealed objectively similar training recommendations for both male and female participants.

For *(i) exercise selection and structure*, ChatGPT recommended exercises in two (GPT3.5 and GPT4.0_{ADV}) or three (GPT4.0_{INT}) exercise groups, using a split-routine approach. For *(ii) training*

frequency, ~3 sessions/week (GPT3.5), 4 sessions/week ($GPT4.0_{ADV}$), and 5 sessions/week ($GPT4.0_{INT}$), were proposed. For *(iii) load intensity*, GPT3.5 (70–75% to 75–80% to 80–85%), $GPT4.0_{INT}$ (60–70% to 70–80% to 80–90%), and $GPT4.0_{ADV}$ (70–80% to 80–90% to 90–95%) provided subtly different progressions. For *(iv) sets and repetitions*, GPT3.5 proposed 2–4 sets and 4–8 repetitions for most exercises, while $GPT4.0_{INT}$ and $GPT4.0_{ADV}$ proposed 3–5 sets and 5–12 repetitions, and 3–6 sets and 3–12 repetitions, respectively. For *(v) exercise tempo*, the 'pause' duration between eccentric and concentric phases differed in GPT3.5 (2/0/2), $GPT4.0_{INT}$ (2/1/2), and $GPT4.0_{ADV}$ (3/0/1/0, 2/0/1/0, and 1/0/1/0, in each phase). For *(vi), rest interval*, GPT3.5 proposed predominantly between 90–120-s, while $GPT4.0_{INT}$ proposed 60–90-s (for high repetition exercise), 90–120-s (medium repetition), and 2–3-min (low-medium repetitions). In comparison, $GPT4.0_{ADV}$ proposed 60–300-s rest intervals depending on training phase and exercise (main vs supplementary).

DISCUSSION

ChatGPT generated relevant content for resistance training programming (Table 1). GPT3.5, $GPT4.0_{INT}$ and $GPT4.0_{ADV}$ proposed three 4-week phases (S1). Some subtle differences were observed between the responses in terms of exercise variables such as exercise selection, frequency, repetitions, and intensities. GPT4.0 (intermediate and advanced) provided additional information, reflecting a better understanding of training prescription. Furthermore, GPT4.0 was adept in tailoring training programmes to accommodate different resistance training competency levels. The generated training programmes generally considered training principles (e.g., progressive overload and variation), comparable with information contained within established guidelines and peer-reviewed resources, and articulated using standard academic language. These outputs indicated some degree of appropriate prioritisation by the technology, in terms of sourcing information.

Manipulation of volume and load intensity are key considerations in designing resistance training programmes [8] and exercise volume-induced changes in steroid hormones like testosterone and cortisol also influence strength gains [9, 10]. It is encouraging that both ChatGPT versions proposed three training phases with varying foci regarding training variables to facilitate strength development [11]. ChatGPT also incorporated both 'main' and 'supplementary' exercises, while proposing appropriate exercises that targeted major muscle groups, such as upper and lower body push-pull variations, which can stimulate hormonal responses that in turn facilitate muscular growth and strength [12]. ChatGPT also employed a split-routine to target specific muscle groups on separate days, a common practice in strength training [12]. The advanced programme proposed a block periodisation, which is common practice for well-trained individuals [12] and included unilateral (for engagement of stabilising muscles) and loaded bodyweight exercises. These routines and strategies adhere to the training principles (e.g., progressive overload, variation, specificity) that allows a training stimulus to remain optimal over time [12]. This prescription offers end-user more comprehensive and relevant information when prompted appropriately.

ChatGPT generally recommended 3–5 sessions of resistance training in a week (S1). $GPT4.0_{ADV}$ stated that "four training sessions provides a balance between training volume and recovery for an advanced trainee" (S2). Higher training frequency (≥ 3 sessions/week) augments total weekly training volume, and positively impacts muscular strength [13]. The NSCA recommends 3–4 sessions/week for intermediate and 4–7 sessions/week for advanced trainers [12]. This frequency provides sufficient time for recovery and adaptation, whilst optimising hypertrophy and strength [14, 15]. Interestingly, only $GPT4.0_{ADV}$ considered "active recovery" sessions, citing the promotion of blood flow and removal of waste products (S2). GPT models showed variable load intensity prescriptions, ranging from 60–95% (S1) to allow "proper progressive overload" (S2), with loads varied in main/supplementary exercises. These recommendations are aligned with conventional resistance training [12] but omit emerging trends like low-load prescriptions (< 60%) or blood flow restriction for muscle hypertrophy [8]. Varying repetition ranges for exercises were recommended by different versions of GPT, which generally, aligned with established research on muscle hypertrophy and strength development [12]. For example, the proposed heavy loads (> 85%) with fewer repetitions were specific to achieve training goals (e.g., maximal strength) (S1). Depending on the stated objective, $GPT4.0_{ADV}$ proposed medium repetitions during the initial training period (accumulation), reducing to low-medium during "intensification", and low during "realisation" phase. GPT3.5 proposed low-medium range repetitions (4–8 reps) for most exercises, while $GPT4.0_{INT}$ proposed a relatively medium range (5–12 reps). Medium repetition ranges (e.g., 6–12 reps) may facilitate hypertrophy, and lower repetitions (e.g., 1–6 reps) enhance strength [12]. ChatGPT proposed a multiple-set system tailored to an individual's training level for optimal strength gains (S1). Indeed, intermediate-level individuals benefit from a medium weekly dosage of 5–9 sets, while advanced individuals benefit from both medium and high (≥ 10 sets) weekly sets [14].

$GPT4.0_{ADV}$ recommended additional volume and eccentric loading to enhance hypertrophy and prepare muscle tissues for heavier loads. Indeed, muscular strength can be optimised through training volume and "time under tension" or tempo [13, 16, 17]. As exercise tempo can affect training volume [14], differences in total time under tension, [e.g., 2/0/2 (GPT3.5) and 3/0/3/0 (e.g., $GPT4.0_{ADV}$)] can possibly impact movement and influence training adaptation. Even though both eccentric and concentric training are necessary to optimise hypertrophy [8], current evidence supports strength training protocols with medium eccentric and fast concentric actions (e.g., 2-4/0/1/0) for optimising dynamic strength development in trained and untrained individuals [18]. ChatGPT did provide a debatable assertion that "muscle damage is a key driver for hypertrophy" (S2). These responses again reinforce the importance of human interpretation of responses, prior to application.

Prescribed rest intervals reflected the specific training goals (S1). For example, GPT4.0 (intermediate and advanced) contained appropriate rest interval durations of 60–300 s dependent on exercise repetitions, phase, and exercise types (S1). GPT3.5 proposed 90–120 s for all three phases, even when the training focus was strength development. This prescription deviates from the specificity concept to enhance training gains, which is likely suboptimal, given short rest intervals (e.g., 60–90 s) are usually applied to enhance hypertrophic responses [12] while longer rest intervals (2–5 min) facilitate greater recovery and enable heavier loads to be lifted [12]. Therefore, this indicates a more appropriate prescription from the latest GPT model, compared with earlier iterations.

Currently, ChatGPT supports autodidactic self-learning, but responses need to be carefully appraised. ChatGPT's justifications such as "efficient" use of training time, as well as considerations of active recovery, nutrition, and hydration are noteworthy (S2), as these elements were not outlined in the prompts. This detail indicates a broader awareness of the subject matter than what was detailed in the prompt. A weekly routine that encompasses a well-rounded approach (including proper exercises, structured routines, adequate recovery etc.) is essential for optimising training effectiveness. Furthermore, ChatGPT delivered information in a language comparable to academic sources. However, the suggested guidelines (S1) and rationales (S2) appear to have overlooked some alternative training methods, loading strategies, and set configurations [19]. For example, the potential to induce substantial strength gains through cluster sets, variable resistance training, and blood flow restriction. Other methods, such as supersets and drop sets, which are time-efficient and effective to induce strength gains, were also omitted. This exclusion indicates a possible lack of alignment with contemporary, evolving training methodologies. Future prompts may need to be refined to consider emerging research and suggested programmes should be scrutinised by a topic expert. While only male responses are reported, the prompts for a female subject received comparable recommendations to the male subject regarding training phases, weekly structure, and session routines. This lack of distinction may be due to the disproportionately low number of female training studies and source material available for ChatGPT to draw upon. We strongly support future research focused on female training programmes and acknowledge that females may require different prescription needs to males [20].

In this article, we exclusively examined artificial intelligence-generated training programmes, while thoroughly considering literature and guidelines, leaving the potential impact of personal trainer's recommendations and supervisions unassessed. Also, we did not explore whether ChatGPT can synthesise contextual information, for example, modifying training based on physical readiness. Nevertheless, ChatGPT offered no potential real-time adjustments, or revisions to training protocols, based on feedback or individual progression. Intuitively, practitioners with a sound understanding of resistance training remain best suited to adjusting these variables. Currently, we propose that ChatGPT cannot replace the judgement and empathy of a human practitioner and gaps in acknowledging recent advancements in related research are evident.

CONCLUSIONS

ChatGPT generated realistic information for resistance training, guided by user prompts. However, the suggested programme may require modification. GPT4.0_{ADV} provided greater detail and consideration of training status when prescribing training. As artificial intelligence technologies develop over time, future versions may enhance the user experience. Further exploration and validation of ChatGPT-generated training programmes in real-world settings and with actual athletes is warranted to ascertain their practical utility.

Practical Applications

- ChatGPT can accelerate idea generation and detailed resistance training prescription.
- ChatGPT produced credible information, which may be suitable for general exercise guidance. However, additional professional assistance appears necessary for optimal outcomes.
- During isolating circumstances such as the COVID-19 pandemic, the use of a ChatGPT 'chatbot' for training prescription may help bridge information gaps.
- ChatGPT should be used as a supplementary tool (not a replacement) and combining artificial intelligence with human expertise may optimise exercise prescription effectiveness.

Conflict of interest declaration

The authors declare no conflict of interest.

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SUPPLEMENTARY FILE 1. ChatGPT generated texts for the 12 weeks intermediate and advanced resistance training programmes.

GPT3.5	GPT4.0	GPT4.0
Intermediate programme	**Intermediate programme**	**Advanced programme**
PROMPT 1	**PROMPT 1**	**PROMPT 1**
Weeks 1–4: Foundation Phase	**Weeks 1–4 (High Volume)**	**Weeks 1–4 (Accumulation Phase)**
		(Increasing volume)
Day 1: Upper Body	Day 1 (Chest, Shoulders, Triceps):	Frequency: 4 days a week.
Bench Press: 3 sets of 6–8 reps	Bench Press: 3 sets of 12 reps	
Bent-over Rows: 3 sets of 6–8 reps	Overhead Press: 3 sets of 12 reps	Day 1: Lower Body
Overhead Shoulder Press: 3 sets of 6–8 reps	Dumbbell Fly: 3 sets of 12 reps	Squat – 4 sets × 8 reps
Bicep Curls: 2 sets of 10 reps	Lateral Raise: 3 sets of 12 reps	Deadlift – 3 sets × 8 reps
Tricep Dips: 2 sets of 10 reps	Tricep Pushdown: 3 sets of 12 reps	Bulgarian Split Squats – 3 sets × 10 reps per leg
		Romanian Deadlifts – 3 sets × 10 reps
Day 2: Lower Body	Day 2 (Legs, Abs):	Calf Raises – 4 sets × 12 reps
Squats: 3 sets of 6–8 reps	Squat: 3 sets of 12 reps	
Deadlifts: 3 sets of 6–8 reps	Deadlift: 3 sets of 12 reps	Day 2: Upper Body
Leg Press: 3 sets of 8–10 reps	Leg Press: 3 sets of 12 reps	Bench Press – 4 sets × 8 reps
Leg Curls: 2 sets of 10 reps	Calf Raises: 3 sets of 12 reps	Barbell Rows – 4 sets × 8 reps
Calf Raises: 2 sets of 12 reps	Plank: 3 sets of 60 seconds	Overhead Press – 3 sets × 10 reps
		Pull-Ups – 3 sets × 8 reps (add weight if necessary)
PROMPT 2	Day 3 (Rest)	Dips – 3 sets × 10 reps (add weight if necessary)
Day 1: Upper Body	Day 4 (Back, Biceps):	Day 3: Rest or Active Recovery
Load: 70–75%; Rest: 2 mins; Tempo: 2:0:2	Pull-up: 3 sets of 12 reps	(e.g., light cardio, stretching)
Load: 70–75%; Rest: 2 mins; Tempo: 2:0:2	Bent Over Row: 3 sets of 12 reps	
Load: 70–75%; Rest: 2 mins; Tempo: 2:0:2	Lat Pulldown: 3 sets of 12 reps	Day 4: Lower Body
Load: 60–65%; Rest: 1.5 mins; Tempo: 2:0:2	Barbell Curl: 3 sets of 12 reps	Front Squat – 3 sets × 8 reps
Load: Bodyweight; Rest: 1.5 mins; Tempo: 2:0:2	Hammer Curl: 3 sets of 12 reps	Sumo Deadlift – 3 sets × 8 reps
		Lunges – 3 sets × 10 reps per leg
Day 2: Lower Body	Day 5 (Rest)	Glute Bridge – 3 sets × 12 reps
Load: 70–75%; Rest: 2 mins; Tempo: 2:0:2		Seated Calf Raise – 4 sets × 12 reps
Load: 70–75%; Rest: 2 mins; Tempo: 2:0:2	Day 6 & 7 (Repeat Day 1 & 2)	
Load: 65–70%; Rest: 1.5 mins; Tempo: 2:0:2		Day 5: Upper Body
Load: 60–65%; Rest: 1.5 mins; Tempo: 2:0:2	**PROMPT 2**	Incline Bench Press – 4 sets × 8 reps
Load: 60–65%; Rest: 1 min; Tempo: 2:0:2		Pull-Ups (wide grip) – 4 sets × 8 reps
	Load intensity: 60–70% of one-rep max (1RM)	Dumbbell Shoulder Press – 3 sets × 10 reps
	Rest interval: 60–90 seconds between sets	Barbell Curl – 3 sets × 10 reps
	Exercise tempo: 2–1–2 (2 seconds for the eccentric or lowering phase, 1 second pause, 2 seconds for the concentric or lifting phase)	Skull Crushers – 3 sets × 10 reps
		Day 6 & 7: Rest or Active Recovery
	Day 1 (Chest, Shoulders, Triceps):	
	Bench Press: 3 sets of 12 reps	**PROMPT 2**
	Overhead Press: 3 sets of 12 reps	
	Dumbbell Fly: 3 sets of 12 reps	Intensity: ~70–80% of 1RM
	Lateral Raise: 3 sets of 12 reps	
	Tricep Pushdown: 3 sets of 12 reps	Rest Interval: 60–90 seconds between sets
	Repeat for days 2–7 as above.	Tempo: 3–0–1–0 (3 seconds eccentric, 0 pause at the bottom, 1 second concentric, 0 pause at the top)

SUPPLEMENTARY FILE 1. Continue.

GPT3.5	GPT4.0	GPT4.0
Intermediate programme	**Intermediate programme**	**Advanced programme**
Cont. **PROMPT 1**	***Cont.*** **PROMPT 1**	***Cont.*** **PROMPT 1**
Weeks 5–8: Strength Building Phase	**Weeks 5–8 (Moderate Volume/High Intensity)**	**Weeks 5–8: Intensification Phase** (Increasing intensity)
Day 1: Upper Body Bench Press: 4 sets of 5–6 reps Pull-Ups/Assisted Pull-Ups: 4 sets of 6–8 reps Seated Shoulder Press: 4 sets of 5–6 reps Barbell Bicep Curls: 3 sets of 8 reps Skull Crushers: 3 sets of 8 reps	Increase weights and lower reps to focus on strength. Day 1 (Chest, Shoulders, Triceps): Bench Press: 4 sets of 8 reps Overhead Press: 4 sets of 8 reps Dumbbell Fly: 4 sets of 8 reps Lateral Raise: 4 sets of 8 reps Tricep Pushdown: 4 sets of 8 reps	Frequency: 4 days a week. Day 1: Lower Body Squat – 5 sets × 5 reps Deadlift – 4 sets × 5 reps Bulgarian Split Squats – 4 sets × 8 reps per leg Romanian Deadlifts – 4 sets × 8 reps Calf Raises – 5 sets × 10 reps
Day 2: Lower Body Back Squats: 4 sets of 5–6 reps Romanian Deadlifts: 4 sets of 5–6 reps Leg Press: 3 sets of 6–8 reps Leg Extensions: 3 sets of 8 reps Standing Calf Raises: 3 sets of 10 reps	Day 2 (Legs, Abs): Squat: 4 sets of 8 reps Deadlift: 4 sets of 8 reps Leg Press: 4 sets of 8 reps Calf Raises: 4 sets of 8 reps Plank: 4 sets of 60 seconds	Day 2: Upper Body Bench Press – 5 sets × 5 reps Barbell Rows – 5 sets × 5 reps Overhead Press – 4 sets × 6 reps Weighted Pull-Ups – 4 sets × 6 reps Weighted Dips – 4 sets × 8 reps
Cont. **PROMPT 2** Day 1: Upper Body Load: 75–80%; Rest: 2 mins; Tempo: 2:0:2 Load: Bodyweight; Rest: 2 mins; Tempo: 2:0:2 Load: 75–80%; Rest: 2 mins; Tempo: 2:0:2 Load: 65–70%; Rest: 1.5 mins; Tempo: 2:0:2 Load: 65–70%; Rest: 1.5 mins; Tempo: 2:0:2	Day 3 (Rest) Day 4 (Back, Biceps): Pull-up: 4 sets of 8 reps Bent Over Row: 4 sets of 8 reps Lat Pulldown: 4 sets of 8 reps Barbell Curl: 4 sets of 8 reps Hammer Curl: 4 sets of 8 reps	Day 3: Rest or Active Recovery Day 4: Lower Body Front Squat – 4 sets × 6 reps Sumo Deadlift – 4 sets × 6 reps Lunges – 4 sets × 8 reps per leg Glute Bridge – 4 sets × 10 reps Seated Calf Raise – 5 sets × 10 reps
Day 2: Lower Body Load: 75–80%; Rest: 2 mins; Tempo: 2:0:2 Load: 75–80%; Rest: 2 mins; Tempo: 2:0:2 Load: 70–75%; Rest: 1.5 mins; Tempo: 2:0:2 Load: 65–70%; Rest: 1.5 mins; Tempo: 2:0:2 Load: 65–70%; Rest: 1 min; Tempo: 2:0:2	Day 5 (Rest) Day 6 & 7 (Repeat Day 1 & 2) ***Cont.*** **PROMPT 2** Load intensity: 70–80% of 1RM Rest interval: 90–120 seconds between sets Exercise tempo: 2–0–2 Day 1 (Chest, Shoulders, Triceps): Bench Press: 4 sets of 8 reps Overhead Press: 4 sets of 8 reps Dumbbell Fly: 4 sets of 8 reps Lateral Raise: 4 sets of 8 reps Tricep Pushdown: 4 sets of 8 reps Repeat for days 2–7 as above.	Day 5: Upper Body Incline Bench Press – 5 sets × 5 reps Pull-Ups (wide grip) – 5 sets × 6 reps Dumbbell Shoulder Press – 4 sets × 6 reps Barbell Curl – 4 sets × 8 reps Skull Crushers – 4 sets × 8 reps Day 6 & 7: Rest or Active Recovery ***Cont.*** **PROMPT 2** Intensity: ~80–90% of 1RM Rest Interval: 2–3 minutes between sets Tempo: 2–0–1–0

SUPPLEMENTARY FILE 1. Continue.

GPT3.5	GPT4.0	GPT4.0
Intermediate programme	**Intermediate programme**	**Advanced programme**
Cont. **PROMPT 1**	***Cont.*** **PROMPT 1**	***Cont.*** **PROMPT 1**
Weeks 9–12: Intensification Phase	**Weeks 9–12 (Low Volume/Very High Intensity)**	**Weeks 9–12: Realization Phase** (Testing new strength levels)
Day 1: Upper Body	Further increase weights and decrease reps to peak strength.	Frequency: 4 days a week.
Incline Bench Press: 4 sets of 4–5 reps		
Bent-over Rows (Barbell/D'bell): 4 sets of 4–5 reps		
Arnold Press: 3 sets of 6 reps	Day 1 (Chest, Shoulders, Triceps):	Day 1: Lower Body
Barbell Curls: 3 sets of 6 reps	Bench Press: 5 sets of 5 reps	Squat – 6 sets × 3 reps
Tricep Rope Pushdowns: 3 sets of 6 reps	Overhead Press: 5 sets of 5 reps	Deadlift – 5 sets × 3 reps
	Dumbbell Fly: 5 sets of 5 reps	Bulgarian Split Squats – 4 sets × 6 reps per leg
Day 2: Lower Body	Lateral Raise: 5 sets of 5 reps	Romanian Deadlifts – 4 sets × 6 reps
Front Squats: 4 sets of 4–5 reps	Tricep Pushdown: 5 sets of 5 reps	Calf Raises – 5 sets × 8 reps
Sumo Deadlifts: 4 sets of 4–5 reps		
Bulgarian Split Squats: 3 sets of 6 reps per leg	Day 2 (Legs, Abs):	Day 2: Upper Body
Hamstring Curls: 3 sets of 6 reps	Squat: 5 sets of 5 reps	Bench Press – 6 sets × 3 reps
Seated Calf Raises: 3 sets of 8 reps	Deadlift: 5 sets of 5 reps	Barbell Rows – 6 sets × 3 reps
	Leg Press: 5 sets of 5 reps	Overhead Press – 5 sets × 4 reps
Cont. **PROMPT 2**	Calf Raises: 5 sets of 5 reps	Weighted Pull-Ups – 5 sets × 4 reps
	Plank: 5 sets of 60 seconds	Weighted Dips – 5 sets × 6 reps
Day 1: Upper Body		
Load: 80–85%; Rest: 2 mins; Tempo: 2:0:2	Day 3 (Rest)	Day 3: Rest or Active Recovery
Load: 80–85%; Rest: 2 minutes; Tempo: 2:0:2		
Load: 70–75%; Rest: 1.5 mins; Tempo: 2:0:2	Day 4 (Back, Biceps):	Day 4: Lower Body
Load: 70–75%; Rest: 1.5 mins; Tempo: 2:0:2	Pull-up: 5 sets of 5 reps	Front Squat – 5 sets × 4 reps
Load: 70–75%; Rest: 1.5 mins; Tempo: 2:0:2	Bent Over Row: 5 sets of 5 reps	Sumo Deadlift – 5 sets × 4 reps
	Lat Pulldown: 5 sets of 5 reps	Lunges – 4 sets × 6 reps per leg
Day 2: Lower Body	Barbell Curl: 5 sets of 5 reps	Glute Bridge – 4 sets × 8 reps
Load: 80–85%; Rest: 2 mins; Tempo: 2:0:2	Hammer Curl: 5 sets of 5 reps	Seated Calf Raise – 5 sets × 8 reps
Load: 80–85%; Rest: 2 mins; Tempo: 2:0:2		
Load: 70–75%; Rest: 1.5 mins; Tempo: 2:0:2	Day 5 (Rest)	Day 5: Upper Body
Load: 70–75%; Rest: 1.5 mins; Tempo: 2:0:2		Incline Bench Press – 6 sets × 3 reps
Load: 65–70%; Rest: 1 min; Tempo: 2:0:2	Day 6 & 7 (Repeat Day 1 & 2)	Pull-Ups (wide grip) – 6 sets × 4 reps
		Dumbbell Shoulder Press – 5 sets × 4 reps
		Barbell Curl – 5 sets × 6 reps
	Cont. **PROMPT 2**	Skull Crushers – 5 sets × 6 reps
	Load intensity: 80–90% of 1RM	Day 6 & 7: Rest or Active Recovery
	Rest interval: 2–3 minutes between sets	
	Exercise tempo: 1–0–1	***Cont.*** **PROMPT 2**
	Day 1 (Chest, Shoulders, Triceps):	Intensity: ~90–95% of 1RM for main lifts, ~80–85% for accessory lifts
	Bench Press: 5 sets of 5 reps	
	Overhead Press: 5 sets of 5 reps	Rest Interval: 3–5 minutes for main lifts, 2–3 minutes for accessory lifts
	Dumbbell Fly: 5 sets of 5 reps	
	Lateral Raise: 5 sets of 5 reps	
	Tricep Pushdown: 5 sets of 5 reps	Tempo: 1–0–1–0
	Repeat for days 2–7 as above.	
Remarks	**Remarks**	**Remarks**

SUPPLEMENTARY FILE 1. Continue.

GPT3.5	GPT4.0	GPT4.0
Intermediate programme	**Intermediate programme**	**Advanced programme**
Cont. **PROMPT 1 & 2**	***Cont.*** **PROMPT 1**	***Cont.*** **PROMPT 1**
General Guidelines: – Perform the program 3 days a week, with at least one rest day between sessions. – Increase the weight when you can complete the recommended reps and sets with proper form. – Rest 2–3 minutes between sets of compound exercises and 1–2 minutes for isolation exercises. – Focus on proper form and technique to prevent injuries and maximize gains. – Incorporate a 5–10 minute warm-up and cool-down routine before and after each workout. Track your progress to monitor improvements and make necessary adjustments. Remember, adequate nutrition and sufficient rest are essential for recovery and muscle growth. Listen to your body, and if you ever experience pain or discomfort, consult with a fitness professional or healthcare provider.	Note: Always start each workout with a good warm-up, and finish the workout with a cool-down. Adjust weights as needed to ensure they're challenging but don't compromise form. Ensure rest days are taken to promote muscle recovery and growth. Lastly, it's essential to pair this program with a balanced diet that supports your training goals. Please consult with a fitness professional to ensure that these exercises and the program are suitable for your particular circumstances. Before starting any new exercise program, it's always a good idea to consult with a healthcare professional. ***Cont.*** **PROMPT 2** Note: The 1RM should be updated periodically, possibly every 4 weeks, to account for strength increases. Exercise tempo should be adjusted according to individual comfort and safety. Always ensure proper form to prevent injury. Remember that recovery is crucial – do not skip rest days. Consult a professional for individual advice. Again, ensure that you're using proper form and technique throughout the program, and if you have any concerns or questions, consult with a certified fitness professional or healthcare provider.	Note: Warm-up appropriately before each session. Increase the weight if the prescribed reps feel easy. Ensure proper form and technique. Consider adding in supplemental work (abdominal exercises, rotator cuff exercises) and stretching/mobility work on active recovery days. Ensure adequate nutrition and sleep to support recovery. After this 12–week program, you may want to consider taking a deload week, where volume and intensity are reduced to allow for optimal recovery before starting another program or cycle. ***Cont.*** **PROMPT 2** These percentages are starting points. It's essential to listen to one's body and adjust based on how the training feels. If someone finds that a set at a particular percentage feels too easy or hard, adjusting the weight accordingly is important. Proper warm-up, form, and technique are paramount, especially when working with higher intensity loads.

Note: All the texts within this table were generated by ChatGPT. During prompt 2, GPT-3.5 provided the same training programme as prompt 1, which we removed to improve readability, and only the additional variables were shown. GPT-3.5 also provided the same "general guidelines" in prompt 1 and prompt 2. *Training tempo:* a sequence 3:1:2:0 (or 3/1/2/0 or 3-1-2-0) reflects an exercise performed as 3 seconds eccentric, 1 second pause before the next motion, 2 seconds concentric, and 0 or no pause before the next repetition. *Abbreviations:* GPT: Generative Pre-trained Transformer; rep/reps: repetition(s); mins: minutes.

SUPPLEMENTARY FILE 2. GPT3.5 and GPT4.0 generated text specifying reasons of the suggested programme variables.

Intermediate training programme	Intermediate training programme	Advanced training programme
GPT3.5	**GPT4.0**	**GPT4.0**
Foundation Phase (Weeks 1–4) – 6–8 reps: Moderate rep range to build a foundation of strength and muscular endurance. – 70–75% Load Intensity: A balance between challenging the muscles and allowing for proper progression. – 2 minutes Rest Interval: Sufficient time to recover between sets and perform the exercises with adequate intensity. – Tempo: 2:0:2 (2 seconds eccentric, 0–second pause, 2 seconds concentric): Emphasizes controlled movements and muscle tension. Strength Building Phase (Weeks 5–8) – 5–6 reps: Lower rep range for increased intensity to promote strength gains. – 75–80% Load Intensity: Higher intensity to challenge the muscles further as strength improves. – 2 minutes Rest Interval: Sufficient rest for heavy lifting and recovery between sets. – Tempo: 2:0:2: Slower eccentric phase enhances time under tension for greater strength development. Intensification Phase (Weeks 9–12) – 4–5 reps: Lower rep range to focus on maximal strength development. – 80–85% Load Intensity: High intensity to stimulate strength gains in the intermediate lifter. – 2 minutes Rest Interval: Allows for proper recovery between heavy sets. Tempo: 2:0:2: Controlled tempo to maintain muscle tension during the challenging lifts.	1. Periodisation Periodisation is used to structure training into different phases over the course of the 12 weeks. Each phase focuses on different aspects of strength training (high volume, moderate intensity, and high intensity), allowing for gradual adaptation and reducing the risk of overtraining. 2. High Volume (Weeks 1–4) Lower intensity (60–70% 1RM) and higher volume (more repetitions) is used initially to build muscular endurance, improve technique, and prepare the body for the higher intensities to come. 3. Moderate Volume/High Intensity (Weeks 5–8) The intensity is increased to 70–80% of 1RM and volume is reduced to allow for greater strength and muscle size gains, and to further prepare the body for the very high intensity in the final phase. 4. Low Volume/Very High Intensity (Weeks 9–12) In this phase, intensity is further increased to 80–90% of 1RM and volume is further reduced. This focuses on maximizing muscle strength. 5. Rest Intervals: Shorter rest intervals are used during the high-volume phase to keep the intensity moderate and ensure the body is adequately stressed by the higher number of reps. As the program transitions to higher intensity, the rest periods between sets are extended to allow for sufficient recovery and enable the individual to lift more weight.	1. Periodization: Reason: Allows for systematic programming to avoid plateaus and manage fatigue. Benefits: Maximizes adaptation, minimizes overtraining, and allows for targeted training outcomes (e.g., hypertrophy, strength, power). 2. Load Intensity (% of 1RM): Weeks 1–4 (70–80%): Reason: Focus on moderate weight to allow for higher volume and focus on technique. Benefits: Lays a foundation, promoting hypertrophy and preparing tissues for heavier loads. Weeks 5–8 (80–90%): Reason: Increase in weight to shift focus towards pure strength adaptations. Benefits: Neuromuscular adaptations, increased motor unit recruitment. Weeks 9–12 (90–95%): Reason: Heavy weights to realize strength gains and test new strength levels. Benefits: Peak strength adaptation and increased CNS efficiency. 3. Rest Intervals: Weeks 1–4 (60–90 seconds): Reason: Shorter rest due to moderate loads; sufficient for ATP-PCr energy system recovery. Benefits: Enhances hypertrophic adaptations and keeps workout duration reasonable. Weeks 5–8 & 9–12 (2–5 minutes): Reason: As intensity increases, more recovery time is needed between sets. Benefits: Allows for full recovery and maximal effort in subsequent sets, optimizing strength gains.

SUPPLEMENTARY FILE 2. Continue.

Intermediate training programme	Intermediate training programme	Advanced training programme
GPT3.5	**GPT4.0**	**GPT4.0**
	6. Exercise Tempo: A slower tempo (2–1–2) is used during the high volume phase to increase time under tension, improving muscular endurance and promoting technique. As the program progresses to higher intensities, the tempo is increased slightly to allow for heavier weights to be lifted. 7. Exercise Selection: The program includes compound movements, which work multiple muscle groups at once. This is efficient and effective for improving overall strength. Exercise order is arranged in a way that larger muscle groups are worked before smaller ones. 8. 1RM Updates: Regularly updating the 1RM ensures the training load is adjusted as strength increases. This keeps the intensity appropriate for the desired training effect. 9. Rest and Recovery: Rest days are crucial for muscle recovery and growth. They also prevent overtraining and reduce the risk of injuries. Remember, while this plan provides a general guideline, it should be individualized to meet specific needs and goals. The person should also consider their diet, hydration, and sleep as these factors greatly affect the outcome of the training program. Always consult a professional to ensure a safe and effective training plan.	4. Tempo (eccentric-pause-concentric-pause): Weeks 1–4 (3–0–1–0): Reason: Emphasis on eccentric phase promotes muscle damage, a key driver for hypertrophy. Benefits: Maximizes time under tension (TUT) and promotes hypertrophy. Weeks 5–8 & 9–12 (2–0–1–0 & 1–0–1–0): Reason: As weight increases, it's challenging to maintain a slow eccentric; focus shifts to moving the weight effectively. Benefits: Allows for handling of heavier loads and optimal force production. 5. Exercise Selection: Reason: Compound movements are prioritized as they recruit multiple muscle groups, allowing for heavier loads and maximizing strength adaptations. Benefits: Functional strength gains, comprehensive muscle development, and efficient use of training time. 6. Frequency (4 days a week): Reason: Provides a balance between training volume and recovery for an advanced trainee. Benefits: Allows sufficient stimuli for adaptation while ensuring adequate recovery, reducing the risk of overtraining. 7. Active Recovery Days: Reason: Active recovery can promote blood flow and aid in the removal of waste products. Benefits: Speeds up recovery, mitigates soreness, and reduces the risk of overuse injuries. These variables, when combined systematically, aim to provide an effective platform for advanced strength adaptations while managing fatigue and injury risk.

Note: All the texts within this table were generated by ChatGPT. *Training tempo:* a sequence 3:1:2:0 (or 3/1/2/0 or 3-1-2-0) reflects an exercise performed as 3 seconds eccentric, 1 second pause before the next motion, 2 seconds concentric, and 0 or no pause before the next repetition. *Abbreviations:* GPT: Generative Pre-trained Transformer; rep/reps: repetition(s); mins: minutes;

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Using artificial intelligence for exercise prescription in personalised health promotion: A critical evaluation of OpenAI's GPT-4 model

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ABSTRACT: The rise of artificial intelligence (AI) applications in healthcare provides new possibilities for personalized health management. AI-based fitness applications are becoming more common, facilitating the opportunity for individualised exercise prescription. However, the use of AI carries the risk of inadequate expert supervision, and the efficacy and validity of such applications have not been thoroughly investigated, particularly in the context of diverse health conditions. The aim of the study was to critically assess the efficacy of exercise prescriptions generated by OpenAI's Generative Pre-Trained Transformer 4 (GPT-4) model for five example patient profiles with diverse health conditions and fitness goals. Our focus was to assess the model's ability to generate exercise prescriptions based on a singular, initial interaction, akin to a typical user experience. The evaluation was conducted by leading experts in the field of exercise prescription. Five distinct scenarios were formulated, each representing a hypothetical individual with a specific health condition and fitness objective. Upon receiving details of each individual, the GPT-4 model was tasked with generating a 30-day exercise program. These AI-derived exercise programs were subsequently subjected to a thorough evaluation by experts in exercise prescription. The evaluation encompassed adherence to established principles of frequency, intensity, time, and exercise type; integration of perceived exertion levels; consideration for medication intake and the respective medical condition; and the extent of program individualization tailored to each hypothetical profile. The AI model could create general safety-conscious exercise programs for various scenarios. However, the AI-generated exercise prescriptions lacked precision in addressing individual health conditions and goals, often prioritizing excessive safety over the effectiveness of training. The AI-based approach aimed to ensure patient improvement through gradual increases in training load and intensity, but the model's potential to fine-tune its recommendations through ongoing interaction was not fully satisfying. AI technologies, in their current state, can serve as supplemental tools in exercise prescription, particularly in enhancing accessibility for individuals unable to access, often costly, professional advice. However, AI technologies are not yet recommended as a substitute for personalized, progressive, and health condition-specific prescriptions provided by healthcare and fitness professionals. Further research is needed to explore more interactive use of AI models and integration of real-time physiological feedback.

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INTRODUCTION

The growth of artificial intelligence (AI) has generated countless possibilities in various fields, including healthcare and fitness [1–4]. With the ability to process vast amounts of data and generate personalised recommendations, AI has emerged as a captivating and promising tool in exercise prescription [1]. However, despite the rapid development and implementation of AI-driven fitness applications, the ability of these technologies to deliver personalised, effective and, most importantly, safe exercise regimens for individuals with specific health conditions involving varying severity or co-morbidities remains largely unexplored. Adherence to physical activity (PA) guidelines is indisputably a key determinant of health, with a profound impact on the prevention and

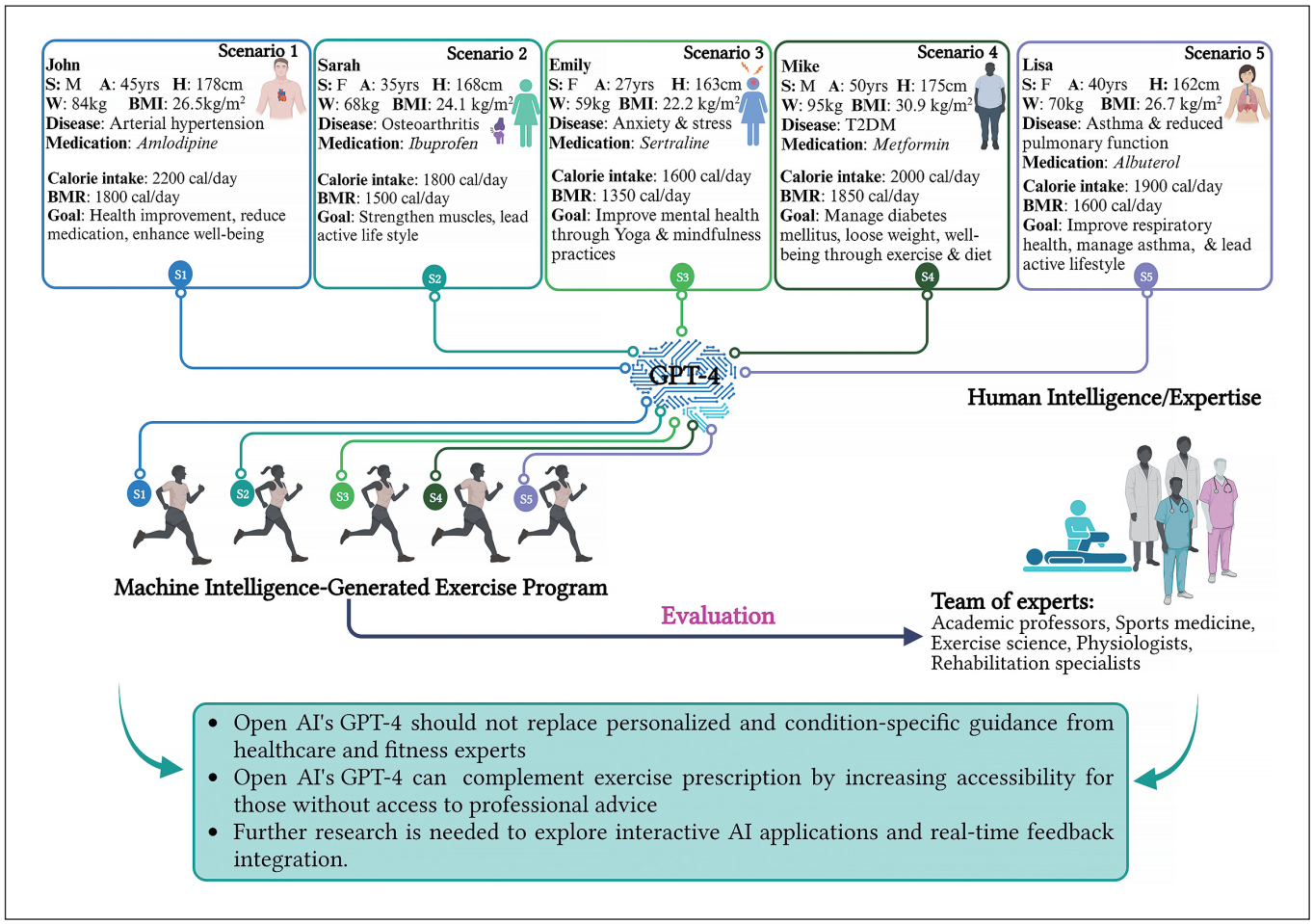


FIG. 1. Graphical Abstract AI-sports

management of a range of health conditions, including cardiovascular diseases [5], respiratory diseases [6], diabetes mellitus [7], mental health disorders (eg; anxiety [8, 9], depression [10]), and Alzheimer disease [11, 12]. However, exercise prescription is not a one-size-fits-all approach or solution [13]. Numerous factors, such as individual health status, age, lifestyle, and personal fitness goals, influence the effectiveness and safety of an exercise regimen, thereby necessitating personalised exercise programs [13]. While a multitude of fitness applications aiming to offer a degree of individualisation in exercise prescription have entered the market [14, 15], they often fail to encompass the complexity of specific health conditions and the influence of medications [16], and an individual's psychosocial factors [17]. Accordingly, this scenario represents a notable limitation of these applications in their current form, potentially restricting their safe usability for individuals with specific health concerns.

The emergence of AI chatbots shows promise in filling the need for more automated exercise prescriptions. By integrating large-scale data analytics with machine learning algorithms, these AI-powered systems can generate personalised recommendations in various fields, including nutrition [18], mental health [19, 20], and fitness [4, 21]. Nevertheless, these technologies also harbour their own set of limitations [22–24], such as the lack of empathetic human touch, privacy concerns, and challenges in transforming broad guidelines into individualised recommendations.

The aim of the study was to critically assess the efficacy of exercise prescriptions generated by OpenAI's Generative Pre-Trained Transformer 4 (GPT-4) model. These prescriptions, tailored for five hypothetical individuals with diverse health conditions and fitness goals, were subjected to evaluation by several leading experts in exercise prescription.

MATERIALS AND METHODS

This research was a simulation study designed to assess the potential of OpenAI's GPT-4 model in producing individualised exercise programs for hypothetical patients with diverse health conditions and fitness goals. The study comprised four main phases: i) Scenario Creation, ii) GPT-4 prompt, iii) Exercise program generation (presented in the results section) and iv) Expert evaluation (presented in the Discussion section).

Scenario Creation

Five hypothetical patient profiles were created by the principal investigators (ID, HBS, KC in the authors' list) with formal training and expertise in sports medicine, exercise science, and exercise physiology (Figure 1). The profiles included variables such as sex, age, height, weight, body mass index (BMI), medical conditions, medications intake, mean calorie intake, basal metabolic rate (BMR, estimated using the Harris-Benedict equations for females [25] and males [26]), motivations (ie; specific fitness goals), level of PA, and profession or occupation. The scenarios were intentionally diverse to represent a wide range of health conditions and fitness objectives prevalent in the general population.

GPT-4 Prompt

We tasked GPT-4 with crafting a consistent fitness program suited for five distinct scenarios. The unified directive was as follows: *"Create a well-rounded 30-day fitness program table that seamlessly integrates workout sessions and rest days. To optimize the effectiveness of this routine, pay special attention to the FITT concept, which dictates the Frequency (how often), Intensity (how hard), Time (how long), and Type (what kind) of exercises to incorporate. Use the rate of perceived exertion (RPE) scale, a measure of how intense the exercise feels, to tailor the program to individual comfort levels. For the cardiovascular exercises, apply heart rate (HR) monitoring, a reliable method to adjust the exercise intensity, ensuring it is just right for each individual. For the strength-building exercises, adopt the one repetition maximum (1RM) principle, a standard that indicates the maximum weight someone can lift for one repetition, to set the appropriate workout intensity."*

Exercise Program Generation and Evaluation

Upon generating the programs, a comprehensive evaluation was undertaken by a panel of experts in exercise prescription. This distinguished panel comprised academic professors and doctors from the fields of sports medicine, exercise science, clinical exercise physiology, and rehabilitation.

The evaluators were from a diverse set of twenty countries, including: Australia, the Basque Country, Brazil, Chile, China, France, Iran, Italy, Japan, Jordan, Malaysia, Mexico, Norway, Poland, Qatar, Syria, Switzerland, Tunisia, the United Kingdom, the United Arab Emirates, and the United States of America. This broad geographic representation ensured a reflection of varied national norms and practices in exercise prescription. Each evaluator held a Ph.D. in exercise science and/or medicine and occupied a significant role in academia, with many serving as editors, associate editors or editor-in-chief for one or more esteemed academic journals in the field.

To mitigate subjective judgments and enhance the study's objectivity, we intentionally selected evaluators from different countries, backgrounds, and educational experiences. We aimed to minimize subjectivity, ensuring a comprehensive and accurate evaluation by consolidating feedback from all contributors. Furthermore, to bolster the study's reliability, we followed the criteria of "Defining the Role of Authors and Contributors" as outlined by the International Committee of Medical Journal Editors guidelines [27]. Only those who made substantial contributions to the study were listed as authors. Contributors who made less significant contributions or improvements to the manuscript were duly acknowledged.

In the methodology of our research, we adopted an innovative approach consistent with the study's pioneering spirit. While conventional methodologies favour traditional evaluation scales, we intentionally chose a quasi-qualitative direction. This decision was made to benefit from the collective expertise and insights of our international panel of experts in exercise prescription. Rather than restricting ourselves to a quantitative scale, our methodology leaned towards this richer approach, aptly aligning with the unique scope of our study. It is pertinent to note a potential limitation here: the absence of a traditional scale, which some might anticipate in evaluations of this nature. Under the supervision of lead investigators (ID, HBS, and KC in the authors' list), our evaluation strategy was subjected to a rigorous three-stage refinement process. The initial phase involved sharing a draft with co-authors for feedback. This was followed by stages of iterative refinement based on collective insights, and the process concluded with a final review for accuracy and completeness. For brevity, certain GPT-4 outputs were reformatted into 10 tables without any other modification.

Single-Prompt Approach: Our Methodology Behind AI Interaction

In the process of generating exercise programs using the GPT-4 model, our approach was designed to mirror a typical patient experience with ChatGPT. The prompts provided to the AI model contained basic information that any sports enthusiast or beginner might readily find on the internet. This methodology was chosen to simulate a real-world scenario where individuals might seek exercise advice without the benefit of multiple rounds of expert interaction.

As sports scientists and exercise medicine specialists, we recognize that iterative interactions with the model, refining and adjusting inputs, could potentially yield a more precise and tailored exercise program. Such a program would inevitably reflect the unique background, expertise, and preferences of a practicing sports scientist. However, two primary reasons guided our decision against this approach: i) Iterative interactions were not the primary objective of our study. Our focus was on assessing the model's capability to generate exercise prescriptions based on a singular, initial interaction, akin to a typical, lay person user experience; and ii) the scope and length of our study, already extensive (with over 7700 words and 10 tables), limited the feasibility of conducting multiple rounds of interactions and evaluations for each scenario. Engaging in such a detailed iterative process would have been feasible for a single case report, but not for the five diverse scenarios we chose to present in this study. For clarity and precision, it is vital to note that the creation of the scenarios and the responses from GPT-4 occurred on 25 June 2023. Given that ChatGPT's performance can evolve over time, underscoring this specific date ensures the context and accuracy of our study are well-documented.

RESULTS

Scenario 1

A. Author Prompt

John – Cardiovascular Health Focus

- Sex: Male
- Age: 45 years
- Height: 178 cm
- Weight: 84 kg
- BMI: 26.5 kg/m^2
- Medical Condition: Arterial hypertension
- Medications: John is currently taking *Amlodipine*, a calcium channel blocker, to manage his blood pressure.
- Mean calorie intake: 2,200 calories per day
- BMR: Approximately 1,800 calories per day
- Motivation: Improve cardiovascular health, reduce reliance on medication, and enhance overall well-being through regular PA.

B. GPT-4 response

Given John's hypertensive condition and goals, a combination of cardio, strength, and flexibility training would be beneficial. Cardio exercise helps lower blood pressure, strength training can aid in weight management, and flexibility exercises improve overall body functions. All exercises should be performed at a moderate intensity (RPE 4–6 out of 10). Here is a sample 30-day exercise program (Table 1). This program alternates between cardio- and strength-training days, with one day of rest every third day. The "strength training (whole body)" refers to a mix of exercises that target all major muscle groups. For example, it could include squats, lunges, push-ups, seated rowing, and core exercises. As always, John should consult with his doctor before starting this or any other exercise program. He should also monitor his blood pressure regularly and pay attention to how he feels during exercise, especially if he experiences any discomfort or unusual symptoms.

TABLE 1. Scenario 1: John's 30-day exercise program generated by generative pre-trained transformer 4.

Day	Exercise	Duration (min)	Intensity
1	Walking	30	Moderate (60–70% MHR)
2	Strength Training (Whole body)	30	Moderate (60–75% 1RM)
3		Rest day	
4	Cycling	30	Moderate (60–70% MHR)
5	Strength Training (Whole body)	30	Moderate (60–75% 1RM)
6		Rest day	
7	Walking	30	Moderate (60–70% MHR)
8	Strength Training (Whole body)	30	Moderate (60–75% 1RM)
9		Rest day	
10	Cycling	30	Moderate (60–70% MHR)
11	Strength Training (Whole body)	30	Moderate (60–75% 1RM)
12		Rest day	
13	Walking	30	Moderate (60–70% MHR)
14	Strength Training (Whole body)	30	Moderate (60–75% 1RM)
15		Rest day	
16	Cycling	30	Moderate (60–70% MHR)
17	Strength Training (Whole body)	30	Moderate (60–75% 1RM)
18		Rest day	
19	Walking	30	Moderate (60–70% MHR)
20	Strength Training (Whole body)	30	Moderate (60–75% 1RM)
21		Rest day	
22	Cycling	30	Moderate (60–70% MHR)
23	Strength Training (Whole body)	30	Moderate (60–75% 1RM)
24		Rest day	
25	Walking	30	Moderate (60–70% MHR)
26	Strength Training (Whole body)	30	Moderate (60–75% 1RM)
27		Rest day	
28	Cycling	30	Moderate (60–70% MHR)
29	Strength Training (Whole body)	30	Moderate (60–75% 1RM)
30		Rest day	

1RM: One repetition maximum. MHR: Maximal heart rate.

C. Interaction with GPT-4

C.1. Author Prompt

Could you consider elaborating on the workout sessions, perhaps by detailing changes in intensity within a single session? Also, it might be beneficial to incorporate the RPE scale as an additional measure of exercise intensity.

C.2. GPT-4's response

Absolutely, I can definitely provide a more detailed session breakdown. I will provide an example for a walking session and a strength training session (Table 2).

Scenario 2

A. Author Prompt

Sarah – Musculoskeletal Strength Emphasis

- Sex: Female
- Age: 35 years
- Height: 168 cm
- Weight: 68 kg
- BMI: 24.1 kg/m^2
- Medical condition: Osteoarthritis
- Medications: Sarah occasionally takes over-the-counter anti-inflammatory medication, like *Ibuprofen*, to alleviate joint discomfort.
- Mean calorie intake: 1,800 calories per day
- BMR: Approximately 1,500 calories per day
- Motivation: Strengthen muscles to support joints, overcome physical challenges, and lead a more active lifestyle.

B. GPT-4's response

Given Sarah's osteoarthritis condition, a combination of strength training, low-impact cardio, and flexibility exercises would be beneficial. Strength training can help strengthen the muscles surrounding her joints, which can alleviate some of the pressure and reduce pain. Low-impact cardio exercises are easier on the joints while helping improve cardiovascular health. Flexibility exercises can help maintain joint mobility and function. Here is a sample 30-day exercise program, including more detailed session breakdowns for strength and cardio sessions (Table 3). "Strength Training (Whole body)" refers to a mix of exercises that target all major muscle groups, focusing on controlled movements to avoid joint strain. For example, it could include leg press, seated row, chest press, lat pull-down, and core exercises.

For strength training and swimming (Table 4), let's use a similar structure to the previous detailed sessions but adapted for Sarah. Sarah should adjust the intensity of her exercises based on her own perceived exertion, comfort level, and any joint pain. As always, she should consult with her doctor before starting this or any other exercise program, especially due to her osteoarthritis condition.

Scenario 3

A. Author Prompt

Emily – Mental Health and Flexibility Target

- Sex: Female
- Age: 27 years
- Height: 163 cm
- Weight: 59 kg
- BMI: 22.2 kg/m^2
- Medical condition: Anxiety and stress-related issues

TABLE 2. Scenario 1: Interaction with generative pre-trained transformer 4 (GPT-4).

Detailed walking session generated by GPT-4			
Time (min)	**Activity**	**Intensity**	**Rate of perceived exertion**
0–5	Warm-up: slow walking	Low (50% MHR)	2 to 3
5–10	Walking	Moderate (60% MHR)	4 to 5
10–20	Walking	Moderate-High (70% MHR)	6 to 7
20–25	Walking	Moderate (60% MHR)	4 to 5
25–30	Cool-down: slow walking	Low (50% MHR)	2 to 3
Detailed strength training session (whole body) generated by GPT-4			
Time (min)	**Exercise**	**Sets × Reps (second); Intensity**	**Rate of perceived exertion**
0–5	Warm-up: light cardio, dynamic stretching	Not applied; Low	2 to 3
5–10	Squats	3 × 10; Moderate (60% 1RM)	5 to 6
10–15	Lunges	3 × 10; Moderate (60% 1RM)	5 to 6
15–20	Push-ups	3 × 10; Moderate (60% 1RM)	5 to 6
20–25	Seated Row	3 × 10; Moderate (60% 1RM)	5 to 6
25–30	Core exercises (eg; planks)	3 × 30; Moderate	5 to 6
30–35	Cool-down: static stretching	Not applied; Low	2 to 3

1RM: One repetition maximum. MHR: Maximal heart rate.

TABLE 3. Scenario 2: Sarah's 30-day exercise program generated by generative pre-trained transformer 4.

Day	Exercise	Duration (min)	Intensity
1	Strength Training (Whole body)	45	Moderate (60–75% 1RM)
2		Rest day	
3	Low-impact Cardio (Swimming)	30	Moderate (60–70% MHR)
4		Rest day	
5	Strength Training (Whole body)	45	Moderate (60–75% 1RM)
6		Rest day	
7	Low-impact Cardio (Swimming)	30	Moderate (60–70% MHR)
8		Rest day	
9	Strength Training (Whole body)	45	Moderate (60–75% 1RM)
10		Rest day	
11	Low-impact Cardio (Swimming)	30	Moderate (60–70% MHR)
12		Rest day	
13	Strength Training (Whole body)	45	Moderate (60–75% 1RM)
14		Rest day	
15	Low-impact Cardio (Swimming)	30	Moderate (60–70% MHR)
16		Rest day	
17	Strength Training (Whole body)	45	Moderate (60–75% 1RM)
18		Rest day	
19	Low-impact Cardio (Swimming)	30	Moderate (60–70% MHR)
20		Rest day	
21	Strength Training (Whole body)	45	Moderate (60–75% 1RM)
22		Rest day	
23	Low-impact Cardio (Swimming)	30	Moderate (60–70% MHR)
24		Rest day	
25	Strength Training (Whole body)	45	Moderate (60–75% 1RM)
26		Rest day	
27	Low-impact Cardio (Swimming)	30	Moderate (60–70% MHR)
28		Rest day	
29	Strength Training (Whole body)	45	Moderate (60–75% 1RM)
30		Rest day	

1RM: One repetition maximum. MHR: Maximal heart rate.

TABLE 4. Scenario 2: Interaction with generative pre-trained transformer 4 (GPT-4).

Detailed strength training session generated by GPT-4			
Time (min)	**Exercise**	**Sets × Reps (seconds); Intensity**	**Rate of perceived exertion**
0–5	Warm-up: light cardio, dynamic stretching	Not applied; Low	2 to 3
5–10	Leg Press	3 × 10; Moderate (60% 1RM)	5 to 6
10–15	Seated Row	3 × 10; Moderate (60% 1RM)	5 to 6
15–20	Chest Press	3 × 10; Moderate (60% 1RM)	5 to 6
20–25	Lat Pull-Down	3 × 10; Moderate (60% 1RM)	5 to 6
25–30	Core exercises (eg; planks)	3 × 30; Moderate	5 to 6
30–45	Cool-down: static stretching	Not applied; Low	2 to 3
Detailed swimming session generated byGPT-4			
Time (min)	**Activity**	**Intensity**	**Rate of perceived exertion**
0–5	Warm-up: slow swimming	Low (50% MHR)	2 to 3
5–10	Swimming	Moderate (60% MHR)	4 to 5
10–20	Swimming	Moderate-High (70% MHR)	6 to 7
20–25	Swimming	Moderate (60% MHR)	4 to 5
25–30	Cool-down: slow swimming	Low (50% MHR)	2 to 3

1RM: One repetition maximum. MHR: Maximal heart rate.

TABLE 5. Scenario 3: Emily's 30-day exercise program generated by generative pre-trained transformer 4.

Day	Exercise	Duration (min)	Intensity
1	Yoga	45	Moderate
2	Low-impact Cardio (Walking/Cycling)	30	Moderate (60–70% MHR)
3	Yoga	45	Moderate
4		Rest day	
5	Yoga	45	Moderate
6	Low-impact Cardio (Walking/Cycling)	30	Moderate (60–70% MHR)
7	Yoga	45	Moderate
8		Rest day	
9	Yoga	45	Moderate
10	Low-impact Cardio (Walking/Cycling)	30	Moderate (60–70% MHR)
11	Yoga	45	Moderate
12		Rest day	
13	Yoga	45	Moderate
14	Low-impact Cardio (Walking/Cycling)	30	Moderate (60–70% MHR)
15	Yoga	45	Moderate
16		Rest day	
17	Yoga	45	Moderate
18	Low-impact Cardio (Walking/Cycling)	30	Moderate (60–70% MHR)
19	Yoga	45	Moderate
20		Rest day	
21	Yoga	45	Moderate
22	Low-impact Cardio (Walking/Cycling)	30	Moderate (60–70% MHR)
23	Yoga	45	Moderate
24		Rest day	
25	Yoga	45	Moderate
26	Low-impact Cardio (Walking/Cycling)	30	Moderate (60–70% MHR)
27	Yoga	45	Moderate
28		Rest day	
29	Yoga	45	Moderate
30	Low-impact Cardio (Walking/Cycling)	30	Moderate (60–70% MHR)

MHR: Maximal heart rate.

TABLE 6. Scenario 3: Interaction with generative pre-trained transformer 4 (GPT-4).

Time (min)	Activity	Intensity	Rate of perceived exertion
	Detailed yoga session generated by GPT-4.		
0–10	Warm-up: simple stretching and breathing exercises	Low	2 to 3
10–35	Yoga: sequences including a mix of standing, seated and supine postures	Moderate	5 to 6
35–45	Cool-down: relaxation and meditation	Low	2 to 3
	Detailed low-impact cardio session (walking/cycling) generated by GPT-4		
0–5	Warm-up: slow walking/cycling	Low (50% MHR)	2 to 3
5–10	Walking/Cycling	Moderate (60% MHR)	4 to 5
10–20	Walking/Cycling	Moderate-High (70% MHR)	6 to 7
20–25	Walking/Cycling	Moderate (60% MHR)	4 to 5
25–30	Cool-down: slow walking/cycling	Low (50% MHR)	2 to 3

MHR: Maximal heart rate.

– Medications: Emily is under the care of a psychiatrist and is prescribed Sertraline, an antidepressant, to manage her anxiety.
– Mean calorie intake: 1,600 calories per day
– BMR: Approximately 1,350 calories per day
– Motivation: Improve mental health, find inner peace, and achieve emotional balance through yoga and mindfulness practices.

B. GPT-4's response

Given Emily's goal of improving mental health and increasing flexibility, incorporating practices such as yoga, meditation, and low-intensity cardio exercise such as walking, or cycling can be highly beneficial. Yoga has been shown to improve flexibility, balance, and strength, while also having positive effects on mental health, reducing anxiety, and stress. Here is a sample 30-day exercise program, including more detailed session breakdowns for yoga and cardio sessions (Table 5).

For yoga and walking/cycling (Table 6), let's use a similar structure to the previous detailed sessions but adapted for Emily. Emily should adjust the intensity of her exercises based on her own perceived exertion, comfort level, and any mental or physical discomfort. As always, she should consult with her doctor before starting this or any other exercise program, especially given her mental health condition.

Scenario 4

A. Author Prompt

Mike – Diabetes mellitus management and weight loss objective

– Sex: Male
– Age: 50 years
– Height: 175 cm
– Weight: 95 kg
– BMI: 30.9 kg/m^2
– Medical Condition: Type 2 diabetes mellitus
– Medications: Mike takes *Metformin*, an oral diabetes medication, to manage his blood sugar levels.
– Mean calorie intake: 2,000 calories per day
– BMR: Approximately 1,850 calories per day
– Motivation: Manage diabetes effectively, lose weight to improve overall health, and enhance well-being through PA and proper nutrition.

B. GPT-4's response

Given Mike's objective of managing his diabetes mellitus and losing weight, a combination of regular cardio exercise, strength training, and dietary adjustments can be very beneficial. Regular PA can help improve insulin sensitivity, while a healthy diet can help manage blood glucose levels and contribute to weight loss. Here is a sample 30-day exercise program, including more detailed session breakdowns for strength and cardio sessions (Table 7). "Strength Training (Whole body)" refers to a mix of exercises that target all major muscle groups, focusing on controlled movements to avoid joint strain. For example, it could include leg press, seated row, chest press, lat pull-down, and core exercises.

For strength training and cardio sessions (Table 8), let's use a similar structure to the previous detailed sessions but adapted for Mike.

Scenario 5

A. Author Prompt

Lisa – Respiratory Health and Pulmonary Function

– Sex: Female
– Age: 40 years
– Height: 162 cm
– Weight: 70 kg
– BMI: 26.7 kg/m^2
– Medical condition: Asthma and reduced pulmonary function
– Medications: Lisa uses an inhaler (Albuterol) as a bronchodilator to manage her asthma symptoms.
– Mean calorie intake: 1,900 calories per day
– BMR: Approximately 1,600 calories per day
– Fitness objective: Improve respiratory health and lung capacity, manage asthma effectively, and lead an active lifestyle.

B. GPT-4's response

Given Lisa's objective of improving her respiratory health and pulmonary function, a combination of regular low-impact cardio and resistance exercises, along with breathing exercises, could be very beneficial. Regular PA can help increase lung capacity, improve cardiovascular health, and enhance overall fitness. Additionally, specific breathing exercises can help strengthen the respiratory muscles, increase lung function, and manage asthma symptoms effectively. Here is a sample 30-day exercise program for Lisa (Table 9). "Resistance Training (Whole body)" refers to a mix of exercises that target all major muscle groups, focusing on controlled movements and proper breathing techniques to avoid respiratory strain.

Here is a detailed breakdown of Lisa's resistance training session (Table 10). For each exercise, Lisa should take slow, deep breaths, exhaling on the exertion phase and inhaling on the return. If she feels any shortness of breath, she should slow down or take a break, and use her inhaler if necessary. Breathing exercises are also an integral part of her program. Pursed-lip breathing involves inhaling through the nose and exhaling slowly through puckered lips, while diaphragmatic breathing focuses on fully engaging the diaphragm, not just the chest, during breaths. These exercises can help increase lung capacity and improve respiratory muscle function, thereby helping to manage asthma symptoms. As always, she should consult with her doctor before starting this or any other exercise program, especially given her asthma condition.

DISCUSSION

The primary objective of this study was to critically assess the efficacy of exercise prescriptions generated by OpenAI's GPT-4 model for five hypothetical patient profiles with diverse health conditions

TABLE 7. Scenario 4: Mike's 30-day exercise program generated by generative pre-trained transformer 4.

Day	Exercise	Duration (min)	Intensity
1	Cardio (Walking/Running)	30	Moderate (60–70% MHR)
2	Strength Training (Whole body)	45	Moderate (60–75% 1RM)
3		Rest day	
4	Cardio (Walking/Running)	30	Moderate (60–70% MHR)
5	Strength Training (Whole body)	45	Moderate (60–75% 1RM)
6		Rest day	
7	Cardio (Walking/Running)	30	Moderate (60–70% MHR)
8	Strength Training (Whole body)	45	Moderate (60–75% 1RM)
9		Rest day	
10	Cardio (Walking/Running)	30	Moderate (60–70% MHR)
11	Strength Training (Whole body)	45	Moderate (60–75% 1RM)
12		Rest day	
13	Cardio (Walking/Running)	30	Moderate (60–70% MHR)
14	Strength Training (Whole body)	45	Moderate (60–75% 1RM)
15		Rest day	
16	Cardio (Walking/Running)	30	Moderate (60–70% MHR)
17	Strength Training (Whole body)	45	Moderate (60–75% 1RM)
18		Rest day	
19	Cardio (Walking/Running)	30	Moderate (60–70% MHR)
20	Strength Training (Whole body)	45	Moderate (60–75% 1RM)
21		Rest day	
22	Cardio (Walking/Running)	30	Moderate (60–70% MHR)
23	Strength Training (Whole body)	45	Moderate (60–75% 1RM)
24		Rest day	
25	Cardio (Walking/Running)	30	Moderate (60–70% MHR)
26	Strength Training (Whole body)	45	Moderate (60–75% 1RM)
27		Rest day	
28	Cardio (Walking/Running)	30	Moderate (60–70% MHR)
29	Strength Training (Whole body)	45	Moderate (60–75% 1RM)
30		Rest day	

1RM: One repetition maximum. MHR: Maximal heart rate.

TABLE 8. Scenario 4: Interaction with generative pre-trained transformer 4 (GPT-4).

Detailed strength training session generated by GPT-4			
Time (min)	**Exercise**	**Sets × Reps (second); Intensity**	**Rate of perceived exertion**
0–5	Warm-up: light cardio, dynamic stretching	Not applied; Low	2 to 3
5–10	Leg Press	3 × 10; Moderate (60% 1RM)	5 to 6
10–15	Seated Row	3 × 10; Moderate (60% 1RM)	5 to 6
15–20	Chest Press	3 × 10; Moderate (60% 1RM)	5 to 6
20–25	Lat Pull-Down	3 × 10; Moderate (60% 1RM)	5 to 6
25–30	Core exercises (eg; planks)	3 × 30; Moderate	5 to 6
30–45	Cool-down: static stretching	Not applied; Low	2 to 3
Detailed cardio session (walking/running) generated GPT-4			
Time (min)	**Activity**	**Intensity**	Rate of perceived exertion
0–5	Warm-up: slow walking	Low (50% MHR)	2 to 3
5–10	Walking/Running	Moderate (60% MHR)	4 to 5
10–20	Walking/Running	Moderate-High (70% MHR)	6 to 7
20–25	Walking/Running	Moderate (60% MHR)	4 to 5
25–30	Cool-down: slow walking	Low (50% MHR)	2 to 3

1RM: One repetition maximum. MHR: Maximal heart rate.

TABLE 9. Scenario 1: Lisa's 30-day exercise program generated by generative pre-trained transformer 4.

Day	Exercise	Duration (min)	Intensity
1	Cardio (Swimming)	30	Moderate (60–70% MHR)
2	Breathing Exercises (Pursed-lip and Diaphragmatic Breathing)	15	Low
3	Resistance Training (Whole body)	45	Moderate (60–75% 1RM)
4	Rest day		
5	Cardio (Cycling)	30	Moderate (60–70% MHR)
6	Breathing Exercises (Pursed-lip and Diaphragmatic Breathing)	15	Low
7	Resistance Training (Whole body)	45	Moderate (60–75% 1RM)
8	Rest day		
9	Cardio (Swimming)	30	Moderate (60–70% MHR)
10	Breathing Exercises (Pursed-lip and Diaphragmatic Breathing)	15	Low
11	Resistance Training (Whole body)	45	Moderate (60–75% 1RM)
12	Rest day		
13	Cardio (Cycling)	30	Moderate (60–70% MHR)
14	Breathing Exercises (Pursed-lip and Diaphragmatic Breathing)	15	Low
15	Resistance Training (Whole body)	45	Moderate (60–75% 1RM)
16	Rest day		
17	Cardio (Swimming)	30	Moderate (60–70% MHR)
18	Breathing Exercises (Pursed-lip and Diaphragmatic Breathing)	15	Low
19	Resistance Training (Whole body)	45	Moderate (60–75% 1RM)
20	Rest day		
21	Cardio (Cycling)	30	Moderate (60–70% MHR)
22	Breathing Exercises (Pursed-lip and Diaphragmatic Breathing)	15	Low
23	Resistance Training (Whole body)	45	Moderate (60–75% 1RM)
24	Rest day		
25	Cardio (Swimming)	30	Moderate (60–70% MHR)
26	Breathing Exercises (Pursed-lip and Diaphragmatic Breathing)	15	Low
27	Resistance Training (Whole body)	45	Moderate (60–75% 1RM)
28	Rest day		
29	Cardio (Cycling)	30	Moderate (60–70% MHR)
30	Breathing Exercises (Pursed-lip and Diaphragmatic Breathing)	15	Low

1RM: One repetition maximum. MHR: Maximal heart rate.

TABLE 10. Scenario 5: Detailed resistance training session generated by generative pre-trained transformer 4.

Time (min)	Exercise	Sets × Reps (second); Intensity	Rate of perceived exertion
0–5	Warm-up: light cardio, dynamic stretching	Not applied; Low	2 to 3
5–10	Leg Press	3 × 10; Moderate (60% 1RM)	5 to 6
10–15	Seated Row	3 × 10; Moderate (60% 1RM)	5 to 6
15–20	Chest Press	3 × 10; Moderate (60% 1RM)	5 to 6
20–25	Lat Pull-Down	3 × 10; Moderate (60% 1RM)	5 to 6
25–30	Core exercises (eg; planks)	3 × 30; Moderate	5 to 6
30–45	Cool-down: static stretching	Not applied; Low	2 to 3

1RM: One repetition maximum.

and fitness goals. Our focus was on assessing the model's capability to generate exercise prescriptions based on a singular, initial interaction, akin to a typical user experience. This evaluation was conducted by leading experts in the field of exercise prescription. Through this assessment, we aimed to understand the potential and limitations of AI, specifically GPT-4, in crafting personalised exercise programs, juxtaposing its outputs against the insights and expertise traditionally provided by human professionals in the domain.

Evaluation and Review: Scenario 1 – John

John's case represents a typical middle-aged individual suffering from arterial hypertension, aiming to enhance his cardiovascular health and reduce medication dependence through a structured exercise program. The generated 30-day regimen shows a commendable application of the FITT principles, incorporating a blend of cardiovascular exercises, resistance training, and flexibility routines, all of which are demonstrably beneficial for hypertensive individuals and cardiovascular health in general [28, 29].

The program's heavy reliance on moderate-intensity workouts is in line with recommendations for hypertensive patients, reducing the likelihood of excessive spikes in blood pressure during exercise [30]. This approach aligns with a conservative strategy that prioritizes understanding how the individual reacts to moderate-intensity exercise before exploring more intense options.

While emerging research indicates that high-intensity interval training (HIIT) could offer substantial cardiovascular and hypertensive benefits [31, 32], the decision to exclude HIIT from the initial 30-day program is consistent with a personalized and cautious approach. A human practitioner might rationalize current decisions based on prior experiences, successes, and failures. For instance, if a practitioner had previously observed a patient with similar health conditions to John experiencing adverse reactions to HIIT, they might be more cautious and exclude HIIT from John's program. In this context, the exclusion of HIIT seems prudent, reflecting the practitioner's accumulated knowledge and experience over time. The ChatGPT-formulated program, while based on prevalent information from the Internet, might prioritise information that is more abundant or historically prevalent over newer, potentially more relevant expert insights. This means that while GPT-4 can access a vast amount of information, it may not always prioritise the most recent or expert-endorsed data. As a result, traditional practices with a wealth of online information might be favoured over newer, updated methodologies. This highlights a limitation in the model's ability to discern the quality and relevance of the information it accesses.

Introducing HIIT might be a valuable addition to the program in the future, but only after careful monitoring and assessment of the individual's response to the initial regimen. This approach recognises that exercise prescription can be subjective and based on individual preferences and needs, and emphasises the importance of personalised care in managing hypertensive conditions. A prudent approach also acknowledges the nuanced judgment that human practitioners bring to exercise prescription, balancing evidence-based practice with individualized care.

While the HR-based approach for managing workout intensity is noteworthy, it would have been beneficial to incorporate specific HR zones for each cardio session [33]. For hypertensive patients like John, individualized HR zone training could prove particularly beneficial [34]. A common approach to establishing these zones involves first estimating the predicted maximal HR (MHR) (usually using the "220-age" formula [35]). Then, the Karvonen formula [36] [target training HR = resting HR + (0.6 × (MHR – resting HR))] is applied, followed by calculating different training zones as percentages of this target HR [37]. However, one should be cautious with the use of predicted MHR calculations as several estimation issues should not be overlooked; ideally, the real MHR of the individual should be measured by qualified practitioners [38]. Indeed, if a patient estimates his/her HR zones using an inappropriate formula, it could lead to an inadequate exercise intensity. GPT-4 did not mention any details about how to calculate those HR zones, and predicted MHR. This shortcoming might not only hinder the effectiveness of the exercise program but also pose potential health risks, particularly for individuals with underlying medical conditions such as arterial hypertension. Accurate calculation and understanding of HR zones are essential for tailoring an exercise regimen that is both safe and effective for the individual's specific needs and goals.

Resistance training has sensibly been included in the program, aligning with research demonstrating its positive impact on blood pressure and cardiovascular health [39, 40]. Nonetheless, high-resistance strength training may lead to a significant transient increase in systolic and diastolic blood pressure [41]. Consequently, a program design leaning towards lower resistance with higher repetitions might be a safer alternative for John, while still providing the benefits of resistance training, without the associated acute blood pressure elevation [42].

An evident progression in terms of exercise duration is a strong point of the program. However, it appears to overlook the progression in other crucial areas, such as workout intensity, the weight used in resistance training, and the complexity of exercises. A program incorporating systematic progression in these elements might stimulate continual adaptation and improvement, offering greater benefits for John. Another element seemingly absent from the program is RPE monitoring [43]. RPE could provide a more reliable measure of workout intensity than HR alone [44].

The program effectively integrates rest days, underscoring their importance in facilitating recovery and adaptation. However, the current placement of these rest days, predominantly after strength training sessions, raises questions. Strategically positioning rest days after high-intensity workouts or after a series of consecutive moderate-intensity sessions can optimize muscle recovery and reduce the risk of overtraining [45]. Thus, re-evaluating the sequencing of rest days in this context may enhance the overall effectiveness of the program. Furthermore, it is essential to note the increase in

self-paced exercise recommendations, especially for specific populations. Such exercises allow individuals to adjust their workout intensity based on their comfort and capability, which can be particularly beneficial for those taking certain medications that might affect their physiological response to exercise. The GPT-4 program also seemed limited by meeting (but not surpassing) the PA guideline of 150 minutes per week of moderate to vigorous PAs. The necessity of two rest days per week should also be questioned as some patients may prefer to exercise in smaller "doses" more than 5 times per week.

Additionally, the importance of isometric exercises and varied contraction regimens cannot be overlooked. Different health disorders may necessitate specific contraction patterns to maximize therapeutic benefits. A recent network meta-analysis reported that isometric exercise may provide the largest improvement in resting blood pressure [40]. For instance, isometric exercises, which involve muscle contractions without any significant movement, can be especially beneficial for individuals with certain joint or muscular disorders. The program could benefit from integrating these exercises, tailoring the contraction regimen to the specific needs of the individual's health condition. However, patients should be warned to avoid the Valsalva manoeuvre while performing isometric exercises to avoid any overload on the cardiovascular system, and there were no cautions highlighted on this aspect by the proposed program [46].

Lastly, even if not prompted for, we believe that the proposed program should have touched on the patient diet with at least a list of food to avoid in association with arterial hypertension. Indeed, any clinical practitioner if confronted with suggesting an exercise-based program for hypertensive patient will most probably consider associated risk factors like nutrition or sleep.

The exercise program generated by GPT-4 exhibits a solid grasp of fundamental exercise prescription principles. However, there is room for refinement in areas like individualised intensity measures, systematic progression, nuanced application of resistance training guidelines, and incorporation of RPE as a training monitoring tool. With these adjustments, the program could potentially provide further health benefits for John.

Evaluation and Review: Scenario 2 – Sarah

Sarah, a 35-year-old female diagnosed with osteoarthritis, aspires to augment her musculoskeletal strength, mitigate her joint discomfort, and live a more active lifestyle. The AI model's proposed exercise plan, designed around FITT principles, provides a suitable starting point for such objectives. However, the program warrants a more detailed critique, particularly in the context of osteoarthritis management and the potential therapeutic benefits of PA.

First, the model appropriately prescribes strength-training exercises, a cornerstone of non-pharmacological management for osteoarthritis [47]. Not only does resistance training improve muscular strength, but enhances joint stability and function and alleviating osteoarthritis symptoms [47]. However, the prescription lacks specificity in terms of the intended load, repetitions, and sets for each exercise, which might influence the therapeutic effectiveness.

To illustrate, a typical exercise prescription for someone like Sarah might specify that she should perform leg presses at 60% of her 1RM, for three sets of 10 repetitions, with a 2-minute rest in-between. This level of detail ensures that the exercise is tailored to her current strength level, providing a clear progression path. However, there was no instruction for how she safely determine her 1RM for each exercise. The AI's recommendation might have been more generic, indicating leg presses while specifying the weight, repetitions, and sets. Such a lack of specificity, while more general and perhaps easier to adopt, could however lead to suboptimal results and even potential injury if Sarah were to guess the appropriate weight or overexert herself.

Secondly, the AI model rightly includes flexibility exercises in the program, which are crucial for maintaining joint range of motion and reducing stiffness associated with osteoarthritis [48]. However, the model could have suggested exercises for enhancing proprioception and neuromuscular control exercises, such as balance and agility training, which are often compromised in individuals with osteoarthritis [49].

Regarding cardiovascular exercises, the model adequately introduces low-intensity activities (ie; walking). This aspect is critical considering the load-bearing nature of osteoarthritis, particularly as it affects Sarah's lower limbs. Nevertheless, the model could have suggested non-weight-bearing activities such as cycling, which may reduce joint stress while promoting cardiovascular fitness [50].

Furthermore, the program could have been further individualised by considering the affected joints and tailoring the exercises accordingly. For example, if Sarah's osteoarthritis affects her knees, certain exercises such as deep squats shall be avoided due to excessive knee joint load [51, 52]. It is worth noting that while there were traditional reservations about land-based exercises for individuals with osteoarthritis, recent research and clinical practices have evolved. Land-based exercises, when performed with proper technique and under expert guidance, can be beneficial for osteoarthritis patients [51, 52]. They can help improve joint mobility, muscle strength, and overall functional capacity. Moreover, these exercises can be adapted to ensure minimal joint stress while maximizing therapeutic benefits. For instance, shallow squats or partial range movements can be introduced to reduce undue strain on affected joints. The key lies in the correct execution, progression, and individualisation of these exercises to suit the specific needs and limitations of the osteoarthritis patient. This aspect is overlooked by the model.

The information provided to Sarah also recommended an evaluation by a doctor before commencing her exercise program. This recommendation, which is based on older guidelines, is incorrect since new guidelines support that low-risk patients do not require a screening examination or "medical clearance" to begin an exercise program [53]. Of course, Sarah could pursue this for added

safety, but the GPT-4 model could have presented this option without making it a barrier for those with poor access to care.

Finally, the model logically incorporates rest days into the program, which are essential for promoting tissue healing and recovery, thus reducing the risk of exacerbating Sarah's osteoarthritis symptoms. However, Sarah could engage in light activities such as stretching or yoga on "rest" days, thus promoting flexibility and relaxation, adding value without hampering recovery. A more detailed, tailored, and multifaceted approach that considers the specific nuances of her osteoarthritis could better cater to Sarah's needs, and help her achieve her fitness objectives more effectively and safely.

Evaluation and Review: Scenario 3 – Emily

Emily is a 27-year-old individual grappling with anxiety and stress-related issues, and managed with *Sertraline*, an antidepressant. Her objective was to enhance her mental health, find inner peace, and achieve emotional balance through yoga and mindfulness practices. Given the increasing popularity and frequent requests for yoga as a potential solution for such health conditions, the AI model suggests a detailed yoga-centric program, constructed in adherence to FITT principles. While Yoga is a commonly sought-after intervention, it is essential to acknowledge that many other behavioral and cognitive interventions have shown beneficial results. However, an in-depth analysis reveals some areas for potential improvement. Moreover, the program seems to overlook crucial factors like sleep quality and chronotype, which play a pivotal role in managing anxiety and stress.

The model appropriately emphasizes yoga and meditation, both of which are well-documented for their beneficial effects on mental health, stress reduction, and overall well-being [54]. The model correctly provides for a gradual increase in session length, permitting Emily to adapt progressively to the increasing demands of the program. However, the program could have been improved by providing specific details about the yoga poses and sequences suitable for stress relief, such as restorative or Yin Yoga, along with guidance on proper alignment and modification options to suit Emily's comfort and skill level. This outcome may be possible through links to other resources and instructional videos. In addition, as with scenario 2, the GPT-4 model recommended a medical evaluation before beginning the exercise program which is not necessary in an otherwise healthy young adult.

The prescription of daily yoga sessions, coupled with mindfulness exercises, shows the model's understanding of the importance of consistency in mental health-related physical activities. However, the prescription does not adequately account for the rest and recovery necessary to prevent potential physical and mental fatigue associated with daily yoga practices [55]. Incorporating rest days or days with lighter, restorative practices could potentially improve this aspect of the program.

The model's program also includes a 'perceived exertion' measure, the Borg scale, to self-assess the intensity of the yoga sessions. While this can be a useful tool for cardio-based workouts, it may not be entirely suitable for yoga, where intensity can vary significantly based on the type of Yoga and individual postures [55]. A more appropriate measure might be a discomfort or difficulty scale, particularly suited to the slower pace and holding postures of yoga. Lastly, the model does not incorporate any form of aerobic or strength training exercises in Emily's program [56, 57]. While her primary focus is on mental health and yoga practices, including these forms of exercises could provide complementary benefits. Aerobic exercises, for instance, are known to promote endorphin release, aiding in mood improvement [58], while strength training can boost self-esteem and body image, providing an additional mental health benefit [59].

Evaluation and Review: Scenario 4 – Mike

Mike, a 50-year-old male diagnosed with type 2 diabetes mellitus, aims to manage his disease effectively, lose weight, and improve his overall health and well-being. The AI model recommends a program that combines cardiovascular, strength, and flexibility exercises, abiding by FITT principles. While this program aligns with general recommendations for managing diabetes mellitus and promoting weight loss, it lacks specificity and a more nuanced understanding of exercise prescription in the context of diabetes management.

The model correctly prescribes cardiovascular exercise as a vital component of Mike's program, given its well-documented benefits in enhancing insulin sensitivity, aiding glucose control, and promoting weight loss [60]. However, it misses a crucial point: the potential rare risk of hypoglycemia during and following prolonged aerobic exercise for individuals on glucose-lowering medications [61, 62]. It is essential for patients like Mike, especially if they are on *metformin*, to be cognizant of exercise-induced glucose fluctuations. While *metformin* generally reduces the risk of exercise-induced hypoglycemia due to its mechanism of action on liver glucose production, some nuances need to be considered. A more personalised approach for *metformin* users would involve monitoring blood glucose levels pre- and post-exercise to understand any unexpected fluctuations. Individuals should also be made aware of the potential for gastrointestinal side effects of *metformin*, which might be exacerbated with exercise. As always, it is advisable to consult with a healthcare provider for individualised guidance, especially when initiating a new exercise regimen while on *metformin* [63, 64]. Furthermore, the inclusion of high-intensity low-volume endurance exercises, such as HIIT or moderate-intensity interval training, could offer additional benefits, and should be considered in the exercise prescription.

Strength training exercises are appropriately included, given their role in improving muscle mass and insulin sensitivity, thereby aiding in glucose control [65]. However, the program could be improved by specifying the types of resistance exercises to be performed, their order, and the amount of resistance (in relation to Mike's actual or estimated 1RM), how to safely determine one's 1RM, and facilitating the safe and effective execution of these exercises.

The program's inclusion of flexibility exercises, which aid in maintaining joint health and overall mobility, is also commendable [48].

However, the model does not provide any specific stretching exercises or guidance on how these should be performed, which could be helpful for preventing potential injury [66].

Given that both hypoglycemia and hyperglycemia can occur in individuals with diabetes mellitus due to exercise, education about self-monitoring of blood glucose and guidance on appropriate responses to abnormal glucose levels are vital [67].

Furthermore, the model could have incorporated education about the signs of hypoglycemia and foot care in the exercise program, given that individuals with diabetes mellitus are often at increased risk of hypoglycemia and foot ulcers [68].

Evaluation and Review: Scenario 5 – Lisa

Lisa, a 40-year-old female with asthma and impaired pulmonary function, has the objective of improving her respiratory health and lung capacity. The AI model proposes a program combining cardiovascular, strength, and flexibility exercises, framed within the FITT guidelines. The program captures a broad range of PAs beneficial for overall health; however, the specificity and customization to cater to Lisa's specific condition seem to be lacking in certain aspects.

The model appropriately prescribes cardiovascular exercise, given its proven benefits in enhancing overall cardiorespiratory fitness [69]. However, the program could have been enhanced by incorporating exercises particularly known for their positive impacts on respiratory health [70]. Additionally, considering the benefits of eccentric exercises, which are less stressful on the cardiorespiratory system compared to other contraction regimens, their inclusion could be advantageous for Lisa. The inclusion of strength training is also pertinent, considering that improved muscle strength can help reduce the overall work of breathing and enhance Lisa's exercise tolerance [71]. Still, a more targeted approach could involve additional exercises to specifically strengthen her respiratory muscles, such as incentive spirometry or breathing against resistance [72–74], beyond the pursed-lip and diaphragmatic breathing suggested by GPT-4.

The model's program incorporates flexibility exercises, beneficial in maintaining overall joint and muscle health [75]. However, the program could be better tailored by suggesting exercises to help enhance thoracic mobility and lung function.

The model correctly uses RPE as a method for Lisa to gauge her exertion levels. However, given Lisa's condition, additional guidance on recognising the signs of exercise-induced bronchoconstriction [76], such as unusual shortness of breath, chest tightness, or prolonged recovery, would have been beneficial.

Furthermore, the program does not address the importance of a proper warm-up and cool-down in preventing exercise-induced bronchoconstriction symptoms, an oversight that could negatively influence Lisa's exercise experience and potentially increase the likelihood of drop out due to exercise related issues/discomfort. The model also did not review proper use of a rescue inhaler for symptom exacerbations during exercise, or the potential need for prophylactic use before exercise. However, the model did recommend that Lisa ensure her inhaler remains near-by, showing that the model considered her condition and the potential associated issues that the patient might encounter while exercising.

Overall Evaluation and Interpretation

This study has explored the application of the GPT-4 model for personalised exercise prescription for five-example patient profiles with various health conditions. The GPT model's responses were able to produce generally safe exercise programs grounded in the general FITT principles and RPE guidelines, demonstrating the potential utility of AI in generating basic exercise recommendations. However, the analysis revealed several limitations and areas for improvement.

The programs suggested by ChatGPT placed a significant emphasis on safety, often prescribing moderate-intensity exercises rather than high-intensity ones. While such a cautious approach is understandable, especially when introducing a new exercise regimen to individuals, it might be perceived as overly conservative. For a short-term intervention over a month, this level of caution in the initial weeks might be excessive. Ideally, after establishing a baseline in the first couple of weeks, the intensity could be gradually increased. Starting from the third or fourth week, a small increase in intensity could better balance the goals of improving the patient's health condition while still maintaining safety. However, this progression also depends on the level of fitness of the individual prior to the program (see more below). For an extremely unfit individual, a more prolonged preparation/transition period is warranted to diminish the likelihood of dropping out.

The model's inability to monitor an individual's physiological response and adjust in real-time is a limitation. While some emerging technologies are beginning to offer this capability, ChatGPT, as used here, cannot independently provide real-time feedback or adjustments. This caution restricts the potential for optimal progress and adaptation, which are essential for health improvement and the attainment of specific goals [77].

Furthermore, the program's lack of preliminary patient assessment is a notable shortcoming. If the AI system were to request information such as the subject's current physical condition, exercise experience, or the length of time suffering from the health condition, it could plan the exercise in a more individualised and effective manner. In practice, a human practitioner, with the ability to assess the individual's responses in real-time, would likely provide a more varied, dynamic, and intense program, challenging the individual while ensuring safety and effectiveness with an appropriately individualized prescription [77].

The monotony in program design, highlighted in the present analysis, points towards the lack of variability, another crucial principle of training, in the GPT's prescribed plans. While maintaining a certain level of consistency is necessary for habit formation and gradual adaptation, introducing regular changes in exercise modes, intensities, and volumes is essential to prevent plateaus in progress, enhance motivation, and foster long-term adherence [78].

It seems that unless a patient is extremely unfit, the exercise regimens developed by GPT-4 generally will allow individuals to maintain their current health status but fall short of providing the necessary stimuli for significant advancements in their health or fitness. Moreover, the holistic approach that leads to personalisation and creativity in exercise prescription goes beyond what AI-generated plans can currently offer. This approach considers unique user attributes and adapts in ways that resonate with their individual preferences and needs. Even with technological advances, the prospect of AI systems fully replacing human expertise in the exercise prescription field seems distant as of now (ie; August 2023).

Despite its shortcomings, the GPT model's approach offers a cost-effective alternative for individuals who cannot afford personal trainers, yet it falls short of replicating the personalised service and expertise that human trainers provide. While "digital humans" may provide a potential solution to facilitate knowledge transfer, motivation, and adherence, this study was designed only to evaluate the content and appropriateness of the exercise recommendations. Although not directly investigated in our study, the authors contend that the nuances of human interaction, motivation, and real-time adjustment of training plans based on various factors likely remain unparalleled by AI in its current state. As indicated in the methodology section, the decision to conduct this analysis without significant interaction with the GPT model was deliberate and guided by several considerations. Primarily, the study sought to assess ChatGPT's ability to tailor training programs to specific patient requirements, simulating the experience of a typical untrained layperson lacking sports science knowledge. This approach allowed for critical evaluation of the program from the perspective of sports medicine and sports science specialists. Further iterative interactions with AI and text expansion were beyond the scope of this study. We also assert that a broader investigation of multiple health profiles would yield more valuable insights than a narrow focus on a single profile with numerous interactions. To ensure clarity, some evaluations, which are common between scenarios, were addressed in the overall evaluation to avoid redundancy. Despite these limitations, the study-maintained objectivity by adopting an observational approach, avoiding external factors that might alter the model's responses. This method facilitated a clear differentiation between the model's inherent knowledge and potential external influences.

Future research should explore the interactive capabilities of AI models, including ChatGPT, to determine if they can be harnessed to enhance the specificity and effectiveness of prescribed exercise programs. Such studies may also consider focusing on individual patient cases with multiple interactions to assess the model's ability to generate better-tailored training programs over successive rounds of interaction. As AI technology advances, subsequent iterations of these models may overcome existing limitations, enabling patients and fitness professionals to utilize these tools for crafting more accessible, personalized, and effective exercise prescriptions. Such advancements can serve as valuable aids, amplifying the expertise of fitness professionals rather than replacing them. This evolution emphasizes the potential of AI to act as a collaborative tool, enriching the human element in the domain of fitness and health.

Limitations

In evaluating the application of AI, specifically the GPT-4 model, for crafting personalized exercise programs, this study has uncovered both promising avenues and significant limitations. These constraints in addition to the limitations of our study must be carefully considered when interpreting the findings.

One primary limitation was the static nature of the interaction with the AI model. While personal and medical details were provided, along with a request for a 30-day exercise program, the model's ability to refine and expand upon its initial recommendations through continued dialogue was not fully investigated. This approach led to somewhat generic recommendations, reflecting the study's focus on a wide array of medical conditions rather than an in-depth exploration of a single case. Other studies should consider examining iterative interactions with AI throughout the course of an exercise program.

Our patient profiles were intentionally simplistic with a singular medical condition. Thus, our results may not be fully generalizable to the performance of AI in formulating an exercise program for patients with multiple co-morbidities or more severe medical disorders. This opportunity represents an important area of future research.

Another significant limitation is the quasi-qualitative aspect of the study. While this approach offers some advantages, especially given the pioneering nature of our research, the lack of a recognised and validated scale is a substantial concern for such evaluations. This gap underscores the necessity for future research dedicated to the development of validated scales for evaluating chatbots-generated programs, whether in sports medicine or broader medical science contexts.

Furthermore, the current technological limitations of AI, which preclude real-time physiological monitoring and responsive adjustments to exercise prescriptions, were evident in the model's tendency to prescribe extremely conservative programs. Without real-time feedback, safety has been prioritised over challenge and progression, potentially hindering fitness and health improvements. This limitation is underscored by the contrast with other technological advancements, such as the "precision motion technology "offered by "Hinge Health" [79], which can provide the real-time monitoring currently beyond the reach of AI models like GPT-4.

The lack of specificity and customisation in addressing individual health conditions and goals also emerged as a limitation, impacting the effectiveness of the programs and possibly influencing users' motivation and adherence. Additionally, the study's omission of factors such as ethnicity, exercise preferences, profession, lifestyle habits, and other personal details further constrained the model's ability to provide tailored fitness programs. Although attempts were made to incorporate these details, the model's unresponsiveness led the authors to retain the original approach.

The model's inclination towards safety over progression, while understandable given the lack of real-time feedback, could obstruct significant long-term fitness and health gains, especially for individuals with specific health conditions requiring more targeted programming. Finally, there are some individuals that will need the continuous presence of a coach next to them to exercise [80, 81], and for these persons AI may never provide an adequate solution.

Future research should pursue more dynamic and interactive designs, exploring continued dialogue with AI models, and integration of real-time physiological monitoring technology. Such advancements could enhance the specificity and effectiveness of AI-generated exercise programs, opening new possibilities for personalised fitness and health interventions.

CONCLUSIONS

Our findings indicate that AI technology (in this case OpenAI's GPT-4) can provide a baseline for safe exercise recommendations. However, the technology may not offer the degree of complexity and adaptability required for optimized long-term fitness and health improvement. The exercise regimens developed by GPT-4 permit individuals with average fitness to maintain their current health status, but do not provide sufficient stimuli for obtaining substantial advancements in their health or fitness. This conclusion highlights the irreplaceable value of human expertise and experience in the realm of exercise prescription. Live fitness specialists, with their ability to dynamically adjust and personalise workout plans, maintain a decisive advantage over current AI technology in fostering meaningful physiological adaptations and health benefits.

Chatbots AI technology, as it stands (mid 2023), appears to possess limitations that restrict its potential within the realm of personalised exercise prescription. Despite offering generally safe recommendations, AI-generated fitness plans seem to lack the personalisation, creativity, and variability that a fitness specialist can provide. For instance, a fitness specialist could consider the patient's fluctuating energy levels and tailor the intensity of workouts, accordingly, ensuring that the user remains engaged and motivated while avoiding burnout. This level of holistic approach leads to personalization and creativity beyond what AI-generated plans can offer, as it takes into account unique user attributes and adapts in ways that resonate with their individual preferences and needs. Even with technological advances, the full replacement of human expertise in the exercise prescription field by AI systems seems distant. This study highlights areas of improvement for AI systems, such as enhancing real-time interactions, improving personalisation and adaptability algorithms, and integrating a greater degree of progression in exercise intensity and variability in training. Only through such advancements can AI provide exercise recommendations that come closer to matching the quality and effectiveness of a live fitness specialist.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The data that support the findings of this study are openly available upon request from the corresponding author.

Conflict of interest declaration

The authors declare no conflict of interest. The authors wish to affirm that this research was executed with complete academic integrity, free of any commercial or financial biases. Specifically, while we employed the paid version of ChatGPT 4.0 for its advanced capabilities in exercise prescription, this was not done with any intention to promote or encourage its use. Our choice of this platform was strictly to assess its potential in the realm of exercise prescription, without any sponsorship or incentives from the developers or associated entities of ChatGPT. No author has affiliations with OpenAI or any other commercial entities related to the content of the manuscript. Our sole commitment remains to transparent, unbiased evaluations that serve to advance the intersection of sports medicine and artificial intelligence.

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Authors' contributions

I.D: conception and design.

I.D, H.B.S, A.E.O, J.M.G, C.C.T.C, J.A.W, N.G, O.H, R.A.A, L.F.R.S, M.R, L.L.P.D, R.L.V, M.T, L.J.M.D.S, K.T, H.C, M.Z, Ö.E, S.S, M.B.A, H.H.S, H.E, I.M, P.Z, S.S, B.K, I.M.A, Ø.S, J.A.D, K.C: analysis and interpretation of the data. I. D and H.B.S: drafting of the paper.

I.D, H.B.S, A.E.O, J.M.G, C.C.T.C, J.A.W, N.G, O.H, R.A.A, L.F.R.S, M.R, L.L.P.D, R.L.V, M.T, L.J.M.D.S, K.T, H.C, M.Z, Ö.E, S.S, M.B.A, H.H.S, H.E, I.M, P.Z, S.S, B.K, I.M.A, Ø.S, J.A.D, K.C: revising it critically for intellectual content.

All authors gave their final approval to the version that will be published.

Declaration

We want to emphasize that the interpretation of the results in this study is somewhat subjective, as it relies on the authors' experience with advanced language models. Although we endeavoured to maintain objectivity, it is important to acknowledge that different reviewers may hold varying interpretations of the findings. In an effort to mitigate this limitation, we sought the feedback of a substantial number of highly active experts in sports medicine, exercise sciences, and exercise physiology, as indicated in the author list. Their insights provided a nuanced critical evaluation of chatGPT-4's capabilities in exercise prescription for individuals living with medical conditions and seeking exercise programs.

In the interest of transparency, it's essential to clarify our authorship criteria. The listed authors are those who have made significant and sustained contributions to the study. Others, who made valuable but intermittent contributions, are duly recognized in the 'Acknowledgements' section. Additionally, while many specialists were approached and expressed initial interest, not all were able to contribute to a degree that warranted formal recognition. Those who neither made significant contributions nor responded within the agreed-upon timeframe have not been acknowledged in the manuscript.

Throughout this endeavor, our primary goal has been to maintain integrity, transparency, and an unwavering commitment to academic rigor.

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The influence of match status on the conditional characteristics of tactical sprint actions in professional soccer players

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ABSTRACT: This study aimed to analyse the influence of the match status on the conditional characteristics of tactical sprint actions among Spanish professional soccer players, considering playing positions. Thirty-two Spanish male professional soccer players from a LaLiga Spanish Second Division (LaLiga SmarthBank) team participated in this study. Actions above 85% of the players' maximum velocity were analysed based on their tactical purpose. These findings provide valuable information regarding the tactical aspects of sprinting in soccer, emphasizing the influence of playing positions and match status on the distribution of tactical sprint actions. No effects of match status were observed for any game phase. However, when tactical actions were individually studied, it was observed that the maximum velocity in Chase actions was higher when the team was winning, while in Press actions, the maximum velocity was higher when the team was losing and in in Run in behind/ Penetrate, the maximum velocity was higher in drawing situations compared to losing situations. No effects of match status on the distance covered during sprinting were observed, and regarding duration, significant differences were only observed in Recovery run actions. In addition, the influence of match status is higher when playing positions are considered, although the within playing positions analysis revealed significant differences only in CM players. These findings provide valuable information for the design of specific training drills considering playing positions, suggesting the need to analyse the previous match in order to structure the training load of the microcycle in a comprehensive manner.

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INTRODUCTION

Time-motion analysis has made it possible to understand the physical profiles of professional players during match play [1, 2], helping practitioners to make objective decisions regarding performance and injury risk [3]. Soccer is characterized by the presence of repetitive high intensity actions (HIA) such as jumps, accelerations or sprints interspersed with low-intensity periods [4]. Specifically, Oliva-Lozano et al. [5] revealed that soccer players perform near to 10 sprints per match and cover approximately 19.5 m per sprint. These data confirm that high-intensity sprinting demands have significantly increased in the last decade [6], greatly impacting soccer preparation [7], so further studies on the topic are warranted.

Previous studies have suggested that sprint actions may be considered a prerequisite for successful performance in soccer. This is based on the fact that linear sprints are the most frequent actions in goal situations, creating a shot and evading an opponent [8, 9]. However, the significance of sprint actions extends beyond these specific scenarios, as they are present in critical situations throughout a soccer match, such as goals [7]. Therefore, in order to enhance preparation, it is necessary to perform studies that integrate the conditional with tactical demands of sprint actions. Specifically, Oliva-Lozano et al. [10] and Ju et al. [11] contextualized sprint actions in professional soccer, focusing on tactical roles and determining the associated conditional demands for each action. However, the majority of previous studies have used absolute values to determine sprint actions. Hence, future studies based on individualized velocity thresholds (e.g., 85% of maximum player's velocity) are required. Moreover, it is important to consider other variables, such as the duration of sprint actions, as highlighted by Oliva-Lozano et al. [10] to optimize the design and prescription of training tasks.

To optimize the aforementioned aims, it is relevant to consider certain contextual variables that influence the sprint demands during matches, with particular emphasis on the match outcome [12–14]. However, prior studies have primarily focused on the final result of the match [10, 11], making it interesting to explore how the match status influences the tactical sprint actions. Another contextual variable with a key role in soccer demands is playing position. In this

regard, Oliva-Lozano et al. [10] conducted a study that revealed the significant influence of playing position on the maximum velocity and distance covered during sprinting by Spanish professional soccer players. Specifically, they found that midfielders achieved lower maximum velocities compared to their counterparts. Additionally, these authors reported differences in the frequency and nature of tactical sprint actions performed by players in different playing positions, which has been confirmed by Ju et al. [11] with English professional soccer players. These insights highlight the importance of considering playing position when analysing tactical sprint actions, which allow for a more comprehensive understanding of the demands placed on players in different roles.

Despite the increase in the research focusing on the contextualized analysis of sprint actions in professional soccer, to our knowledge, no studies considering individualized velocity thresholds to analyse tactical sprint demands have been performed. Thus, this study aimed to analyse the influence of the match status on the conditional characteristics of tactical sprint actions among Spanish professional soccer players, considering playing positions. Based on previous studies [10, 11], we hypothesized that match status substantially influences the conditional variables of the tactical sprint actions, being different according to playing positions.

MATERIALS AND METHODS

Study design

A retrospective, descriptive longitudinal design was applied to analyse the influence of the match status on the characteristics of sprint actions among Spanish professional soccer players, considering playing positions and conditional and tactical variables. An observation instrument based on the integrated approach of Ju et al. [15] was designed to assess all the sprint actions performed by players. The sprint actions were classified as defence, attack, defence-attack transition (DAT) and attack-defence transition (ADT). Specifically, these actions were categorized according to the classification presented in Table 1.

Additionally, playing position and conditional variables (i.e., maximum velocity achieved, duration and distance covered in each sprint action) were considered. Specifically, the players were divided into five positions on the field: central defender (CD), wide defender (WD), central midfielder (CM), wide midfielder (WM) and forward (F). To classify the sprint actions based on match status the score at the time of the action was registered as follows: "losing" (L), "drawing" (D) or "winning" (W).

Participants

Thirty-two professional male soccer players (M_{age}: 27.2 ± 5.6 years, M_{height}: 182.3 ± 4.2 cm, M_{weight}: 78.5 ± 5.5 kg), who competed in LaLiga Spanish Second Division (LaLiga SmarthBank) during the 20/21 season, voluntarily participated in this study. Players belonged to the same squad, which was composed of 6 CD, 3 WD, 11 CM, 8 WM and 4 F. Goalkeepers were excluded due to their specific match demands. A total of 1848 sprint actions in which the players achieved a maximum velocity higher than 85% of their reported maximum velocity during the season were selected [10]. LaLiga provided the data used in the study after participants signed their informed consent. According to the Declaration of Helsinki, the players' and team confidentiality was ensured. The study received full approval by the Ethics Committee of the University of Extremadura; Vice-Rectorate of Research, Transfer and Innovation – Delegation of the Bioethics and Biosafety Commission (Protocol number: 239/2019).

Procedures

All the selected sprint actions were independently analysed by four observers, who were members of professional soccer staffs and had experience in soccer research and video analysis. The observers

TABLE 1. Descriptions of the variables within the integrated approach.

Actions	Description
Defense	
Chase	Player chases an opponent who has the ball.
Press	Player runs directly towards opposition player on/or receiving the ball, or towards space or players not on/receiving the ball (typically blocking passing channels).
Recovery run	Player runs back towards their own goal to be goal side when out of position.
Close Down/Interception	Player cuts out pass.
Collective run	Most of the players on the team move in the same direction simultaneously
Attack	
Run with ball	Player moves with the ball either dribbling with small touches or running at speed with fewer ball touches.
Run in behind/Penetrate	Player attacks space behind, overtakes and/or unbalances the opposition defense (typically ball is behind).
Break into the box	Player enters the opposition's penalty box in an attempt to receive the ball (typically from a cross – ball in front and wide).
Over/Underlap	Player runs from behind to in front of the player on the ball or receiving the ball.
Move to receive/Exploit the space	Player moves to receive a pass from a teammate or to create/exploit space (typically comes short or moves wide to receive ball).

TABLE 2. Action characteristics according to game phase and match status (Coeff ± SE).

	Defense				Attack				DAT				ADT			
	-1	0	1	*df*	-1	0	1	*df*	-1	0	1	*df*	-1	0	1	*df*
Max vel ($km \cdot h^{-1}$)	29.20 ± 0.30	29.20 ± 0.29	29.10 ± 0.32		29.30 ± 0.31	29.40 ± 0.30	29.60 ± 0.34		29.50 ± 0.33	29.50 ± 0.30	29.50 ± 0.35		29.70 ± 0.30	29.50 ± 0.29	29.70 ± 0.32	
Duration (s)	5.81 ± 0.18	5.89 ± 0.14	5.91 ± 0.27		6.14 ± 0.23	6.21 ± 0.17	5.93 ± 0.33		7.45 ± 0.28	7.29 ± 0.19	7.62 ± 0.36		7.22 ± 0.18	7.12 ± 0.14	6.90 ± 0.26	
Distance (m)	34.40 ± 1.11	34.10 ± 0.92	32.80 ± 1.64		37.20 ± 1.39	36.80 ± 1.08	36.20 ± 1.98		46.90 ± 1.69	44.80 ± 1.18	45.40 ± 2.17		44.20 ± 1.13	43.10 ± 0.93	41.70 ± 1.59	

Notes. Coeff = coefficients; SE = Standard Error; Defense = defense phase; Attack = attack phase; TDA = defense-attack transition; TAD = attack-defense transition; df = significant differences; -1 = losing; 0 = drawing; 1 = winning; Max vel = maximum velocity achieved in the actions; Duration = average duration of actions; Distance = meters covered during sprint actions; # = significant differences between losing and drawing; $ = significant differences between losing and winning; ¨ = significant differences between drawing and winning; *p < 0.05, p < 0.01.

underwent training on the interpretation of these types of actions, and they demonstrated high inter-rater reliability (kappa statistic, $\kappa = 0.85$) and an almost perfect intra-rater reliability (kappa statistic, $\kappa = 0.90$). The observers categorized the actions according to the possibilities presented in Table 1, and they reached a final agreement on 97% of the actions. For each sprint action, the following variables were provided: match status, playing position, tactical action, maximum velocity, duration and distance covered.

The optical tracking system ChyronHego (TRACAB, New York, US) was used to collect the match data. This multi-camera tracking system consists of 8 super 4K-High Dynamic Range cameras based on a positioning system (Tracab—ChyronHego VTS) which records and analyses X and Y positions for each player from several angles, providing real-time two-dimensional tracking (tracking data are recorded at 25 Hz). Additionally, a customized report was created using Mediacoach software (LaLiga, Madrid, Spain), which synchronized tracking data with the video footage of each match.

Statistical analysis

All statistical analyses were conducted using RStudio [16]. Considering the characteristics of the sample, organized hierarchically, nested in groups, and with a longitudinal structure, the best procedure to analyse the data is through linear mixed models (LMM). Furthermore, LMM have demonstrated their ability to cope with unbalanced and repeated-measures data [17]. Thus, the cross-classified multilevel models are suitable for data structures that are not purely hierarchical. Consequently, a general multilevel-modelling strategy was applied where fixed and random effects in different steps were included [18]. A two-level hierarchy was modelled for the analysis. The variables related to actions' characteristics (i.e., maximum velocity, duration and distance) were included as dependent variables in the models. Match status (i.e., losing, drawing and winning) and playing position (i.e., CD, WD, CM, WM and F) were the independent variables included as fixed effects. The player variable was considered as the random effect in the analysis. All LMM were performed using the lm4 package from RStudio [19]. Values were represented as coefficients and standard error (coeff ± SE). SE was selected instead of standard deviation (SD) to show the dispersion of the sample mean if we continued to take values. Statistical significance was set at $p < 0.05$ and $p < 0.01$.

RESULTS

Table 2 shows the sprint action characteristics according to game phase and match status. No significant differences were observed between match statuses within each game phase regarding maximum velocity, duration and distance.

Sprint actions' characteristics related to defensive actions are presented in Table 3. The maximum velocity achieved by the Chase action was significantly higher ($p < 0.05$) when the team was winning compared to when the team was losing or drawing. Similarly, the maximum velocity achieved by Press actions was significantly higher ($p < 0.05$) when the team was losing than when the team was drawing. Conversely, the distance covered by Recovery run actions was significantly higher ($p < 0.05$) when the team was losing compared to when the team was winning. No significant differences were observed in the rest of the defensive actions.

Table 4 shows the summary statistics for action characteristics according to attack action type and match status. The maximum velocity achieved by the Run in behind/Penetrate action was significantly higher ($p < 0.01$) when the team was drawing than when the team was losing. No significant differences were observed in the rest of the attacking actions.

Table 5 shows the summary statistics for action characteristics according to playing position and match status. Considering the maximum velocity, when the team was drawing, CM achieved lower

TABLE 3. Sprint actions characteristics related to defense actions (Coeff ± SE).

	Chase				Press				Recovery run				Close Down/Interception				Collective run			
	-1	0	1	*df*	-1	0	1	df	-1	0	1	*df*	-1	0	1	*df*	-1	0	1	*df*
Max vel ($km \cdot h^{-1}$)	29.80 ± 0.32	29.60 ± 0.30	30.40 ± 0.41	$*¨*	29.40 ± 0.32	28.90 ± 0.30	29.20 ± 0.35	#*	29.40 ± 0.30	29.30 ± 0.29	29.10 ± 0.33		29.20 ± 0.32	29.60 ± 0.30	29.60 ± 0.37		29.50 ± 0.35	29.20 ± 0.31	29.40 ± 0.42	
Duration (s)	6.66 ± 0.27	6.64 ± 0.21	7.03 ± 0.51		5.43 ± 0.27	5.81 ± 0.21	5.83 ± 0.37		7.60 ± 0.21	7.35 ± 0.16	7.21 ± 0.29		5.50 ± 0.28	5.46 ± 0.20	5.34 ± 0.41		7.61 ± 0.37	7.15 ± 0.24	7.38 ± 0.53	
Distance (m)	41.20 ± 1.61	40.60 ± 1.29	44.50 ± 2.96		31.10 ± 1.60	32.30 ± 1.28	30.20 ± 2.16		47.60 ± 1.27	45.20 ± 1.03	42.60 ± 1.74	$*	32.00 ± 1.70	32.90 ± 1.25	31.50 ± 2.42		46.50 ± 2.16	42.10 ± 1.44	45.90 ± 3.11	

Notes. Coeff = coefficients; SE = Standard Error; df = significant differences; Max vel = maximum velocity achieved in the actions; Duration = average duration of actions; Distance = meters covered during sprint actions; -1 = losing; 0 = drawing; 1 = winning; # = significant differences between losing and drawing; $ = significant differences between losing and winning; ¨ = significant differences between drawing and winning; *$p < 0.05$, $p < 0.01$.

TABLE 4. Sprint actions characteristics related to offensive actions (Coeff ± SE).

	Run with ball				Run in behind/Penetrate				Break into the box				Over/Underlap				Move to receive			
	-1	0	1	*df*	-1	0	1	*df*	-1	0	1	*df*	-1	0	1	*df*	-1	0	1	*df*
Max vel ($km \cdot h^{-1}$)	29.30 ± 0.35	29.30 ± 0.33	29.60 ± 0.44		29.40 ± 0.33	29.90 ± 0.31	29.50 ± 0.36	#	29.20 ± 0.35	29.10 ± 0.32	29.30 ± 0.41		29.90 ± 0.66	29.10 ± 0.40	29.30 ± 0.83		30.20 ± 1.36	29.10 ± 0.73	-	
Duration (s)	6.64 ± 0.38	6.75 ± 0.29	6.45 ± 0.58		5.78 ± 0.30	6.42 ± 0.24	6.46 ± 0.40		6.82 ± 0.37	6.58 ± 0.27	6.34 ± 0.52		7.83 ± 1.04	7.51 ± 0.50	7.07 ± 1.34		4.52 ± 2.30	6.60 ± 1.15	-	
Distance (m)	41.90 ± 2.22	41.80 ± 1.77	40.70 ± 3.38		35.10 ± 1.77	38.70 ± 1.45	38.20 ± 2.38		40.10 ± 2.19	37.40 ± 1.63	37.60 ± 3.04		50.30 ± 6.01	45.40 ± 2.94	45.60 ± 7.76		29.60 ± 13.3	39.60 ± 6.69	-	

Notes. Coeff = coefficients; SE = Standard Error; df = significant differences; Max vel = maximum velocity achieved in the actions; Duration = average duration of actions; Distance = meters covered during sprint actions; -1 = losing; 0 = drawing; 1 = winning; # = significant differences between losing and drawing; $ = significant differences between losing and winning; ¨ = significant differences between drawing and winning; *$p < 0.05$, $p < 0.01$.

TABLE 5. Sprint actions characteristics according to playing position and match status (Coeff ± SE).

	CD				WD				CM			
	-1	0	1	*df*	-1	0	1	*df*	-1	0	1	*df*
Max vel ($km \cdot h^{-1}$)	29.50 ± 0.35	29.70 ± 0.35	29.90 ± 0.37		29.60 ± 0.40	29.50 ± 0.38	29.90 ± 0.44		29.40 ± 0.32	29.10 ± 0.32	28.70 ± 0.36	#*$¨*
Duration (s)	6.23 ± 0.25	5.91 ± 0.20	6.28 ± 0.34		6.55 ± 0.26	6.75 ± 0.19	6.56 ± 0.42		6.91 ± 0.22	7.12 ± 0.17	6.25 ± 0.32	¨*
Distance (m)	36.50 ± 1.67	35.20 ± 1.42	37.10 ± 2.16		41.60 ± 1.86	42.50 ± 1.53	41.40 ± 2.70		41.30 ± 1.44	39.80 ± 1.25	35.30 ± 2.04	$¨*
	WM				**F**				***df***			
	-1	0	1	*df*	-1	0	1	*df*	-1	0	1	
Max vel ($km \cdot h^{-1}$)	29.60 ± 0.34	29.50 ± 0.33	29.70 ± 0.37		29.30 ± 0.38	29.30 ± 0.36	29.30 ± 0.34			b	beh	
Duration (s)	6.60 ± 0.23	6.74 ± 0.18	6.84 ± 0.34		6.34 ± 0.27	6.18 ± 0.19	6.76 ± 0.35		b*	abcg*i		
Distance (m)	41.10 ± 1.55	41.50 ± 1.34	43.30 ± 2.16		38.60 ± 1.83	37.01 ± 1.43	36.60 ± 2.24		a*b*c*	ab*cg*j*	c*hj*	

Notes. Coeff = coefficients; SE = Standard Error; CD = central defender; WD = wide defender; CM = central midfielder; WM = wide midfielder; F = Forward; df = significant differences; -1 = losing; 0 = drawing; 1 = winning; Max vel = maximum velocity achieved in the actions; Duration = average duration of actions; Distance = meters covered during sprint actions; # = significant differences between losing and drawing; $ = significant differences between losing and winning; ¨ = significant differences between drawing and winning; a = significant differences between CD and WD; b = significant differences between CD and CM; c = significant differences between CD and WM; d = significant differences between CD and F; e = significant differences between WD and CM; f = significant differences between WD and WM; g = significant differences between WD and F; h = significant differences between CM and WM; i = significant differences between CM and F; j = significant differences between WM and F; *$p < 0.05$, $p < 0.01$.

values than CD ($p < 0.01$), and when the team was winning, the maximum velocity achieved by CM was significantly lower ($p < 0.01$) than CD, WD and WM. Considering the duration, when the team was losing the match, the sprint actions performed by CD were significantly shorter ($p < 0.05$) than CM actions. When the team was drawing the match, CD performed actions significantly shorter ($p < 0.01$) than WD, CD and WM. Regarding the distance covered, when the team was losing, CD covered significantly less distance ($p < 0.05$) than the WD, CM and WM. Similarly, when the team was drawing, CD covered significantly less distance during the sprint actions ($p < 0.05$) than the WD, CM and WM. Finally, when the team was winning, WM covered a significantly greater distance ($p < 0.05$) than CD, CM and F. Within playing position analysis revealed that maximum velocity achieved in the sprint actions by CM was significantly higher when they were losing than when they were drawing ($p < 0.05$) or winning ($p < 0.01$). The duration of the actions performed by CM was significantly longer ($p < 0.05$) when they were drawing than when they were winning. In relation to the distance covered during the actions, CM covered a significantly greater distance during the sprint actions ($p < 0.01$) when they were losing than when they were winning the match. No significant differences were observed in the rest of the playing positions.

DISCUSSION

This study aimed to analyse the influence of the match status on the conditional characteristics of tactical sprint actions among Spanish professional soccer players, considering playing positions. This is the first study to investigate the effects of match status on tactical sprint actions in relation to conditional variables. The results obtained show that the conditional characteristics of tactical sprint actions are lightly influenced by match status, although when playing positions are considered this influence is increased.

Soccer is divided into four game phases (i.e., defence, attack, DAT and ADT) that generally have different conditional demands [20]. However, these demands related to sprint actions remain constant regardless of the match status, so an analysis based on specific tactical actions seems necessary. Previous studies have analysed the differences in conditional variables of tactical sprint actions [10, 11], mainly considering playing positions, but none of them have done so taking into account the match status. Regarding defence action, the maximum velocity achieved during Chase actions was higher when the team was winning compared to when the team was losing or drawing. This could be attributed to a greater emphasis on defending a positive result, which would generate a faster Chase action. Additionally, when the team was losing, Press actions required higher maximum velocity than when the team was drawing, likely due to the increased height and intensity in pressure to regain possession quickly and increase the chances of drawing the game. Related to this, a longer duration in Recovery run sprint action was observed when the team was losing compared to when the team was winning. The increase in pressure when the team is losing leads to Recovery run actions starting from positions further away from their own goal, resulting in longer durations. Regarding attack actions, only differences were observed in Run in behind/Penetrate, with a higher maximum velocity when the team was drawing than when the team was losing. These findings suggest the need to structure the training load of the microcycle according to the development of the previous match, which must be comprehensively analysed.

Some authors have observed that playing position has a significant effect on the maximum velocity and distance covered during sprinting [10]. However, these authors selected sprint actions based on an absolute value (i.e., $> 30\ km \cdot h^{-1}$), and did not consider the match status, making comparisons difficult. In our study, it was observed that CD employed shorter durations to cover sprint actions than CM when the team was losing. Similarly, the distance covered by CD in these conditions was lower than WD, CM and WM. These differences could be supported by the fact that when the team was losing, the majority of playing positions focused on attacking, resulting in higher sprint efforts in terms of duration and distance for those playing positions. When the team was drawing, CD achieved higher maximum velocities than CM, while the duration and the distance covered during sprint actions by CD is shorter compared to WD, CM, WM. In this line, lower distances and durations were observed for F compared to WD and CM. Similarly to when the team was losing, higher sprint efforts are imposed on WD, WM and CM, although CD must increase the maximum velocity of this sprint actions to cover gaps left by teammates while attacking. Finally, when the team was winning, lower maximum velocities were observed in CM sprint actions than CD, WD and WM, possibly because these players focus on maintaining their position on the field, avoiding unnecessary risks when they are winning. Likewise, the distance covered by WM was shorter than F, CD and CM, as the sprint actions made by these players may be reduced in an effort to maintain their position on the field. Within playing positions differences were only observed in CM. In this regard, the maximum velocity is higher when drawing or winning compared to when losing, and also higher when drawing compared to when winning. Additionally, the distance and the duration of sprint actions are reduced when the team is winning. This could be due to the specific role of CM, which involves a combination of defensive and attacking actions that are influenced by the match status [21].

This study has some limitations that should be acknowledged. The main one is that only male soccer players were analysed. Thus, further studies involving female players are required. Likewise, a single team was studied; since each team has its specific characteristics, these findings should be considered with caution. However, a recent study compared match demands between First and Second Division teams [22], obtaining similar results, which could indicate the possibility of generalizing our results. Finally, match-related contextual variables such as team formation, team level, style of play or players' age were not considered.

CONCLUSIONS

This study provides a novel approach to achieving a comprehensive analysis of tactical sprint actions in professional soccer by incorporating the effects of match status. In this regard, no effects of match status were observed for any game phase. However, when tactical actions were individually studied, it was observed that the maximum velocity in Chase actions was higher when the team was winning, while in Press actions, the maximum velocity was higher when the team was losing and in Run in behind/Penetrate, the maximum velocity was higher in drawing situations compared to losing situations. No effects of match status on the distance covered during sprinting were observed, and regarding duration, significant differences were only observed in Recovery run actions. However, the influence of match status is greater when playing positions are considered, revealing significant differences in the within playing positions analysis only in CM players. These findings provide valuable information regarding how match status influences sprint actions in professional soccer players. In a practical approach, strength and conditioning coaches must design specific training drills considering playing positions and based on the analysis of the previous match in order to structure the training load of the microcycle in a comprehensive manner, transitional activities being an interesting option [23].

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Unveiling the acute neurophysiological responses to strength training: An exploratory study on novices performing weightlifting bouts with different motor learning models

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ABSTRACT: Currently, there is limited evidence regarding various neurophysiological responses to strength exercise and the influence of the adopted practice schedule. This study aimed to assess the acute systemic effects of snatch training bouts, employing different motor learning models, on skill efficiency, electric brain activity (EEG), heart rate variability (HRV), and perceived exertion as well as mental demand in novices. In a within-subject design, sixteen highly active males (mean age: 23.13 ± 2.09 years) randomly performed snatch learning bouts consisting of 36 trials using repetitive learning (RL), contextual interference (blocked, CIb; and serial, CIs), and differential learning (DL) models. Spontaneous resting EEG and HRV activities were recorded at PRE and POST training bouts while measuring heart rate. Perceived exertion and mental demand were assessed immediately after, and barbell kinematics were recorded during three power snatch trials performed following the POST measurement. The results showed increases in alpha, beta, and gamma frequencies from pre- to post-training bouts in the majority of the tested brain regions (p values ranging from < 0.0001 to 0.02). The CIb model exhibited increased frequencies in more regions. Resting time domain HRV parameters were altered following the snatch bouts, with increased HR ($p < 0.001$) and decreased RR interval ($p < 0.001$), SDNN, and RMSSD (p values ranging from < 0.0001 to 0.02). DL showed more pronounced pulse-related changes ($p = 0.01$). Significant changes in HRV frequency domain parameters were observed, with a significant increase in LFn ($p = 0.03$) and a decrease in HFn ($p = 0.001$) registered only in the DL model. Elevated HR zones (> HR zone 3) were more dominant in the DL model during the snatch bouts (effect size = 0.5). Similarly, the DL model tended to exhibit higher perceived physical (effect size = 0.5) and mental exertions (effect size = 0.6). Despite the highest psycho-physiological response, the DL group showed one of the fewest significant EEG changes. There was no significant advantage of one learning model over the other in terms of technical efficiency. These findings offer preliminary support for the acute neurophysiological benefits of coordination-strength-based exercise in novices, particularly when employing a DL model. The advantages of combining EEG and HRV measurements for comprehensive monitoring and understanding of potential adaptations are also highlighted. However, further studies encompassing a broader range of coordination-strength-based exercises are warranted to corroborate these observations.

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INTRODUCTION

A debate on the most effective motor learning theories for optimizing skill acquisition and neurophysiological adaptation is often "heated" and recurring topic among sports scientists, physical education teachers, and coaches. The most discussed motor learning approaches in sports within this context include repetitive learning (RL), variability of practice (VP), contextual interference (CI), and differential learning (DL), although only the RL and DL have been designed for the field of sports [1].

Historically, the RL model, initially popularized by psychologists' animal experiments, is rooted in the early theories of learning, and often associated with early behaviorist principles of learning. The RL model emphasized the importance of repeating correct trials for optimal skill acquisition. The belief was that through repetition of correct movements, the motor system becomes more efficient, and the learner can perform the specific skills with greater precision and accuracy. However, over time, this theory was challenged. Initially, just following the theory of behaviorism, there was a primary focus on inducing changes in movement behaviors by manipulating external stimuli, including variations in instructions, equipment, environmental factors, etc. [1]. Subsequently, in the second half of the 20th century, the emergence of other models, such as the VP and CI, introduced a shift of variations from the system's environment toward the system itself, represented by the learner. Both models involve task differentiation, with the VP model varying only the variable parameters, such as absolute timing, absolute forces, etc. [2], while the CI model extends this differentiation to "invariants" (i.e., the generalized motor programs (GMP)) for learning multiple movements in parallel and the schedule (i.e., temporal structuring of the learning process) [3, 4].

More recently, in the late 1990s, the DL model emerged [5, 6], asserting that effective learning requires the more general introduction of differences facilitated by the incorporation of stochastic perturbations or noise [7, 8] that is finely tuned to the learner's individual and situational characteristics during the learning process [1, 9]. The origin of this model was derived from a variety of other recent research areas. These research areas primarily include (i) the study of individuality in whole-body movements and their non repeatability [10–12]; (ii) principles related to system dynamics, involving the direct or indirect amplification of observed fluctuations to destabilize the system and initiate controlled or guided self-organizing learning processes; (iii) the application of this approach to large degrees of freedom (DGF) movements and the integration of its principles with other models such as CI [1]; and (iv) the utilization of machine learning, specifically artificial neural network (ANN) training methods for pattern recognition, recently used as a valuable tool for quantifying similarities and classifying skills [13–16].

While the RL model aims to map changing behavior/thinking by repetitively imitating a role model, the DL model, in its most extreme interpretation, involves no repetition and no augmented feedback. In between, the CI model originally involves learning a single movement through repetitions that are interleaved with other movements [17–19]. Meanwhile, the CI model is most often applied to the parallel learning of multiple movements (i.e., more than one fine motor skill is practiced in either random or serial order) but considers movement and target errors as destructive to learning progress. In contrast, DL suggests that only differences (facilitated by adding stochastic perturbations during the acquisition process) allow learning to occur and considers errors, which always occur during RL, as essential fluctuations that have a constructive influence on learning through a real self-organizing process where no concrete information about the solution is provided [5, 6, 20, 21].

In studies investigating the CI effect, structured practice setting usually involves two primary forms, namely, blocked and random practices. In blocked practice, learners perform all trials of a task before transitioning to a different task with diverse variable parameters according to the VP model. This approach minimizes the variability of the invariants and allows for repeated task performance [22]. In contrast, random practice involves interleaving tasks with different GMPs in an unpredictable order, with tasks rarely repeated in consecutive trials [22]. The paradoxical two effects of CI assume that random practices hinder performance during the acquisition phase while providing more permanent benefits during the retention or learning phase compared to blocked practice [3, 4].

The advantages of the second effect, random practice in the retention phase, were most often attributed to either increased elaboration, assumed to involve learners in deeper and more elaborate information processing [23], or increased forgetting and reconstruction, which requires extra retrieval practice and reconstruction of tasks' plans of action [24, 25]. Based on both theoretical positions, during random practice, a more detailed and permanent representation of the task is being memorized, which may be the origin of the enhanced retention performance. Regarding the reduced likely benefits of a random schedule during the acquisition phase, these theoretical positions rely on cognitive load theory [26], suggesting overloading the capacity of working memory during random practice to hinder performance during the acquisition phase, causing the first effect.

Taking into consideration the abovementioned cognitive-psychological explanations regarding the interference phenomenon, further overloaded cognitive abilities are expected following DL compared to CI practice because of higher variety of movements in the DL acquisition sessions. According to the overload of working memory [26], one should anticipate relatively modest improvements in newly acquired skills during the acquisition phase following the DL compared to the CI schedule. Moreover, in the context of DL, the putative correct skill is executed only once, and since the added noise corresponds to erroneous movements, one can anticipate generally poor learning outcomes. Nevertheless, previous studies on DL contradict the cognitive overload theory and non-erroneous movements in the CI model and provide evidence supporting the superiority of stochastic DL

training, already during the acquisition phase, over repetition-based training in the context of learning a single movement [13, 27–32], as well as over CI learning in the context of learning multiple skills simultaneously in football [32] and volleyball [27, 28, 33].

The failure of the CI-related cognitive load theory to corroborate the superiority of DL over CI acquisition practice can be attributed to the fact that the working memory model relied upon by CI was originally developed only for sequential, visual-spatial content [34], a domain where the model still demonstrates its highest reliability. Therefore, a comprehensive explanation based on reliable neurophysiological measurements is still needed to elucidate how DL practice exhibited the largest short-term benefits while being assumed to further overload the learner's cognitive abilities compared to CI and RL.

Neurophysiological evidence from MRI and EEG research supports the theoretical constructs of the different motor learning models. Findings from MRI studies provided evidence for corroborating different brain activations during blocked vs. randomized practice [35, 36]. However, this has only been observed in movements with few DGFs due to restrictions associated with the available measurement devices. Additionally, these studies only compared different levels of CI practice, with no emphasis on comparative measurements between CI and DL. Comparing brain activity immediately after RL, CI, and various forms of DL practice in badminton, previous EEG-based studies have shown distinct patterns [37, 38]. After CI practice, an increase in gamma and beta frequencies has been recorded in the frontal lobe, indicating the engagement of executively controlled cognitive processes [38]. In contrast, following all forms of DL practice, increases in theta and alpha activities were obtained in somatosensory and motor areas, while activation in the frontal cortex was downregulated toward increased theta and decreased beta and gamma frequencies [38]. This shift in the anterior brain areas suggests that the CI model may induce increased cognitive stress in badminton learners when compared to DL [37, 38]. In this context, recent reports have suggested that the wide range of skill variations in DL acts as a mitigating factor, reducing the risk of medium- and long-term frontal lobe overload, which in turn aids in the motor learning of movements with a high DGF [1, 38, 39]. However, additional neurophysiological comparative studies are necessary to validate this explanation across a broader spectrum of sports skills. On the other hand, combining high CI practice (random) with complex and highly demanding motor tasks could exacerbate cognitive load, thereby reducing the likely benefits of random practice. Again, such assumptions need to be proven using reliable neurophysiological measurements during the acquisition of highly demanding sport skills with high DGF.

According to Cioroslan [40], Olympic weightlifting is a sport of outstanding neuromuscular coordination, fine kinesthetic perception, agility, and the ability to perform explosive movements in a specific line of technique with maximum accuracy. Additionally, Olympic weightlifting exercises, acknowledged as the most intense and demanding strength exercises, are among the few exercises with high DGF that involve the entire body musculature by activating complete muscle chains [41]. It is worth noting that the majority of neurophysiological studies have concentrated on endurance-based sports, leaving a notable gap in our understanding regarding brain activity responses to strength-based exercises [42], such as weightlifting (e.g., snatch, clean and jerk). These exercises present an excellent opportunity to empirically investigate motor learning related assumptions discussed above, using reliable neurophysiological measurements. In this context, electroencephalogram (EEG) is a commonly used tool in research to monitor the brain's electrical activity. It effectively provides insights regarding brain waves in different frequency bands, including the theta (4–7.5 Hz), alpha (8–13 Hz), beta (13–30 Hz), and gamma (30–40 Hz) signals, which are the crucial indicators for understanding neurophysiological states or responses to the different motor learning tasks. Furthermore, recent studies have emphasized the role of the autonomic nervous system in regulation of mental stress level, cognitive functioning, and motor performance [43–45]. Indeed, a greater heart rate variability (HRV) has been associated with reduced stress level and enhanced cognitive and motor functioning [43, 45]. Therefore, it is pertinent to incorporate HRV parameters as part of neurophysiological measurements within the domain of motor control.

Nonetheless, only a few studies have concurrently assessed both EEG and HRV while also controlling the rates of perceived exertion (RPE) during exercises with high DGF performed following different motor learning approaches. For instance, John and Schöllhorn [46, 47] compared the effects of RL and DL approaches, including instructed and self-created variable learning, for learning rope skipping movements on multiple neurophysiological responses. The authors suggested that combining rope skipping with additional cognitively demanding tasks in the form of the DL approach might lead to short-term cognitive overload. They also emphasized the promising, yet inconclusive advantages of DL over RL and highlighted the need for further research, including the CI approach, to comprehensively explore the reciprocal influence of cardiac and central nerve strains in greater detail.

In view of the limited evidence regarding the impact of different motor learning approaches on technical efficiency and the accompanying neurophysiological aspects, specifically in response to strength-based exercise, the present study aimed to investigate the acute systemic effects of three different motor learning models, namely, the RL, CI, and DL, on the technical efficiency the Olympic snatch, along with EEG brain activity and HRV responses in novice learners. This research seeks to provide a more comprehensive understanding of the complex interplay between cognitive load, practice variations, and brain and autonomic nervous system activation patterns. Such insights are crucial for customizing training interventions and optimizing skill acquisition and performance in diverse motor learning contexts.

We hypothesize that, due to the increased mental effort involved in the execution of various coordination tasks, the DL approach would

engage more regions of the cortex than RL and CI, particularly at the somatosensory and motor areas. In contrast, CI would specifically involve executive processing in the frontal area. Additionally, we hypothesize that DL would lead to a lower activation of the sympathetic system through a decrease in the high-frequency band and an increase in the low-frequency band, as well as a greater perceived exertion and mental demand.

MATERIALS AND METHODS

Participants

The sample size was calculated *a priori* based on procedures suggested by Beck [48] and using the software G*power [49]. Values were set at 0.05 for α and 0.95 for power. Based on the studies of John & Schöllhorn [46] and discussions between the authors, the effect size was set to be 0.5 (medium effect). The minimum needed sample size for this study was 10. Sixteen highly active males were recruited to voluntarily participate in this study. After receiving a description of the protocol, potential risks, and benefits of the study, participants gave their written consent to participate in this investigation. The criteria for participant inclusion in the present study are as follows: all participants should be aged between 18 and 29 years old, male, right-handed as assessed by the Edinburgh Handedness Inventory, and should have at least 2 years of experience in fitness and/or CrossFit club (i.e., including at least 6 months of performing barbell-based exercises). Exclusion criteria included previous experience in Olympic weightlifting, current or a history of neurological and/or cardiovascular impairment, eye disorders, psychiatric illnesses, orthopedic diseases, and/or muscular disorders, and the intake of medication that may have influenced EEG brain activity and/or HRV.

The study was conducted according to the Declaration of Helsinki; all subjects were naive to the purpose of the study and were coded with numbers for the anonymity of personal data. The study was approved by the local ethics committee of Faculty 02: Social Sciences, Media, and Sport at Johannes Gutenberg University of Mainz.

Experimental design

A randomized within-subject design was employed to assess the short-term effects of four different motor learning approaches. After a familiarization session, participants reported to the laboratory on four separate occasions, with at least a one-week washout period in between. In a randomized order, during each test session, one single motor learning approach was conducted in a single training bout. This training bout consisted of 36 trials of power-snatch derivates according to one of the four tested motor learning approaches: RL, CI blocked (CIb) or CI serial (CIs), and DL, with a standardized duration of ≈ 3 minutes. The sequence of motor learning approaches was randomized, and all test sessions were performed at the same time of day (in the afternoon, as previously suggested by Ammar et al., [50, 51]) to minimize the effect of diurnal biological variations [52]. The measurements were carried out under laboratory conditions. Changes in brightness, volume, and temperature were standardized and kept to a minimum.

The procedure for each testing session is detailed in Figure 1. Upon arrival at the laboratory, participants completed the short form of the International Physical Activity Questionnaire (IPAQ-SF) and questions 4 and 6 of the Pittsburgh Sleep Quality Index (PSQI). These questionnaires were used to assess their physical activity behaviors and sleep duration and quality over the previous 7 days prior to testing. Spontaneous EEG activity and electrocardiography (ECG) HRV were recorded in a sitting position with eyes open before (PRE-rest, duration: 5 min) and after (POST-rest, duration: 5 min) each

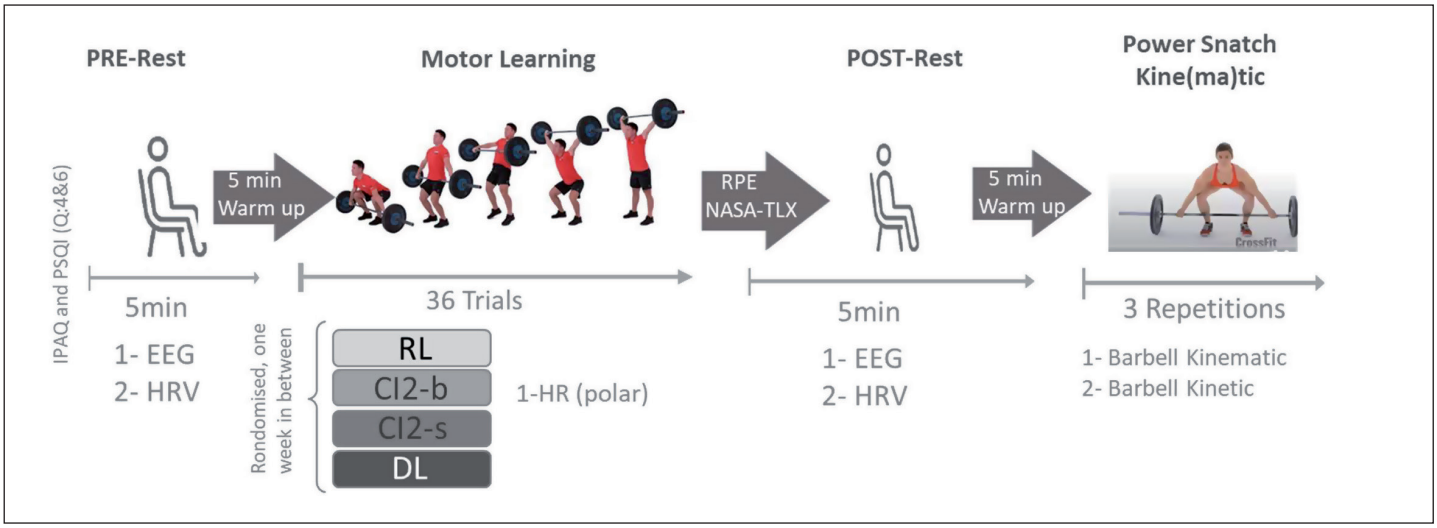


FIG. 1. Study Design.

training session. Ratings of perceived exertion (RPE) and perceived physical and mental demand (NASA Task Load Index_NASA-TLX) were assessed immediately following each training bout. Additional HR was recorded using the Polar H10 HR monitor during each training bout. To determine the effects of the different learning approaches on technical efficiency, barbell kinematics were recorded during three-power snatch trials performed following the POST-rest measurement (Figure 1).

Motor Learning Approaches

The training bouts for the RL approach consisted of 36 repetitions of power snatch. The training bouts of both CI approaches (CIb and CIs) included the power snatch and two power snatch variations: the snatch power jerk (known to improve overhead strength, stability, balance, and barbell control in the catch phase; Soriano et al. [53]) and the high pull snatch (known to improve strength, speed, power, posture, and balance in the extension of the snatch; [54]). Specifically, the training bout of the CIb approach consisted of practising high pull snatch (A), snatch power jerk (B), and power snatch (C) in blocked order: 12 reps (A), 12 reps (B), and 12 reps (C) for a total of 36 trials. The training bout of the CIb approach consisted of practising high pull Snatch (A), snatch power jerk (B), and power snatch (C) in serial order: ABC × 12, with a total of 36 trials.

These three movements were also practiced in serial order (ABC × 12 with a total of 36 trials) during the training bout of the DL approach. However, variations in different movement/environmental parameters, such as foot starting position (for A, B, and C), barbell starting position (for A and C), final positions (for B), eyes closed (for A, B, and C), and/or unstable surface (using the aeris® muvmat [55] for A, B, and C), have been adopted to generate additional movement variability and increase fluctuations. In general, DL training applies movement variations and increased fluctuations to make the system unstable and thereby foster a self-organized learning process by achieving greater effects on brain activity [56]. In addition to the referential condition (no additional movement variability), three levels of movement variability are identified in the present power snatch DL approach: level 1: variation in only one movement/surrounding parameter; level 2: variation in 2 movement/surrounding parameters; and level 3: variation in 3 movement/surrounding parameters.

To homogenize physical (cardiac) exertion in all motor learning approaches according to exhaustion influence on brain activity [57, 58] as well as on the cardiovascular system [59, 60], a nine-second interblock (12 repetitions) rest period was adopted in the RL and both CI conditions (total rest period of 18 seconds during the 36 trials), and a six-second inter-set rest period was adopted in the DL condition (total rest period of 18 seconds during 36 trials). This procedure allowed us to adopt a standardized training bout duration of ≈ 3 minutes. Furthermore, the training bouts for all approaches were performed using the same empty barbell (10 kg).

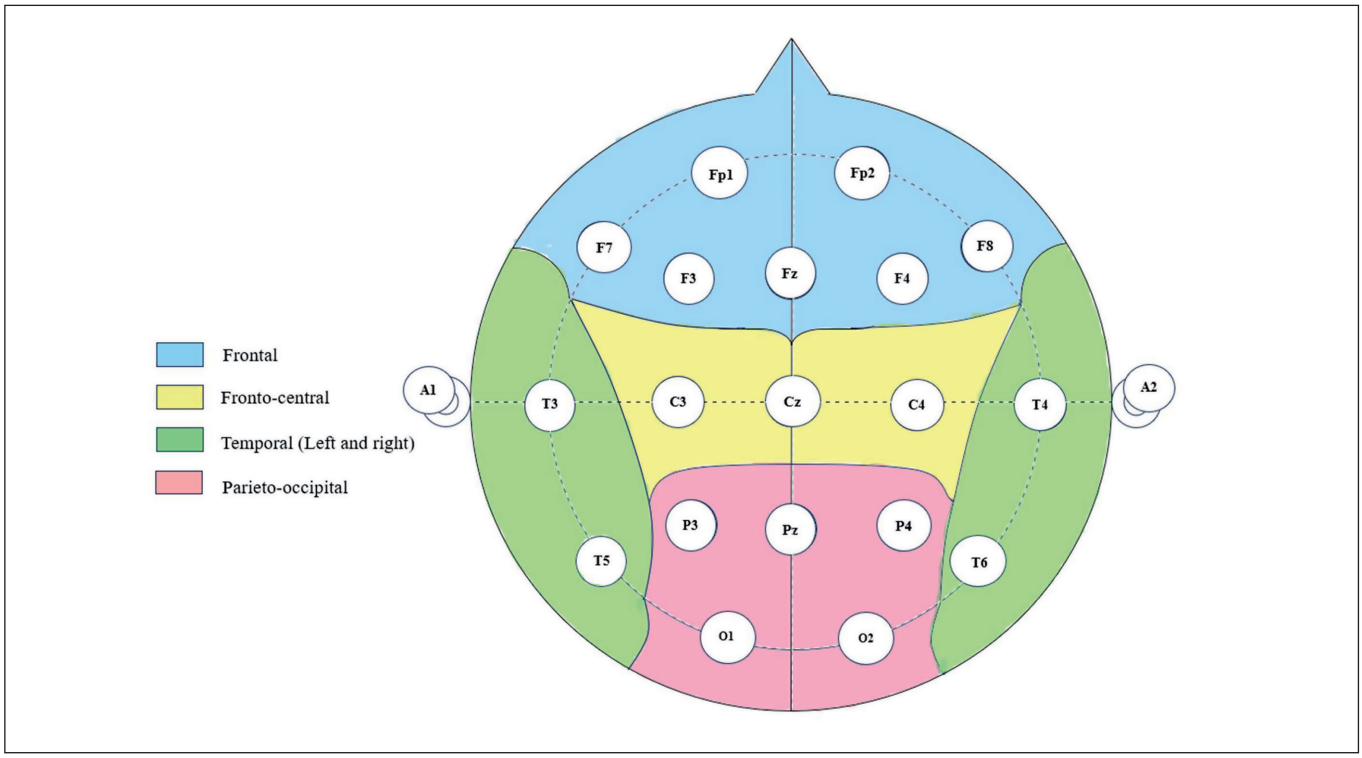


FIG. 2. Scalp location.

Measurements

Physical activity and sleep pattern

The IPAQ-SF has been extensively validated in different cultures and populations [61]. The total weekly PA (MET-min · week-1) was estimated by multiplying the reported weekly time for each IPAQ-SF item (vigorous intensity, moderate intensity, and walking) by their respective metabolic equivalent of task (MET) values [62]. We utilized the original MET values recommended by the official IPAQ guidelines for young and middle-aged adults (18–65 years old): vigorous PA = 8.0 METs, moderate PA = 4.0 METs, and walking = 3.3 METs. Following the IPAQ scoring protocol, participants in the study were categorized into three groups based on their MET–min/wk, which represents the cumulative sum of walking, moderate-intensity physical activities, and vigorous-intensity physical activities: lowly active (< 600 MET–min/wk), moderately active (600 MET–min/wk ≤ PA < 3000 MET–min/wk), and highly active (≥ 3000 MET–min/wk) (http://www.ipaq.ki.se).

Electroencephalogram (EEG) measurement

EEG Data Acquisition

Spontaneous resting EEG was assessed by means of the Micromed SD LTM 32 BS EEG system (Venice, Italy) with a sampling rate of 1024 Hz. This EEG system is composed of 19 electrodes, including Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, and O2, organized according to the international 10–20 system. The 10–20 system is based on the relationship between the electrode location and the subregions of the cerebral cortex. Each point on the system indicates a possible position for the electrode, with a letter to identify the lobes and a number to identify the hemisphere. The 10–20 system ensures accurate electrode placement, enabling researchers to obtain reliable EEG signals. Resting EEG signals were continuously registered before (5 min) and after (5 min) the different training bouts in a sitting position. For all EEG measurements, a homogeneous and low impedance (< 10 kΩ) of the electrodes at all points was sought. Five scalp locations (Figure 2), frontal "F" (Fp1, Fp2, F7, F3, Fz, F4, F8), central "C" (C3, Cz, C4), temporal (left (T-L): T3, T5; right (T-R): T4, T6), and parietal-occipital "P/O" (P3, Pz, P4, O1, and O2), were chosen to gather the signals from the 19 electrodes. The conduction of brain activity was unipolar, with grounding on the nose. Furthermore, a two-channel electrooculogram with electrodes at the medial upper and lateral orbital rims of the right eye was applied. Data were recorded by means of commercially available software (SystemPlus Evolution—Micromed, Venice, Italy).

EEG data preprocessing and analysis

Once the raw signals were recorded, we exported them to MATLAB (EEGLAB-Toolbox 2019 from the University of California, San Diego) for processing.

The data were first visually inspected to remove noisy segments related to electrode placement or technical disturbances. A bandpass filter was set from 2–40 Hz. Independent component analysis (ICA) was performed to deduce recurrent movement from the signal, such as eye blinks, muscle activity, or channel noise. Impaired electrodes were interpolated using a spherical spline interpolation method [63]. A final visual inspection of the time series and the power spectrum was conducted to remove any remaining artifacts.

Spectral power was calculated for each testing session using fast Fourier transformation with a Hanning window, a window size of 4,096 samples (4 s), and a window overlap of 50% for theta [3.5–7.5 Hz], alpha [7.5–12.5 Hz], beta [12.5–30 Hz], and gamma [30–40 Hz]. Band ranges were chosen according to previous studies that involved movement and EEG [38, 47, 55, 64]. The power spectrum was computed for each test session.

Heart rate and heart rate variability (HRV) measurements

Before and immediately after the different training bouts, HRV parameters were measured using a Polar H10 HR monitor with a Pro Strap (Polar Electro Oy, Kempele, Finland). The Polar H10 system measures the electrical signal of the heart in the form of a 1-channel electrocardiogram (ECG) signal using two dry electrodes. From the ECG signal, the R-peaks are detected to derive the RR interval time series. Both the ECG quality [65] and the quality of the derived RR intervals [66, 67] using Polar H10 were previously compared to the gold standard ECG measurement using a 12-channel medical-grade ECG device and found to be excellent.

HRV measurement and analysis

Prior to the test sessions, the Polar H10 electrodes were moistened with room-temperature water prior to being placed on the xiphoid process of the sternum with the chest strap fitted around the participant's chest (just below the chest muscles) [55, 68]. The Android app "Polar Sensor Logger" [69] was used to record the RR intervals. Five minutes of resting in an upright sitting position was recorded. The recorded data were later imported and analyzed in Kubios HRV Standard 3.5.0 analysis software [70]. Using Kubios, artifact removal and detrending were performed as necessary preprocessing steps. Therefore, RR intervals that were larger or smaller than a set threshold compared to the local average were corrected by replacing the identified artifacts with interpolated values using cubic spline interpolation. The threshold was adjusted individually but was generally in the range of 0.25–0.35 seconds (low or medium threshold). Afterwards, the smoothness priors detrending method [71] was used to avoid the effect of slow nonstationary trends in the analysis.

Using the preprocessed RR intervals, several time- and frequency-domain parameters were calculated. From the time domain, we chose the most often used parameters for short-term analysis, which are the mean (MeanRR), the standard deviation (SDNN) and the root mean square of successive differences (RMSSD) of the RR intervals. We also analyzed the lowest (HR-min) and highest (HR-max) values, as well as the average of HR values (HR-mean). To calculate

the frequency parameters, the time series was resampled to 4 Hz, and the FFT spectrum was derived using Welch's periodogram. The low frequency (LF, 0.04–0.15 Hz) and high frequency (HF, 0.15–0.4 Hz) power (n.u.) and the LF/HF ratio were computed from the FFT spectrum. Furthermore, we calculated the coefficient of variation (CVNN) by the formula CVNN = 100 * SDNN/MeanRR. The CVNN tries to minimize the mathematical dependence of the standard deviation from the mean through normalization.

HR zones

HR zones are a useful method for monitoring training intensity [68]. These zones categorize training intensity based on a percentage of the maximum heart rate and are commonly divided into five ranges. For our study, we adhered to the Polar recommendations for each HR zone (https://www.polar.com/blog/running-heart-rate-zones-basics/): Zone 1 (50–60%), Zone 2 (60–70%), Zone 3 (70–80%), Zone 4 (80–90%), and Zone 5 (90–100%). The time spent in each HR zone was calculated as a percentage of the whole training bout time (e.g., spending 1 minute in zone 3 during the three-minute training bouts corresponds to 33.3%). Similar to the HRV, HR zones were controlled using the Polar H10 HR monitor with a Pro Strap (Polar Electro Oy, Kempele, Finland).

Rating of perceived exertion (RPE)

The RPE was obtained immediately following each training bout using an 11-point scale, with scores ranging from 0 (very, very light) to 10 (very, very hard). An intraclass correlation coefficient (ICC) of 0.83 showed that RPE was a good measure of physical effort. It also had good psychometric properties and a strong correlation with a number of other physiological measures of effort [72].

Subjective mental workload

The subjective rating of mental workload was assessed using the NASA-Task Load Index scale (NASA-TLX). The NASA-TLX uses six subscales to assess mental workload, including mental demand, physical demand, temporal demand, performance, effort, and frustration [73]. In the present project, we focused on the analysis of mental demand.

Kinetic and kinematic measures

The three snatch trials performed on a 2.4×0.9 m weightlifting platform were recorded using nine synchronized, commercially available infrared cameras (Oqus 300/310+, Qualisys AB, Sweden) positioned around the platform at a distance of ~6 m from the lifting area, and kinematic data were collected at 250 Hz using Qualisys Track Manager 2.7 (Qualisys AB, Sweden). Two reflective markers were attached to the right and left ends of the barbell, and the calibration was executed beforehand using a calibration wand according to the Qualisys manual.

As suggested by Kipp, Harris, and Sabick [74], during postprocessing, the barbell data were filtered by means of a 2nd-order Butterworth filter with a cutoff frequency of 20 Hz. Computations of vertical and horizontal barbell displacements were conducted using the average location of the right and left markers on the barbell. The average of both markers provides movement of the bar center and avoids the induction of artifacts associated with asymmetrical movement [75, 76]. In the present study, we focused on the peak vertical displacement of the barbell registered during the turnover phase and on three key horizontal displacement values, which include the maximal horizontal displacement during the first pull (mh1) and the second pull (mh2) and the horizontal displacement from the most forward position to the catch position (DXL or loop) [41, 75–77].

Statistical analyses

Mean and standard deviation (SD) values were calculated for each variable. The Shapiro-Wilk W-test was used to verify normal distribution of data. A two-way repeated measures analysis of variance (RMANOVA) (i.e., 4 levels [motor learning approaches] × 2 levels [PRE/POST-training]) was used to analyze the EEG and HRV data, and a one-way analysis of variance (RMANOVA) (4 levels [motor learning approaches: RL, CIb, CIs, and DL]) was used to analyze differences between training practices in terms of HR zones during the training bouts, perceived exertion and perceived mental workload, and barbell displacements. When the main effects were found to be significant, post hoc t tests with Bonferroni correction or Wilcoxon tests were used. The equality of variances (homogeneity) was verified using Bartlett's test. To estimate the meaningfulness of significant differences, effect sizes were calculated as partial eta-squared ($\eta p2$) for the main effects and the interaction between them and as Cohen's d (d) for the paired comparison. Values of 0.01, 0.06, and 0.13 for partial eta-squared and 0.2, 0.5, and > 0.8 for Cohen's d represent small, moderate, and large effect sizes, respectively. To estimate the magnitude of significant differences, difference (Δ) or percent difference (Δ (%)) scores were calculated as follows: Δ = postbouts value – prebouts value; Δ (%) = [((post value – pre value))/(pre value)] × 100. The differences between training models in terms of Δ, particularly for EEG and HRV parameters, were analyzed using a one-way RMANOVA (4 levels [motor learning approaches: RL, CIb, CIs, and DL]). Additionally, relationships between EEG and HRV-related parameters at post-training bouts were analyzed using the Pearson correlation coefficient (r). Significance was accepted for all analyses at the level of $p < 0.05$. Exact p values have been given; results given as "0.000" in the statistics output have been reported as "< 0.0005".

RESULTS

Participants' characteristics

Sixteen highly active athletes (mean age: 23.13 ± 2.09 years) were recruited to participate in this study. Their average body mass index (BMI) was 24.14 ± 2.17, indicating a normal weight range. All participants reported no chronic diseases or sleep disturbances. They reported a fairly good to very good level of sleep quality. Regarding

TABLE 1. The acute effect of the weightlifting training bouts on resting brain activity for theta, alpha, beta, and gamma frequencies.

	Pre-Training bouts				Post-training bouts				ANOVA 2 ways		
	CIb	CIs	DL	R	CIb	CIs	DL	R	Interaction Group × Training (F, p value, ηp^2)	Group effect (F, p value, ηp^2)	Training effect (F, p value, ηp^2)
Theta frequency (N ± SD)											
F	0.85 ± 2.09	0.87 ± 2.45	1.81 ± 2.14	1.43 ± 2.3	1.22 ± 2.36	1.34 ± 2.35	1.46 ± 2.38	1.28 ± 2.93	(F=1.26, p=0.30, ηp^2= 0.06)	(F=0.24, p=0.87, ηp^2= 0.01)	(F=0.17, p=0.68, ηp^2= 0.003)
C	4.66 ± 2	4.77 ± 2.28	5.27 ± 2.16	4.92 ± 2.39	5.08 ± 2.52	5.04 ± 2.69	5.25 ± 2.72	5.15 ± 2.68	(F=0.32, p=0.82, ηp^2= 0.02)	(F=0.10, p=0.96, ηp^2= 0.01)	(F=1.09, p=0.31, ηp^2= 0.02)
T-L	2.87 ± 2.36	2.93 ± 2.21	3.74 ± 2.59	3.57 ± 2.66	3.22 ± 3.34	3.22 ± 3.01	3.47 ± 3.84	3.72 ± 2.96	(F=0.68, p=0.57, ηp^2= 0.03)	(F=0.25, p=0.86, ηp^2= 0.01)	(F=0.20, p=0.66, ηp^2= 0.003)
T-R	3.08 ± 2.79	3.25 ± 2.63	3.59 ± 2.62	3.36 ± 2.81	3.41 ± 3.03	3.17 ± 3.22	3.78 ± 2.98	3.8 ± 2.95	(F=0.46, p=0.71, ηp^2= 0.02)	(F=0.13, p=0.94, ηp^2= 0.01)	(F=1.86, p=0.18, ηp^2= 0.03)
P/O	5.02 ± 2.37	5.25 ± 2.5	5.55 ± 2.56	5.36 ± 2.62	5.38 ± 2.96	5.59 ± 2.98	5.73 ± 3.09	5.83 ± 2.74	(F=0.11, p=0.96, ηp^2= 0.01)	(F=0.10, p=0.96, ηp^2= 0.01)	(F=4.54, p=0.04, ηp^2= 0.08)
Alpha frequency (N ± SD)											
F	-0.16 ± 3.08	-0.45 ± 3.03	0.26 ± 2.92	0.33 ± 3.22	0.58 ± 3.04	0.43 ± 2.74[a]	0.55 ± 2.94	0.67 ± 2.99	(F=1.18, p=0.33 ηp^2= 0.06)	(F=0.10, p=0.96, ηp^2= 0.01)	(F=17.05, p=0.0001, ηp^2= 0.22)
C	4.79 ± 3.35	4.55 ± 3.27	4.97 ± 3.3	4.91 ± 3.48	5.75 ± 3.46[a]	5.4 ± 3.38	5.75 ± 3.27	5.93 ± 3.23[a]	(F=0.17, p=0.91 ηp^2= 0.01)	(F=0.06, p=0.98, ηp^2= 0.003)	(F=45.89, p < 0.0001, ηp^2= 0.43)
T-L	4.24 ± 3.86	4 ± 3.46	4.75 ± 3.54	4.91 ± 3.8	5.05 ± 4.01	4.93 ± 3.6	5.29 ± 4.12	5.54 ± 3.16	(F=0.37, p=0.77, ηp^2= 0.02)	(F=0.14, p=0.94, ηp^2= 0.01)	(F=24.98, p < 0.0001, ηp^2= 0.30)
T-R	4.87 ± 4.37	4.97 ± 3.78	5.11 ± 3.71	5.13 ± 4.11	5.68 ± 4.23	5.24 ± 4.28	5.94 ± 3.38[a]	6.27 ± 3.63[a]	(F=1.05, p=1.95, ηp^2= 0.13)	(F=0.07, p=0.98, ηp^2= 0.004)	(F=18.49, p < 0.0001, ηp^2= 0.36)
P/O	7.55 ± 3.92	7.73 ± 3.86	7.88 ± 3.74	8.05 ± 3.89	8.55 ± 4.04[a]	8.73 ± 3.93[a]	8.93 ± 3.57[a]	9.07 ± 3.42[a]	(F=0.01, p=0.99, ηp^2= 0.0004)	(F=0.06, p=0.98, ηp^2= 0.003)	(F=61.35, p < 0.0001, ηp^2= 0.51)
Beta frequency (N ± SD)											
F	-6.28 ± 2.72	-6.1 ± 2.41	-5.46 ± 2.01	-5.49 ± 2.51	-4.95 ± 2.55[a]	-5.13 ± 1.96[a]	-4.69 ± 2.08	-4.79 ± 2.14	(F=1.19, p=0.32, ηp^2= 0.06)	(F=0.27, p=0.85, ηp^2= 0.01)	(F=53.10, p < 0.0001, ηp^2= 0.47)
C	-2.95 ± 2.27	-2.97 ± 2.06	-2.59 ± 1.92	-2.54 ± 2.42	-1.89 ± 2.2[a]	-2.17 ± 2.08[a]	-1.74 ± 2.06[a]	-1.7 ± 2.13[a]	(F=0.28, p=0.83, ηp^2= 0.01)	(F=0.16, p=0.92, ηp^2= 0.01)	(F=65.27, p < 0.0001, ηp^2= 0.52)
T-L	-4.07 ± 2.53	-3.61 ± 2.16	-2.98 ± 1.66	-2.96 ± 2.23	-2.53 ± 2.63[a]	-2.59 ± 2.17	-2.43 ± 2.48	-1.96 ± 2.14	(F=1.30, p=0.28, ηp^2= 0.06)	(F=0.49, p=0.70, ηp^2= 0.02)	(F=33.15, p < 0.0001, ηp^2= 0.36)
T-R	-3.85 ± 2.7	-3.32 ± 2.41	-3.17 ± 1.78	-3.36 ± 2.57	-2.48 ± 2.33[a]	-2.87 ± 2.66	-2.04 ± 1.79[a]	-2.19 ± 2.16[a]	(F=2.37, p=0.08, ηp^2= 0.11)	(F=0.22, p=0.88, ηp^2= 0.01)	(F=62.25, p < 0.0001, ηp^2= 0.51)
P/O	-2.25 ± 2.36	-2 ± 1.96	-2 ± 1.77	-1.82 ± 2.3	-1.15 ± 2.48[a]	-1.27 ± 2.2[a]	-1 ± 2.07[a]	-0.87 ± 1.87[a]	(F=0.80, p=0.50, ηp^2= 0.04)	(F=0.08, p=0.97, ηp^2= 0.004)	(F=116.54, p < 0.0001, ηp^2= 0.66)
Gamma frequency (N ± SD)											
F	-9.82 ± 2.72	-9.47 ± 2.24	-8.82 ± 1.85	-8.9 ± 2.38	-7.87 ± 2.56[a]	-7.92 ± 1.88[a]	-7.71 ± 2.35	-7.5 ± 1.88[a]	(F=0.70, p=0.56, ηp^2= 0.04)	(F=0.37, p=0.78, ηp^2= 0.02)	(F=51.88, p < 0.0001, ηp^2= 0.46)
C	-7.59 ± 2	-7.34 ± 1.9	-7.15 ± 1.5	-7.02 ± 2.23	-6.07 ± 2.42[a]	-6.2 ± 1.73[a]	-5.85 ± 2.17[a]	-5.74 ± 1.64[a]	(F=0.23, p=0.87, ηp^2= 0.01)	(F=0.22, p=0.89, ηp^2= 0.01)	(F=63.51, p < 0.0001, ηp^2= 0.51)
T-L	-8.06 ± 2.55	-7.3 ± 2.21	-6.45 ± 1.84	-6.49 ± 2.37	-5.81 ± 3.06[a]	-5.75 ± 2.1	-5.88 ± 3.01	-5.05 ± 2.33	(F=1.61, p=0.20, ηp^2= 0.08)	(F=0.80, p=0.5, ηp^2= 0.04)	(F=28.39, p < 0.0001, ηp^2= 0.32)
T-R	-8.1 ± 2.68	-7.37 ± 2.16	-7.04 ± 1.62	-7.5 ± 2.7	-6.13 ± 2.69[a]	-6.39 ± 2.38	-5.46 ± 2.01[a]	-5.69 ± 1.95[a]	(F=1.11, p=0.35, ηp^2= 0.05)	(F=0.48, p=0.70, ηp^2= 0.02)	(F=60.19, p < 0.0001, ηp^2= 0.50)
P/O	-7.39 ± 2.61	-7.08 ± 1.61	-7.02 ± 1.62	-6.88 ± 2.37	-5.87 ± 2.88[a]	-6.05 ± 2.03[a]	-5.75 ± 2.33[a]	-5.52 ± 1.69[a]	(F=0.41, p=0.75, ηp^2= 0.02)	(F=0.14, p=0.94, ηp^2= 0.01)	(F=67.26, p < 0.0001, ηp^2= 0.53)

a: significant difference from pre to post; Frontal (F), Central (C), temporal left (T_Left), temporal right (T_right) and parieto-occipital (P/O) regions; Contextual interference blocked CIb, Contextual interference serial (CIs), differential learning (DL), Repetitive (R) motor learning models.

lifestyle habits, 87.5% were nonsmokers, and 25% abstained from alcohol consumption. Concerning the time spent on all types of sports activities, participants reported weekly averages of 225.9 ± 137.1 minutes in vigorous activity, 108.4 ± 77.9 minutes in moderate activity, and 631.7 ± 629.5 minutes in walking. Overall, they engaged in a weekly average of 966.1 ± 274.5 minutes across all sports activities.

According to the IPAQ-SF, the participants were highly active, with an average of 4325.82 ± 2666.27 MET.

TABLE 2. The acute effect of the weightlifting training bouts on HRV's time and frequency domains parameters

	Pre-training bout				Post-training bout				ANOVA 2 ways		
	CIb	CIs	DL	R	CIb	CIs	DL	R	Interaction Group × Training (F, p value, ηp^2)	Group effect (F, p value, ηp^2)	Training effect (F, p value, ηp^2)
HRV's time domain (N ± SD)											
HR-min	59.76 ± 9.09	62.05 ± 11.46	61.68 ± 8.78	63.05 ± 10.92	72.07 ± 11.83[a]	72.45 ± 12.90[a]	78.39 ± 11.17[a]	80.11 ± 13.42[a]	(F=2.61, p=0.06, ηp^2= 0.12)	(F=0.93, p=0.43, ηp^2= 0.05)	(F=190.48, p=0.0000, ηp^2= 0.76)
HR-max	80.29 ± 15.13	80.94 ± 13.78	78.47 ± 14.06	80.19 ± 14.98	86.50 ± 12.84	86.48 ± 13.53	95.57 ± 11.66[a]	92.87 ± 15.68[a]	(F=3.27, p=0.03, ηp^2= 0.14)	(F=0.35, p=0.79, ηp^2= 0.02)	(F=46.17, p=0.0000, ηp^2= 0.44)
HR-mean	66.74 ± 10.27	68.56 ± 11.89	67.68 ± 9.89	69.62 ± 12.76	77.89 ± 12.29[a]	77.85 ± 12.94[a]	84.47 ± 11.11[a]	85.23 ± 14.24[a]	(F=3.28, p=0.03, ηp^2= 0.14)	(F=0.70, p=0.56, ηp^2= 0.03)	(F=178.15, p=0.0000, ηp^2= 0.75)
Mean RR	925.85 ± 144.78	906.10 ± 161.04	908.42 ± 131.80	893.93 ± 165.93	790.56 ± 122.90[a]	794.81 ± 140.70[a]	725.75 ± 107.10[a]	729.09 ± 156.41[a]	(F=2.14, p=0.11, ηp^2= 0.10)	(F=0.46, p=0.71, ηp^2= 0.02)	(F=186.74, p=0.0000, ηp^2= 0.76)
HRV-SDNN	59.66 ± 21.05	58.05 ± 25.48	53.08 ± 18.06	56.73 ± 18.32	37.30 ± 19.01[a]	38.26 ± 20.72[a]	30.78 ± 25.75[a]	24.70 ± 11.56[a]	(F=0.92, p=0.44, ηp^2= 0.05)	(F=0.90, p=0.45, ηp^2= 0.04)	(F=71.99, p=0.0000, ηp^2= 0.55)
HR-RMSSD	57.41 ± 24.22	55.32 ± 22.99	52.86 ± 23.66	56.16 ± 23.32	38.15 ± 26.10[a]	39.68 ± 24.90[a]	27.30 ± 18.91[a]	25.21 ± 16.24[a]	(F=1.62, p=0.19, ηp^2= 0.08)	(F=0.69, p=0.56, ηp^2= 0.03)	(F=72.87, p=0.0000, ηp^2= 0.55)
Frequency domains and covariance (N ± SD)											
HFn	0.41 ± 0.21	0.42 ± 0.18	0.49 ± 0.23	0.46 ± 0.19	0.40 ± 0.20	0.44 ± 0.22	0.33 ± 0.14[a]	0.41 ± 0.16	(F=2.53, p=0.07, ηp^2= 0.11)	(F=0.12, p=0.95, ηp^2= 0.01)	(F=4.05, p=0.04, ηp^2= 0.06)
LFn	0.41 ± 0.22	0.40 ± 0.19	0.36 ± 0.18	0.39 ± 0.19	0.40 ± 0.20	0.41 ± 0.19	0.49 ± 0.12[a]	0.41 ± 0.14	(F=2.02, p=0.12, ηp^2= 0.09)	(F=0.07, p=0.97, ηp^2= 0.003)	(F=3.22, p=0.08, ηp^2= 0.05)
LFHF	2.01 ± 2.90	1.37 ± 1.25	1.30 ± 1.39	1.27 ± 1.30	1.44 ± 1.18	1.39 ± 1.12	1.98 ± 1.46	1.33 ± 1.04	(F=1.22, p=0.31, ηp^2= 0.06)	(F=0.42, p=0.74, ηp^2= 0.02)	(F=0.05, p=0.83, ηp^2= 0.001)
CVNN	6.40 ± 1.89	6.37 ± 2.51	5.82 ± 1.71	6.34 ± 1.63	4.58 ± 1.99[a]	4.67 ± 2.19[a]	3.97 ± 2.49[a]	3.29 ± 0.93[a]	(F=1.01, p=0.39, ηp^2= 0.05)	(F=0.97, p=0.42, ηp^2= 0.05)	(F=44.26, p=0.0000, ηp^2= 0.43)

a: significant difference from pre to post; Frontal (F), Central (C), temporal left (T_Left), temporal right (T_right) and parieto-occipital (P/O) regions; Contextual interference blocked CIb, Contextual interference serial (CIs), differential learning (DL), Repetitive (R) motor learning models.

Resting EEG

The acute effects of the weightlifting training bouts on resting brain activity are presented in Table 1 for theta, alpha, beta, and gamma frequencies.

Theta

For the theta frequency range, the two-way ANOVA revealed no statistically significant effects for training, group, or their interaction in all studied regions (F, C, T-L, T-R, and P/O), except for a significant training effect found in the P/O region ($p = 0.04$). All pairwise comparisons in the post hoc analyses were not significant (Table 1). Similarly, no significant differences among the groups were identified in the Δ pre-post training bouts.

Alpha

Regarding the alpha frequency, there was no significant interaction (training × group) and no significant main effect of the group across all parameters. However, statistically significant main effects of training were found in all examined regions (F, C, T-L, T-R, and P/O). Particularly, significantly higher values during the post-training bouts compared to the pre-training bouts were found in CIb in the C ($p = 0.02$) and P/O ($p = 0.01$) regions, in CIs in the F ($p = 0.05$) and P/O ($p = 0.02$) regions, in DL in the T-R ($p = 0.05$) and P/O ($p = 0.004$) regions, and in the R group in the C ($p = 0.01$), T-R ($p = 0.001$) and P/O ($p = 0.01$) regions (Table 1). Regarding the Δ pre-post training bouts, a significantly lower increase was observed in CIs compared to the RL group in the T-R region ($p = 0.02$). In contrast, in the frontal region, the CI group showed a trend toward nonsignificant increases compared to DL and RL (ES = 0.5, Figure 3).

Beta

Similarly, in the beta frequency, there was no significant interaction (training × group) and no significant main effect of group across all parameters. However, significant main effects of training were found in all examined regions (F, C, T-L, T-R, and P/O). Particularly, significantly higher values during the post- compared to the pre-training bouts were found in CIb in all regions with $p < 0.001$, in CIs in the F ($p=0.01$), C, and P/O ($p=0.02$) regions, in DL in the C ($p=0.01$), T-R ($p=0.002$), and P/O ($p=0 < 0.0001$) regions, and in the RL group in the C ($p=0.01$), T-R and P/O ($p < 0.001$) regions (Table 1). Regarding the Δ pre-post training bouts, a significantly lower increase was observed in the CIs compared to CIb group in the T-R region

(p=0.01). In the same way, CIb showed a trend toward nonsignificant higher increases compared to DL and RL in frontal (ES=0.7 and 0.6, respectively) and T-L (ES=0.8 and 0.5, respectively) regions and compared to CIs in T-R (p=0.8) and P/O (p=0.6) regions (Figure 4).

Gamma

Equally, the analysis of gamma frequency yielded significant main effects of training in all examined regions (F, C, T-L, T-R, and P/O), with no significant interaction (training × group) and no significant main effect of group across all parameters. Particularly, significantly higher values during the post- compared to the pre-training bouts were found in CIb in all regions (p range between 0.0001 and 0.003), in CIs in the F (p=0.01), C (p=0.03) and P/O (p=0.04) regions, in DL in the C (p=0.01), T-R (p=0.01) and P/O (p=0.004) regions, and in the R group in the F (p=0.004), C (p=0.01), T-R (p=0.001) and P/O (p=0.002) regions (Table 1).

Regarding the Δ pre-post training bouts, significantly lower increases were observed in the DL compared to CIb group in the T-L region (p=0.03). In the same way, CIb showed a trend toward nonsignificant higher increases compared to DL in frontal T-L (ES=0.8) regions and compared to CIs in T-R (p=0.6) and P/O (p=0.5) regions (Figure 5).

Resting HRV

The acute effect of the weightlifting training bouts on the HRV time and frequency domains parameters are presented in Table 2.

Time domain parameters

There was no significant main effect of the group for any of the time domain parameters, while significant main effects of training were registered for all parameters, and a significant interaction (Time × group) was registered in HR-mean and HR-max. Particularly, significantly higher values of HR-min ($p < 0.0001$) and HR-mean ($p < 0.001$) and lower values of mean RR ($p < 0.0001$), SDNN and RMSSD (p values range between < 0.0001 and 0.02) were found during the post- compared to the pre-training bouts in all groups. Higher values of HR-max were registered at post- compared to pre-training bouts only for the DL and R groups ($p < 0.0001$ and p=0.003, respectively) (Table 2).

Regarding the difference between groups in terms of Δ pre-post training bouts (Figure 6), the DL group showed significantly more prominent increases in HR-min (p=0.03), HR-max (p=0.01) and HR-mean (p=0.01) and a decrease in mean RR (p=0.02) compared to the CIs. Additionally, more prominent increases in HR-mean (p=0.02) and HR-min (p=0.02) and a decrease in RMSSD (p=0.04) were recorded in the RL group compared to CIs.

Frequency domain parameters

There was no significant main effect of group or significant interaction (Training × group) in any of the tested parameters. Significant main effects of training were found for HFn, LFn and CVNN (Table 2), with lower CVNN values at post- compared to pre-training bouts in all tested groups (p values range between < 0.0001 and 0.02) and higher LFn values (p=0.03) and lower HFn values (p=0.001) at post-compared to pre-training bouts only in the DL group.

Regarding the difference between groups in terms of Δ pre-post training bouts (Figure 7), the DL group showed significantly more prominent increases in LFn and decreases in HFn compared to the CIb (p=0.03 and 0.04, respectively) and CI groups (p=0.05 and 0.01, respectively). Likewise, a more prominent increase in LFHF was revealed in the DL group compared to CIb (p=0.05).

HR zones of the weightlifting training bouts

Times (in %) spent in HR zones 1, 2, and 3 during the weightlifting training bouts are presented in Figure 8. As this figure shows, less time was spent in HR zone 1 (< 60% of HR max) following DL and RL practices compared to CIb (p=0.01). In contrast, although not significant, during the DL practice, participants tended to spend more time in HR zone 3 (70–80% of HR max) compared to CIb (ES=0.5) and CIs (ES=0.4).

Correlation between HRV and EEG-related parameters

The correlation coefficients between selected HRV and EEG responses to the weightlifting training bouts are presented in Table 3. As this table indicates, pooled HFn was significantly correlated with (i) beta and gamma frequencies in all tested brain regions except the frontal lobe (r=0.3 with p range between 0.01 and 0.03) and (ii) alpha frequencies only in the frontal lobe (r=0.3, p=0.03). Additionally, the LFHF ratio was shown to significantly correlate with alpha and beta frequencies in the frontal and P/O lobes, respectively (r=-0.3 and p=0.03). Looking at the correlation in response to each motor learning model, significant negative correlations were found between SDNN and alpha P/O and beta F with r=-0.5 and p=0.04.

RPE and mental demand

There was no significant main effect of group on perceived exertion and mental demand. Post hoc comparison showed no significant differences between groups in RPE values, with only a trend for higher values in DL compared to CIb and CIs (ES= 0.5) (Figure 9). Regarding mental demand, post hoc comparison showed significantly higher values following DL compared to RL practice (p=0.04) and trends toward higher values compared to both CI practices (ES ranging between 0.5 and 0.6) (Figure 9).

Kinematic variables

The acute effects of the weightlifting training bouts on technical efficiency based on barbel paths and selected barbel displacements are presented in Figure 10. The one-way ANOVA showed no significant main effect of the group in any of the tested parameters (peak vertical displacement; mh1, mh2 and DXL). In the same way, pairwise comparisons showed no significant differences between groups.

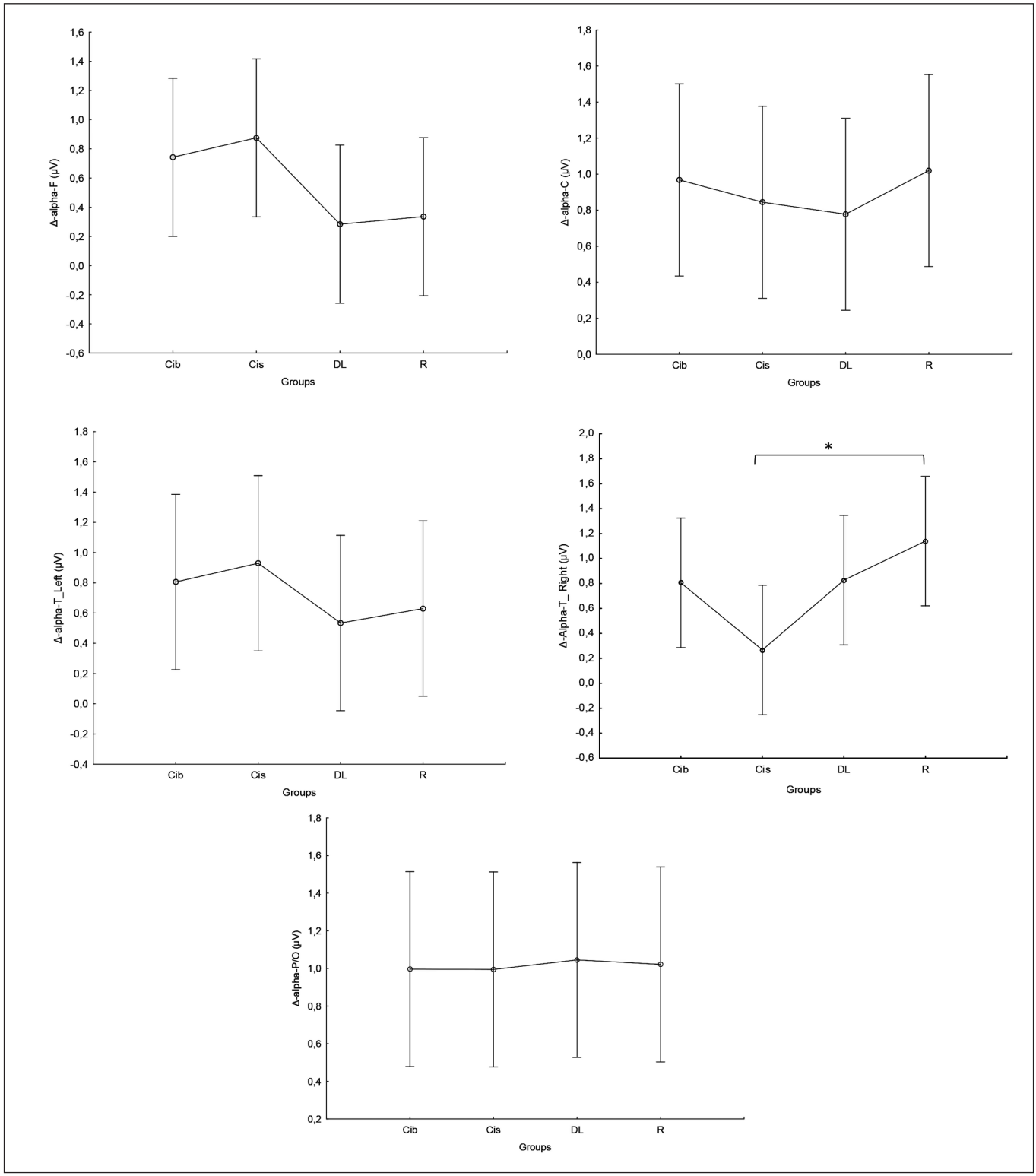


FIG. 3. The acute effect of weightlifting training bouts on Δ (pre-post bouts) alpha frequency recorded at different brain regions. a: significant difference between groups at $p < 0.05$; Frontal (F), Central (C), temporal left (T_Left), temporal right (T_right) and parieto-occipital (P/O) regions; Contextual interference blocked (CIb), Contextual interference serial (CIs), Differential Learning (DL), Repetitive (R) motor learning models.

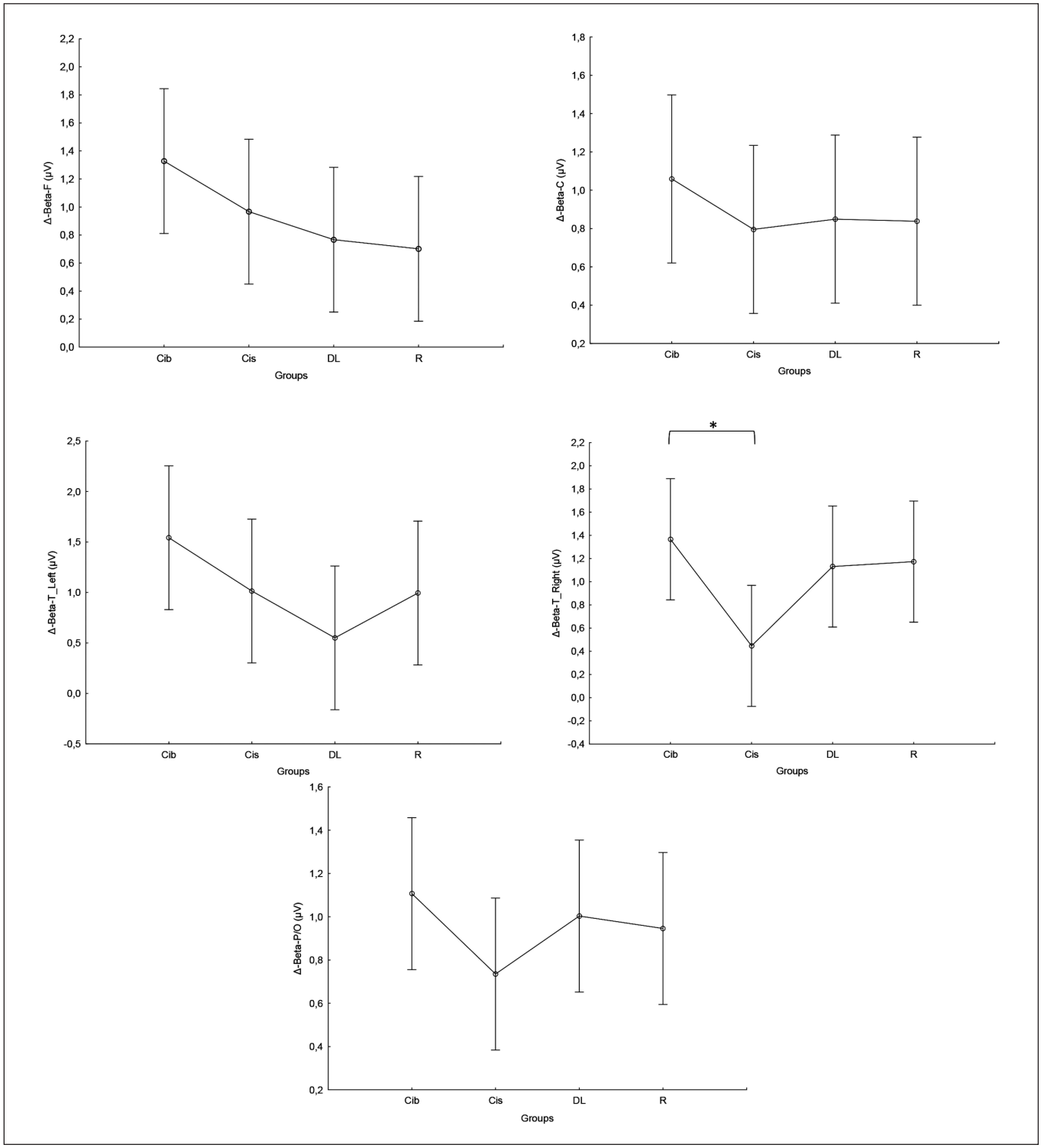


FIG. 4. The acute effect of weightlifting training bouts on Δ (pre-post bouts) Beta frequency recorded at the frontal, fronto-central, temporal (Left and right) and parieto-occipital regions.
a: significant difference between groups at $p < 0.05$; Frontal (F), Central (C), temporal left (T_Left), temporal right (T_right) and parieto-occipital (P/O) regions; Contextual interference blocked CIb, Contextual interference serial (CIs), Differential Learning (DL), Repetitive (R) motor learning models.

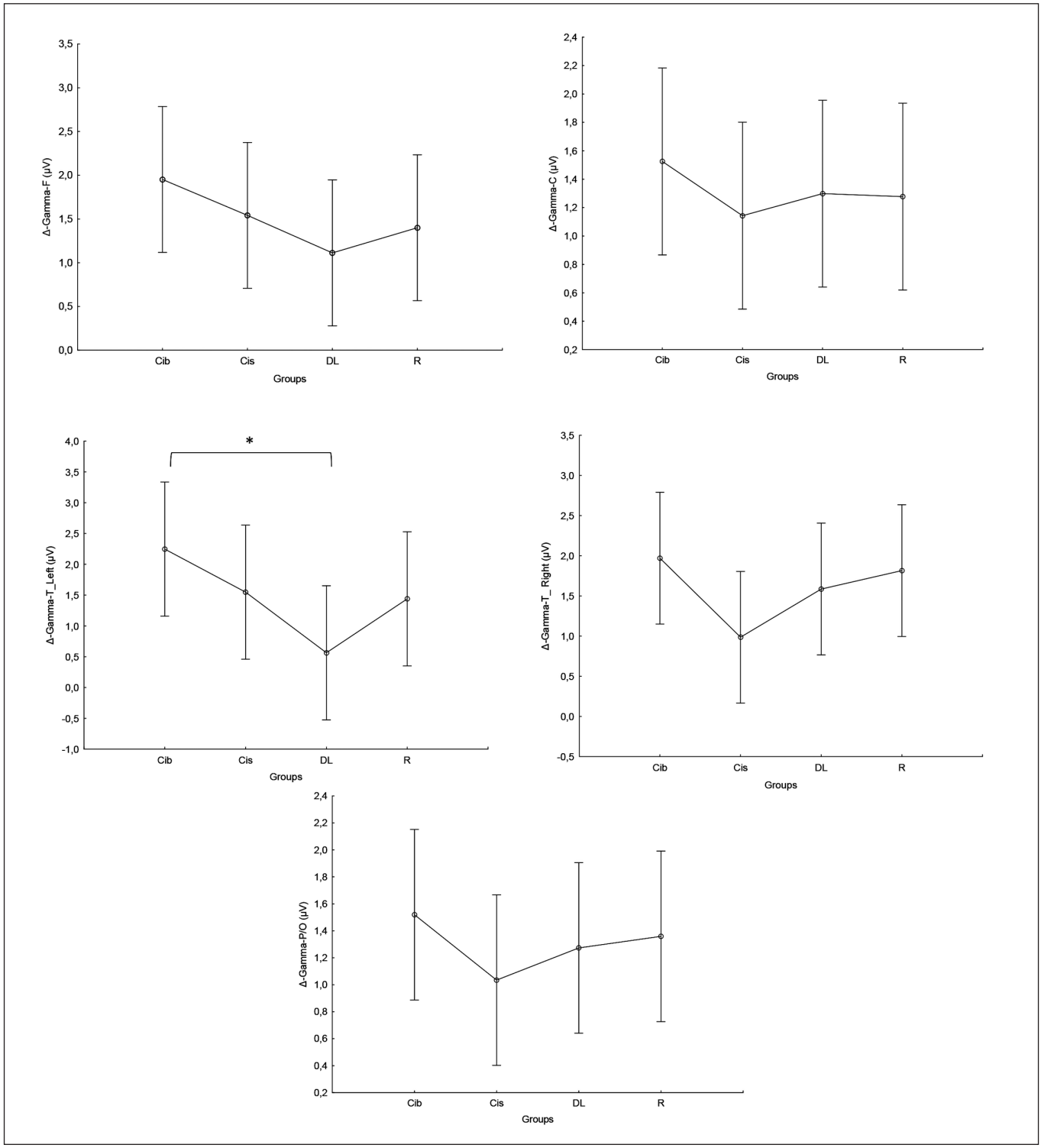


FIG. 5. The acute effect of weightlifting training bouts on Δ (pre-post bouts) Gamma frequency recorded at the frontal, fronto-central, temporal (Left and right) and parieto-occipital regions.
a: significant difference between groups at $p < 0.05$; Frontal (F), Central (C), temporal left (T_Left), temporal right (T_right) and parieto-occipital (P/O) regions; Contextual interference blocked (CIb), Contextual interference serial (CIs), Differential Learning (DL), Repetitive (R) motor learning models.

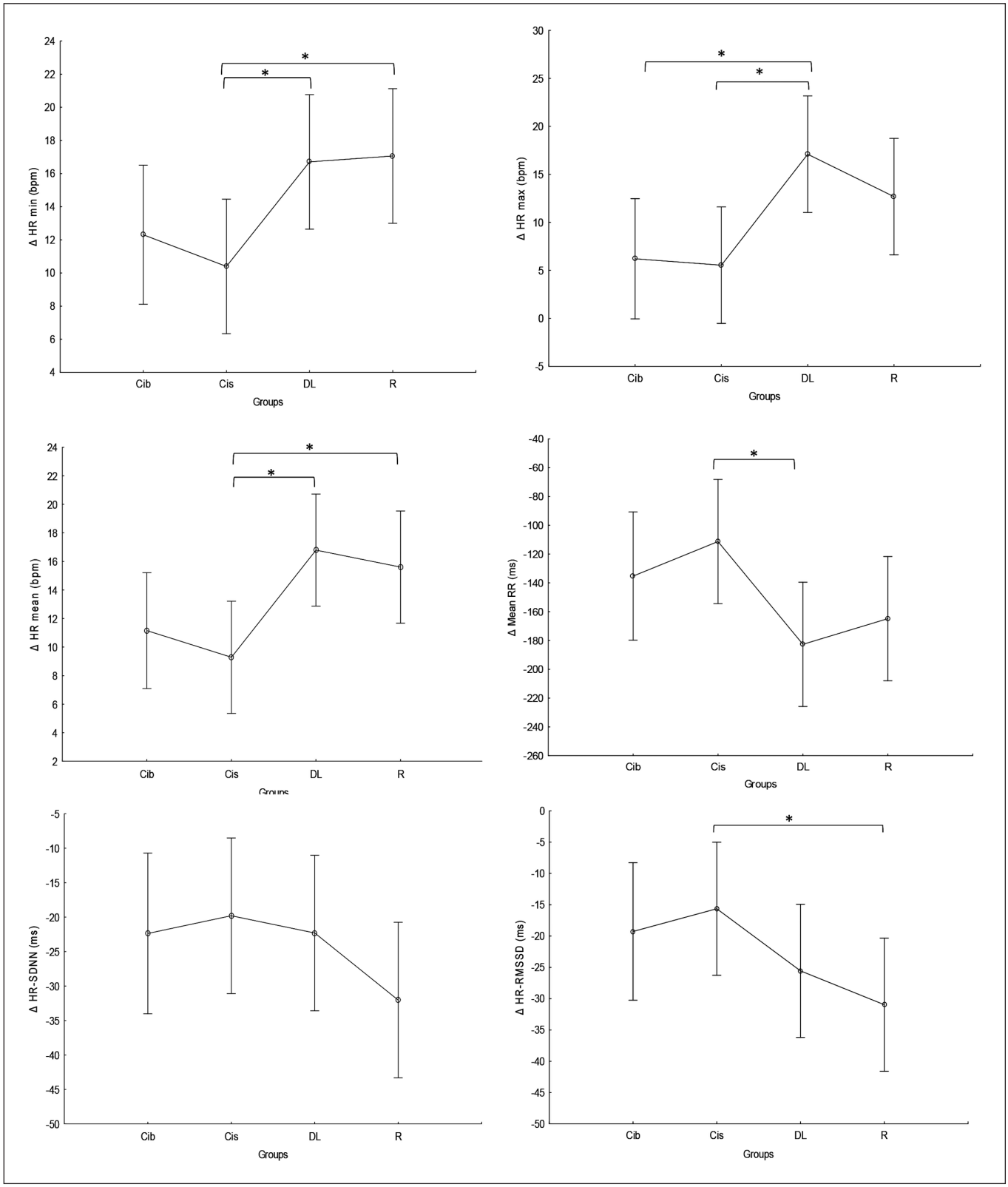


FIG. 6. The acute effect of weightlifting training bouts on Δ (pre-post bouts) HRV´s time domain parameters.
a: significant difference between groups at $p < 0.05$; Minimum heart rate (HR min); Maximum heart rate (HR max); Mean Heart Rate (HR mean); mean RR interval (Mean RR); Standard deviation of NN intervals (SDNN); Root mean square of successive differences (RMSSD); Contextual interference blocked (CIb), Contextual interference serial (CIs), Differential Learning (DL), Repetitive (R) motor learning models.

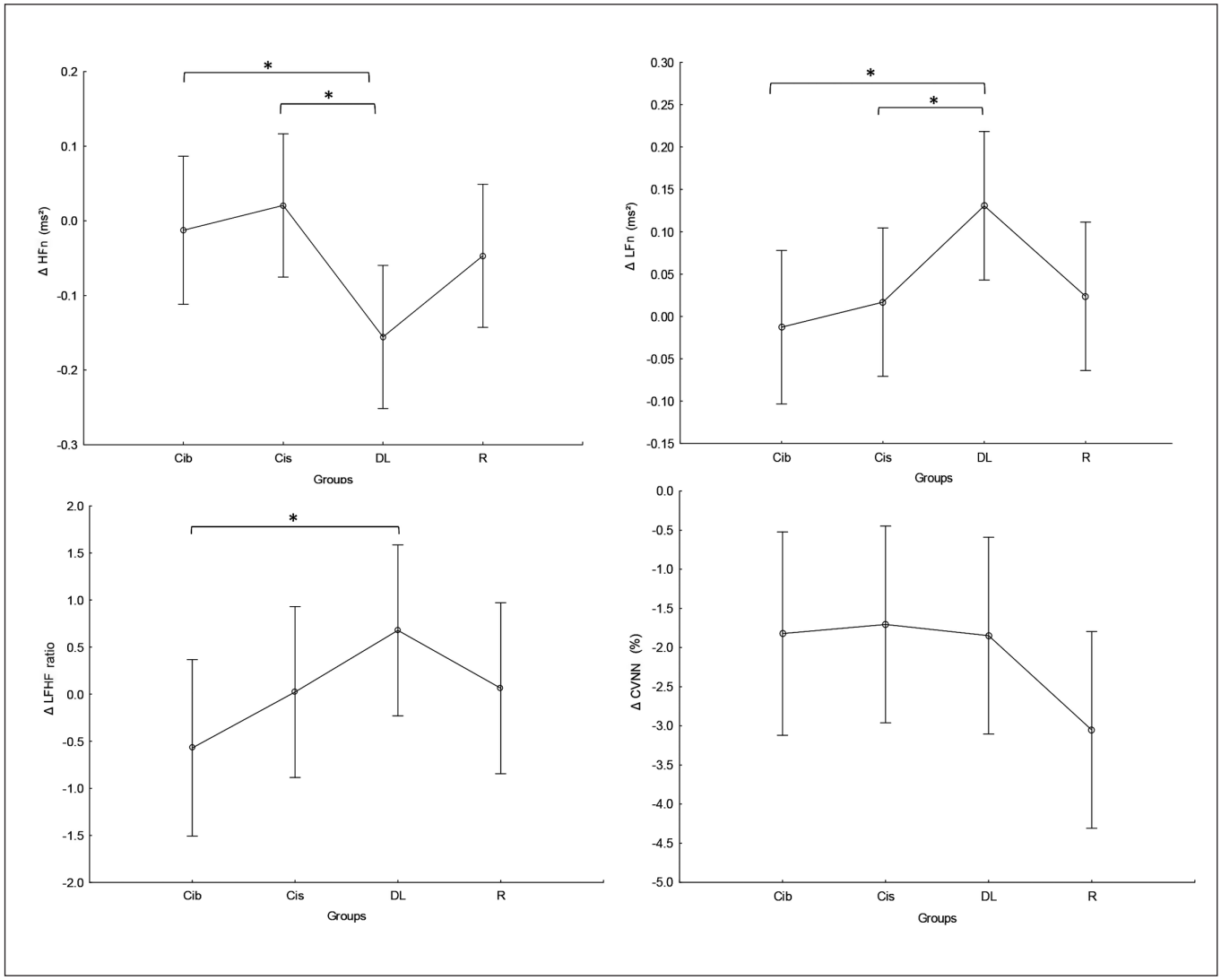


FIG. 7. The acute effect of weightlifting training bouts on Δ (pre-post bouts) frequency domains and covariance.
a: significant difference between groups at $p < 0.05$; HF, high-frequency power; Low-Frequency power (LF); Ratio of Low Frequency to High Frequency (LF/HF ratio); coefficient of variation (CVNN); Contextual interference blocked (CIb), Contextual interference serial (CIs), Differential Learning (DL), Repetitive (R) motor learning models.

DISCUSSION

The purpose of the present study was to assess the acute systemic effects of weightlifting training bouts following different motor learning models on neurophysiological responses and technical efficiency in novices. The main findings revealed increases in alpha, beta, and gamma frequencies following weightlifting training bouts, while theta frequencies remained unaffected. Notably, CIb practice resulted in increased frequencies across the alpha, beta, and gamma bands in 12 out of 15 brain regions (5 regions per band). Subsequently, RL practice showed increases in 10 out of 15 regions, and DL and CIs practice exhibited changes in 8 out of 15 regions. Regardless of the learning models, these changes in brain activity were accompanied by an alteration in HRV's time domain parameters, characterized by increased HR- and decreased RR-related parameters, with more pronounced changes in DL compared to CI. In terms of HRV frequency domain parameters, only DL exhibited increased LFn and decreased HFn. HFn demonstrated positive correlations with beta and gamma frequencies across all tested brain regions except the frontal lobe. In the frontal lobe, this correlation was only significant with the alpha frequency. During the weightlifting training bouts, elevated HR zones (≥ HR zone 3) predominated with DL showing a tendency toward more extended periods in HR zone 3 compared to the other models. Following the weightlifting training bouts, the DL model tended to exhibit higher perceived physical and mental exertions. Concerning the technical efficiency, assessed through selected time discrete and averaged parameters from the barbell trajectory, the current results indicate no significant advantage of one learning model over the other in novices.

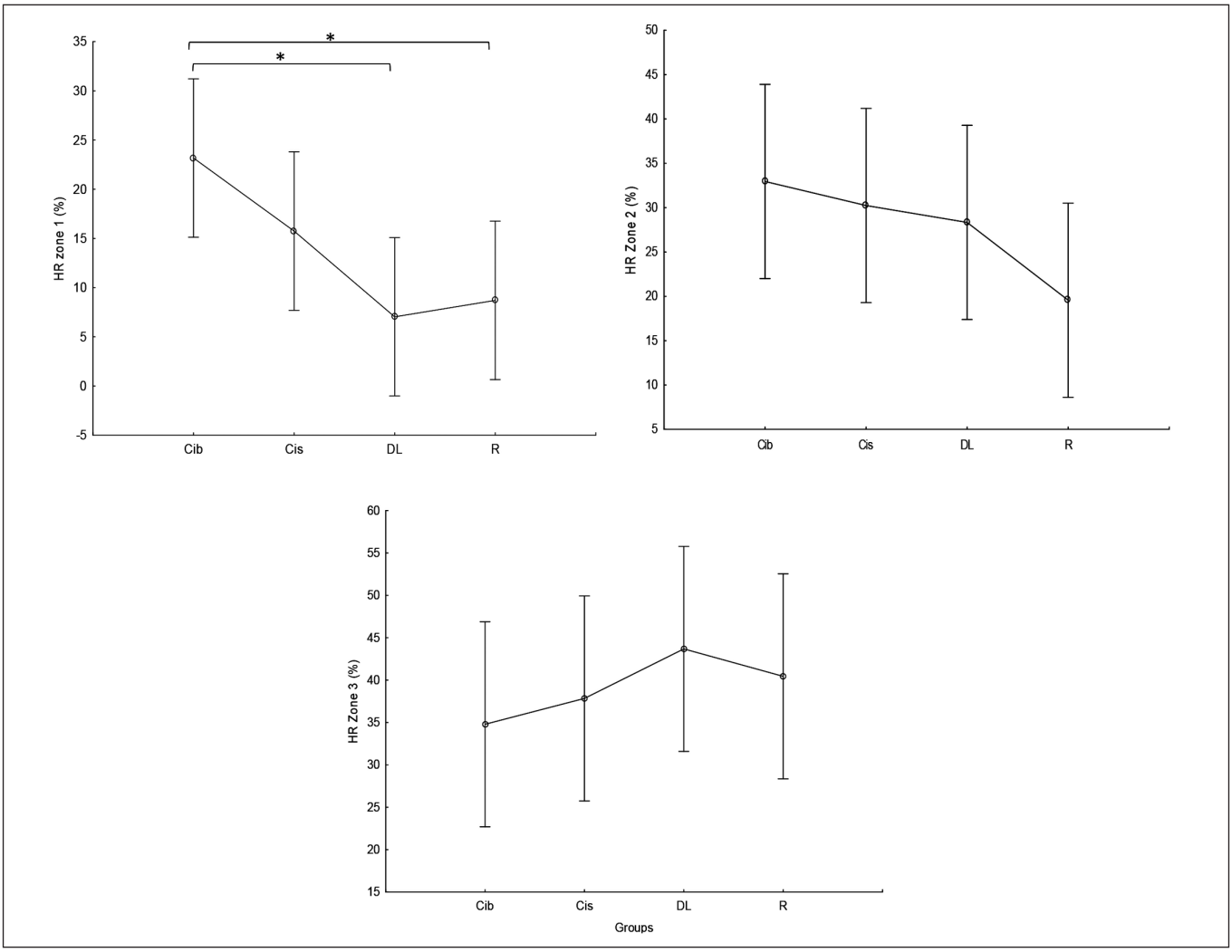


FIG. 8. Proportion of the time spent in HR zones 1 to 3 during the weightlifting training bouts.
a: significant difference between groups at $p < 0.05$; Contextual interference blocked (CIb), Contextual interference serial (CIs), Differential Learning (DL), Repetitive (R) motor learning models.

Resting EEG brain activity can be measured shortly before and after an exercise intervention, which offers a noninvasive, reliable method to study temporary changes in electrocortical activity [78, 79]. Although strength exercise is assumed to induce task-related brain activity [80] and is recognized for its demand on focus and concentration [81, 82], increasing arousal and motivation [83], neurotransmitter release, and blood flow [84], potentially altering associated brain wave patterns, most studies examining the acute effect of exercise on electric brain activity most often focus on endurance-based exercise [42]. A recent systematic review by Hosang et al. [42] reported that out of the 47 included studies, only two [85, 86] evaluated brain activity responses (pre- vs. post-exercise) to strength-based single joint exercises, while the remaining studies focused on cycling and running. Gwin and Ferrsis [85] compared the coherence between contralateral motor cortex EEG signals and lower-limb EMG in the beta and gamma bands during isometric vs. isotonic knee or ankle joint movements. However, the change from pre- to post-resting EEG activity was not controlled. In contrast, Brümmer et al. [86] investigated the change from pre- to post-exercise bouts. However, the explored exercise bouts focused on a strength-based exercise involving only a single joint, controlled by a small muscle group in a seated position (i.e., dynamometer wrist flexion) to minimize coordinative aspects. In our exploration of various databases and sources, we also identified two EEG-based studies on strength training [80, 83] that specifically investigated the electrical activity of the brain during the bench press. Maszczyk et al. [83] identified motivational patterns through frontal alpha asymmetry at an intensity of 60% 1RM and above, while Engchuan et al. [80] observed increases in beta and gamma frequency bands during the bench press. However, careful interpretation is warranted due to certain methodological considerations. In the study by Engchuan et al. [80], EEG activity was recorded from only one location (Fp1), sensitive

TABLE 3. The correlation coefficients between selected HRV and EEG responses to the weightlifting training bouts.

		HRV's time domain			Frequency domains and covariance			
		Mean R-R	**SDNN**	**RMSSD**	**HFn**	**LFn**	**LFHF**	**CVNN**
Alpha frequency	Fp/F	r=-0.17	r=-0.06	r=0.002	**r=0.27**	r=-0.17	**r=-0.27**	r=0.02
		p=0.19	p=0.65	p=0.99	**p=0.03**	p=0.18	**p=0.03**	p=0.87
	C	r=-0.15	r=-0.11	r=-0.05	r=0.22	r=-0.12	r=-0.18	r=-0.06
		p=0.24	p=0.40	p=0.71	p=0.08	p=0.35	p=0.17	p=0.66
	T_links	r=-0.16	r=-0.15	r=-0.08	r=0.21	r=-0.15	r=-0.18	r=-0.10
		p=0.20	p=0.23	p=0.53	p=0.10	p=0.25	p=0.15	p=0.42
	T_recht	r=-0.17	r=-0.13	r=-0.10	r=0.19	r=-0.11	r=-0.20	r=-0.09
		p=0.17	p=0.29	p=0.43	p=0.13	p=0.39	p=0.12	p=0.50
	P/O	r=-0.19	r=-0.13	r=-0.07	r=0.23	r=-0.17	r=-0.22	r=-0.08
		p=0.14	p=0.31	p=0.57	p=0.07	p=0.19	p=0.08	p=0.56
Beta frequency	Fp/F	r=-0.16	r=-0.06	r=-0.01	r=0.22	r=-0.11	r=-0.13	r=-0.004
		p=0.20	p=0.62	p=0.91	p=0.09	p=0.39	p=0.30	p=0.97
	C	r=-0.05	r=-0.04	r=0.03	**r=0.31**	r=-0.18	r=-0.22	r=-0.01
		p=0.69	p=0.78	p=0.82	**p=0.01**	p=0.16	p=0.08	p=0.92
	T_links	r=-0.12	r=-0.15	r=-0.06	**r=0.28**	r=-0.18	r=-0.21	r=-0.12
		p=0.33	p=0.25	p=0.65	**p=0.03**	p=0.16	p=0.10	p=0.36
	T_recht	r=-0.10	r=-0.01	r=0.01	**r=0.29**	r=-0.16	r=-0.24	r=0.03
		p=0.45	p=0.93	p=0.97	**p=0.02**	p=0.19	p=0.06	p=0.84
	P/O	r=-0.07	r=-0.05	r=0.02	**r=0.32**	r=-0.20	**r=-0.27**	r=-0.01
		p=0.60	p=0.73	p=0.91	**p=0.01**	p=0.11	**p=0.03**	p=0.91
Gamma frequency	Fp/F	r=-0.13	r=-0.02	r=0.01	r=0.18	r=-0.08	r=-0.03	r=0.03
		p=0.32	p=0.89	p=0.94	p=0.16	p=0.52	p=0.79	p=0.79
	C	r=-0.01	r=0.07	r=0.10	**r=0.27**	r=-0.15	r=-0.12	r=0.10
		p=0.92	p=0.56	p=0.43	**p=0.03**	p=0.24	p=0.33	p=0.43
	T_links	r=-0.04	r=-0.01	r=0.05	**r=0.27**	r=-0.17	r=-0.14	r=0.02
		p=0.76	p=0.93	p=0.71	**p=0.03**	p=0.19	p=0.26	p=0.89
	T_recht	r=-0.08	r=0.11	r=0.09	**r=0.27**	r=-0.17	r=-0.16	r=0.16
		p=0.54	p=0.39	p=0.47	**p=0.03**	p=0.19	p=0.22	p=0.21
	P/O	r=0.03	r=0.14	r=0.16	**r=0.30**	r=-0.18	r=-0.15	r=0.18
		p=0.81	p=0.27	p=0.22	**p=0.02**	p=0.15	p=0.22	p=0.16

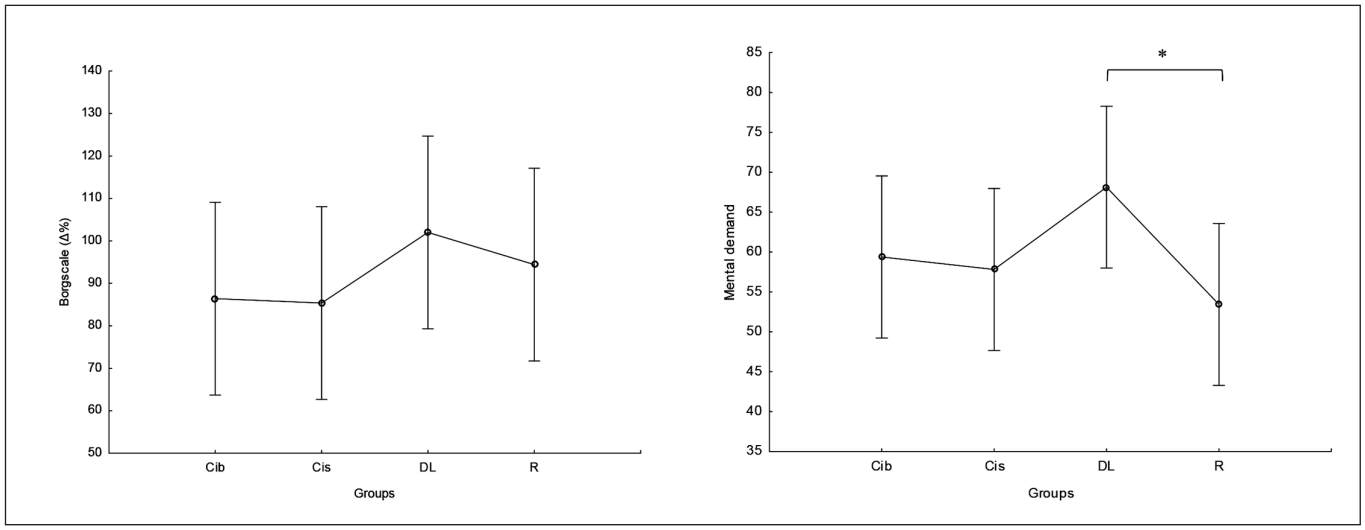


FIG. 9. The acute effect of weightlifting training bouts on perceived exertion and mental demand.
a: significant difference between groups at $p < 0.05$; Contextual interference blocked (CIb), Contextual interference serial (CIs), Differential Learning (DL), Repetitive (R) motor learning models.

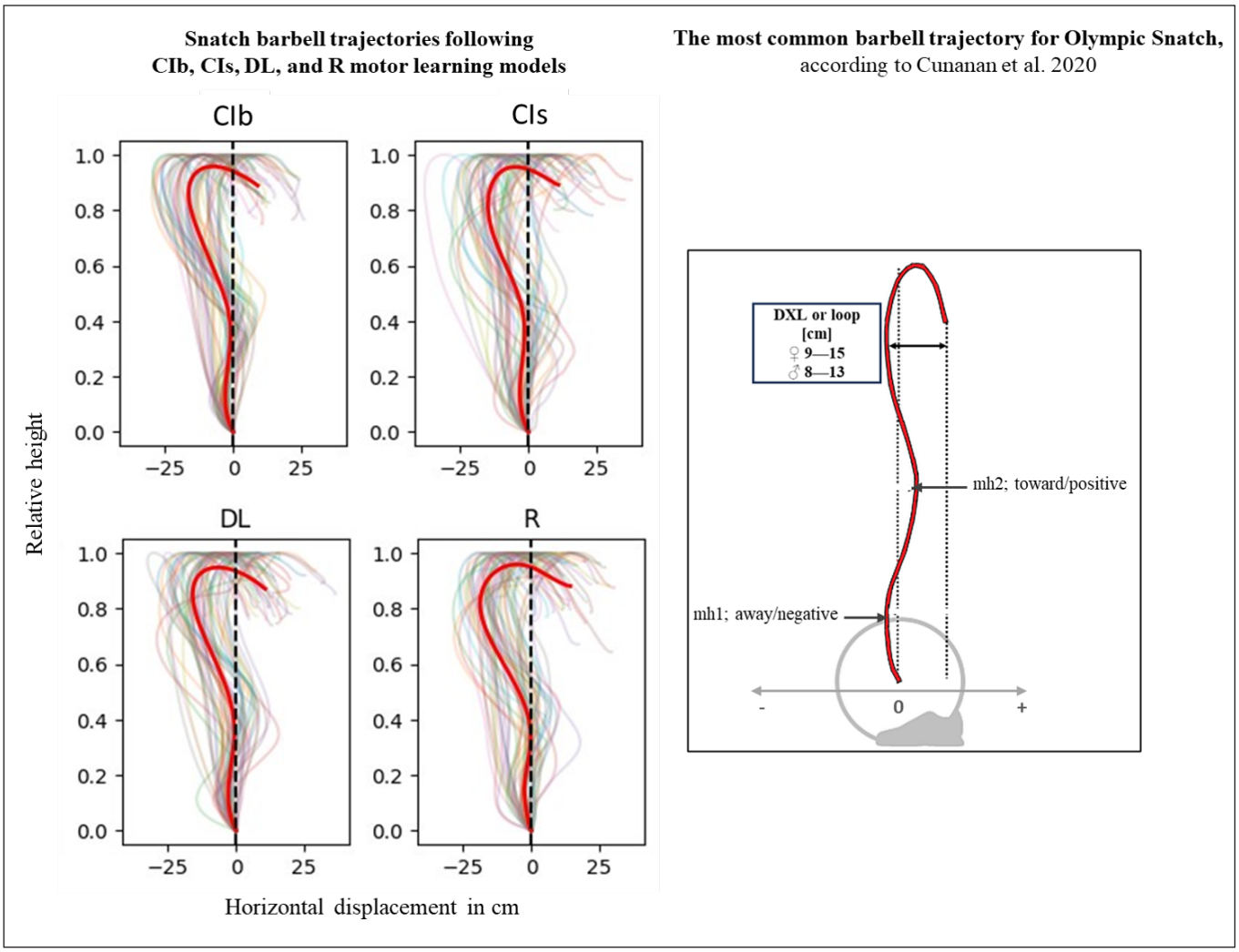


FIG. 10. The acute effects of various weightlifting training bouts on technical efficiency based on barbel paths.

to muscular and ocular artifacts. Additionally, EEG activity was solely recorded during exercise, marked by a broader range of electrical muscle activity (10–200 Hz), potentially influencing gamma bands (30.5–60 Hz). Conversely, Maszczyk et al. [83] employed automatic headcaps with 19 electrodes for EEG recording, but analysed EEG activity exclusively from two locations (F3 and F4). Additionally, it's worth noting that resting EEG activity was recorded for only 15 seconds before each intensity [83], whereas the recommended recording duration for establishing a baseline resting state [87] and achieving reliable results, especially in lower frequency bands, is typically 5–10 minutes. While these studies offered preliminary insights into the acute effects of strength exercise on neurophysiological EEG-based responses, none conducted a comprehensive evaluation of the acute impact on resting brain activity within the context of a strength-dominated whole-body task. Furthermore, none explored the effects of combined coordination-strength-based whole-body tasks. The present study on the latter type of movement represents, therefore, a pioneering effort to comprehensively explore this research field.

The present EEG findings indicate that regardless of the adopted motor learning approach, significant increases in beta and gamma frequencies were found in both the central and P/O regions following the weightlifting training bouts. These findings align with those of Engchuan et al. [80] during bench press movements. Similarly, regardless of the adopted motor learning approach, the present findings report an increase in alpha frequency in the P/O region. An increase in alpha frequency is typically associated with memory consolidation [88], integration of different brain areas [89], and introspection [90], which appears to be particularly pronounced in the studied whole-body movement. According to the dual-mode theory of exercise-related affect [91] and considering limited cortical resources, it is suggested that acute whole-body exercise leads to a redistribution of brain activity, particularly in areas typically associated with preparing and performing motor commands, such as the motor and somatosensory cortex. The present findings only partially support this theoretical framework, specifically in relation to increased motor and somatosensory cortex activities. Indeed, alpha, beta, and gamma frequencies increased after most of the tested weightlifting bouts in the (i) parietal lobe, known to contain the primary

somatosensory cortex crucial for spatial awareness and processing sensory information related to body position and movement; (ii) occipital lobe, which encompasses the primary visual cortex responsible for processing visual information from the eyes; and (iii) central lobe, incorporating the specific C3 and C4 electrodes capturing activity in the primary motor cortex (M1). However, the present findings did not fully support the redistribution of brain activity in response to strength-based exercise, as such frameworks could indicate decreases in other brain regions, such as the frontal lobe, previously suggested by the transient hypo-frontality hypothesis [92]. Indeed, none of the different frequency bands showed a significant decrease in the frontal lobe or in the temporal lobe in response to the present weightlifting exercise.

Particularly, a deeper look at the frontal alpha activities following each of the motor learning models indicates either a significant increase after the CIs model or a stagnation using the CIb, DL, and RL models. Thus, the hypo-frontality hypothesis, previously mentioned by some studies to explain the decrease in frontal alpha activity in response to some endurance-based exercises [86, 93], cannot be supported in response to the conditions presented with combined coordination-strength training. The notable increase in frontal alpha activity following the CI models might be reflective of the execution of cognitive functions such as attentional focus and recovery strategies, known to be a germane process to exercise [42, 94, 95]. Significant increases in central alpha activity have been previously suggested to reflect a decrease in neuro-connectivity from somatosensory afferents [96], as the central sites of the brain have been shown to overlay regions of the sensorimotor cortex that receive afferent feedback [97]. The present findings on alpha frequency did not align with this assumption and showed that the increases in the central region following CIb and RL practices were accompanied by elevated alpha P/O following both models, along with increased alpha T-R following RL. In addition to these increases following the CIb and RL models, alpha frequencies also exhibited increases in P/O following CIs and DL, along with an increase in frontal and T-R regions, respectively, following these two models. These findings are in line with previous studies in endurance-based training reporting enhanced alpha activity both during and post-exercise across the limbic lobe/system (deep in the temporal lobe) [98, 99]. It might be argued that such an increase in gamma activity during exercise results from the cortical inhibition caused by the brainstem and subcortical activation [42, 100, 101]. Taken together, the present study provided evidence that in the P/O region, alpha frequency can increase regardless of the motor learning models applied to whole body movements, while in the other brain regions, changes (increases or stabilizes) depend on the learning model used. These coordination-strength-related findings seem to align with findings from the majority of previous endurance-based studies reporting either an overall increase in alpha activity mainly across the frontal areas [102, 103], with left lateralization [104], central [105, 106] and parieto-occipital [86, 107, 108] regions of the brain, or an absence of significant change in specific regions such as the left prefrontal cortex [109] and the bilateral frontal and temporal or parietal cortex [110, 111].

Regarding the beta frequency band, previous endurance-based exercise revealed primarily increased values, followed by decreased values and no significant changes from pre- to post-exercise [42], with increased activity during and after cyclic endurance activities mainly identified across the frontal [109, 112], central [112], and parietal [113] cortex regions [86] and limbic area [98]. The present coordination-strength-related findings align more with approaches suggesting enhanced beta activity in response to physical exercise by showing that in addition to the abovementioned central and parieto/occipital beta increases registered regardless of the motor learning model, there was also an increase in the remaining tested region for CIb, in the frontal region for CIs, and in the T-R region for DL and RL. Enhanced beta waves from pre- to post-exercise across the sensorimotor areas, reflected in the present study by the increased central and P/O beta activities following all tested strength bouts, can be associated with movement planning and production [114], while the enhanced beta activity across the temporal lobe using CIb (both left and right regions), DL, and R (only the right region), might reflect emotional arousal [115] in response to coordination-strength bouts. However, high-resolution EEG-based studies focusing on the limbic area, known to reflect affection [116], and measuring affective responses to exercise performed using different motor learning approaches are needed to test this hypothesis.

Concerning gamma activity, only a limited knowledge base is available regarding its response to physical exercise. According to the recent systematic review by Hosang et al. [42], out of the 47 included studies, only five explored this frequency band, with two of them testing pre- and post-exercise change and none of them focusing on strength or coordinative-strength exercises. Again, contradictory results between an increase [112] and a decrease [117] in response to endurance exercises in different contexts were reported. Similar to the beta frequency, the present coordination-strength-based findings align more with approaches suggesting enhanced gamma activity in response to physical exercise and showed that in addition to the abovementioned central and parieto/occipital beta increases registered regardless of the motor learning model, there was also an increase in the remaining tested region for CIb, in the frontal region for CIs, and in the T-R region for DL and RL. Such increases in gamma activity in response to strength and/or endurance dominant exercise can be linked to the increased arousal that is germane to exercise performance [118, 119]. However, due to the limited knowledge in this field, further studies measuring gamma activity in response to strength and coordination strength-based exercises are warranted to arrive at firm conclusions.

Lastly, it should be highlighted that the present coordination-strength-based findings showed no change between pre- and post-exercise in terms of theta frequency in any of the tested regions. In response to endurance-based exercise, previous studies showed mainly a significant change from pre- to post-exercise, with either

a decrease across the left prefrontal cortex [109] and the central and parietal cortices [106] or an increase in the bilateral prefrontal cortex [112]. Only one study showed no significant changes in this frequency band. Decreases in theta activity have been suggested to be indicative of impaired cognitive performance immediately after exercise, as well as the absence of relaxation [109, 119]. It can therefore be argued that the absence of a significant decrease in theta activity from pre- to post-weightlifting bouts in the present study can be reflective of reduced cognitive impairment and enhanced relaxation in response to this specific type of exercise. Such an assumption provides an advantage of strength over some endurance-based training (i.e., previously shown to decrease theta activity in different regions of the brain) in terms of exercise-induced low-frequency bands. However, this assumption needs to be corroborated by a range of coordination-strength-based exercises.

In summary, the present study provides a more comprehensive understanding of the profound effect of coordination-strength exercise, specifically weightlifting exercise, on EEG-based brain activity. In addition to the specific mechanisms associated with the different EEG frequency bands mentioned earlier, the relationship between exercise and brain activity in general has been previously associated with alterations in synaptic plasticity neurogenesis in the hippocampus in response to exercise [120]. The actions of signaling molecules such as brain-derived neurotrophic factor and insulin-like growth factor-1 may play pivotal roles in these processes [121–123]. Increased circulating cortisol immediately following exercise has also been shown to affect resting brain oscillatory activity post-exercise [124]. Beyond potential biochemical and hormonal influences, future research should explore mid and long-term effects, as well as individual and situational specificities, to comprehensively understand the complexity of these interactions [1].

To obtain a more comprehensive understanding of the neurophysiological responses to strength and coordination-strength exercise, the present study incorporated resting HRV measurements, known to mainly reflect the function of the autonomic nervous system (ANS) [125], in parallel with EEG recordings. A detailed analysis of the acute effect of coordination-based strength exercise on HRV-related parameters revealed an increase in HR-related parameters (e.g., min, max, and average) and a decrease in RR-related parameters (i.e., mean RR, HRV-SDNN, and RMSSD), with more pronounced changes in DL. In terms of HRV frequency domain parameters, only DL exhibited increased LFn and decreased HFn. These changes suggest the existence of strong physiological strain and a connected regulation of the ANS throughout cardiac vagal control in response to the weightlifting training bouts. In general, HF-HRV is considered to be more prominently dominated by parasympathetic tone [126], while LF-HRV reflects brain activity involved in executive functioning, including working memory utilization [127, 128]. It seems therefore reasonable to assume that strength exercise combined with DL practice seems to reduce parasympathetic resting dominance through lowered vagal modulation and increased sympathovagal balance [129], as well as engage individuals in higher working memory utilization and executive function involvement. These suggestions align with the present results of the perceived mental demand, indicating a trend toward higher mental demand following DL compared to the other learning model (ES ranging between 0.5 and 0.6), which reached significance when compared to RL practice. The extent to which this is related to the increased interaction between the heart and brain, previously reported in DL in combination with rope skipping [47], requires further research. Additionally, more coordination-strength-based studies in different motor learning models are needed to corroborate these suggestions. The present effects of weightlifting-bouts exercise on HRV align with previous findings by de Paula et al. [129], who demonstrated that both aerobic- and strength-based exercise elicited an acute change in HRV characterized by increased HR, LF, and LFHF ratio, along with decreased RR interval and HF. However, this study focused only on individuals with autonomic dysfunction. In healthy adults, previous HRV studies in the field of physical exercise have predominantly focused on the chronic effects of various exercise training regimens on HRV parameters. Notably, strength training has been comparatively less explored in this context and has yielded controversial results [130]. Isometric handgrip training (IHG) and high-intensity strength training fail to induce HRV modifications [131, 132]. Whole-body resistance training in young women has been shown to significantly improve geometric indices [133]. Similarly, 8 weeks of two different resistance training methods (clustering vs. multisets) were found to increase RMSSD and decrease resting heart rate [134]. An increase in autonomically mediated heart rate complexity has also been registered after resistance training, reflecting increased parasympathetic and/or reduced sympathetic cardiac autonomic control [135]. Comparing the chronic effects of high vs. low resistance training intensity, Lin et al. [136] reported improvements in resting heart rate and specific HRV measures in high-intensity training compared to low-intensity training. Again, more strength-based studies in different motor learning models are needed to corroborate these suggestions. Regarding the acute effect, a previous study showed a decrease in RR interval and SDNN and an increase in LF following rope skipping bouts, with a more pronounced effect in the DL group [46]. Lower HRV has been previously correlated with higher cognitive demand, defined by greater executive task strain and particularly sustained attention [137]. Accordingly, the more pronounced decrease in HRV parameters accompanied by the higher mental demand perception and the increase in LF power in response to DL practice registered in the present as well as in the rope skipping study support once more the hypothesis of higher cognitive demands after DL accompanied by specific parasympathetic involvement.

Concerning the correlation between EEG and HRV parameters, previous studies have mainly focused on simultaneously recording EEG and HRV in response to cognitive tasks [138–140]. These studies indicated that executive function is closely related to vagally mediated HRV parameters as a result of the involvement of inhibitory inputs from the prefrontal-subcortical inhibitory circuits to the heart via the vagus nerve [45]. Additionally, in several electrodes, beta

relative power during the working memory task was found to be negatively correlated with resting HF-HRV [139]. These findings suggested HRV as a possible biosignal indicator reflecting brain activity related to working memory [139], executive control functions such as the inhibition of unwanted responses [141], and mental health status in general [44].

Despite these promising findings, limited number of studies that concurrently explore both EEG and HRV responses to physical exercise. For instance, John et al. [142] analyzed the correlation between both measurements during and following (i.e., short-term recovery) incremental exercise testing [142]. The findings showed moderate negative correlations between LF power (ECG) and beta and gamma frequencies (EEG) in total power and the frontopolar, temporal, and occipital lobes with LF power during exercise, which were reduced to only cover the temporal lobe of beta and gamma total spectra. These results are partially in line with the present one, showing a significant positive correlation between HFs and beta and gamma frequencies in 4 out of 5 brain regions (C, T-L, T-R, and P/O) and alpha frequency only in the frontal lobe. These results suggest links between cardiovascular strain induced by strength exercise and motor and sensory demands, followed by an executive function control requirement. A closer examination of the EEG-ECG correlation in each motor learning model revealed a negative correlation between SDNN and alpha frequency in the P/O region and beta frequency in the frontal lobe following DL practice. These findings indicate that the greater decrease in HRV-SDNN in response to coordination-strength exercise under DL practice was associated with greater brain activation in these specific regions.

It's noteworthy that during DL practice, individuals were more engaged in the elevated HR zone (i.e., HR zone 3) and less engaged in the lower HR zone (i.e., HR zone 1) compared to other motor learning models. This heightened HR during DL practice may contribute the trend toward higher perceived exertion following the same training bouts compared to the other practice models (ES = 0.5). These findings suggest an advantage of DL over other models in terms of improved cardiac autonomic modulation and cardiovascular benefits. This aligns with a previous study that reported greater cardiac benefits of high-intensity exercise compared to low-intensity exercise [136]. Notwithstanding this, it is crucial to direct future attention to the observed phenomenon wherein the learning approach leading to the highest increase in physiological and cognitive load—namely DL, together with the CIs presenting the second largest exercise variations—demonstrated the lowest number of significant EEG-related increases. This is noteworthy as such neuro-physiological responses are typically positively correlated [142]. These findings further underscore the interplay between highly coordinated, variable training, whether in combination with endurance [47] or strength training, and its influence on the complex relationship between the heart and brain in unprecedented ways. The potential implications of these findings may extend to the field of therapies, offering novel avenues for exploration in the future.

In terms of technical efficiency, a recent study by Cunanan et al. [143] examining the barbell kinematics of top athletes participating in the 2015 world and 2017 Pan-American weightlifting championships, reported that the most common barbell trajectory for snatch follows the "'away-toward-away-toward' pattern with a loop (the horizontal displacement from the most forward position to the catch position) ranging between 9 and 15 cm for male athletes. This pattern describes that during the lift, the bar first moves away from the body, then toward the body, then away and toward the body again, resulting in negative-positive-negative-positive displacements [41, 52, 76]. The results from this weightlifting study, conducted with novices, indicate that regardless of the motor learning models employed, the averaged barbell paths do not align with the recommended trajectory. Indeed, positive horizontal displacement values are observed only during the "catch phase" at the end of the movement. Similarly, none of the loop values fell within the recommended range of 9–15 cm. Specifically, the absolute DXL values ranged between 23 and 27 cm following the four learning models, with no significant difference between them.

According to the overload of cognitive working memory [26], a worst technical performance was expected following the DL practice due to the higher cognitive demand, evidenced by the more pronounced decrease in HRV, increase in LF, and higher mental demand perception. The absence of significant differences between the different practice models in terms of technical outcomes calls this theory into question. Additionally, the present findings suggest that regardless of the motor learning model, one training bout of 36 total weightlifting trials was not enough to approach the high-performance model of complex Olympic snatch movements, involving the entire body musculature. Whether a certain weight threshold must be surpassed or more substantial increase in exercise noise is necessary for more effective changes requires further research. In the context of classical repetition-based motor learning, higher total trial numbers and/or longer practice durations have been previously recommended to elucidate differences in terms of technical efficiency between practice models [144, 145]. Whether individual characteristics dominate over motor learning models in relation to the acute effect of learning bouts on weightlifting technical efficiency, must be shown by future research. Nevertheless, future studies investigating individual responsiveness to such training bouts and employing artificial intelligence methods, such as machine learning and pattern recognition based on individual vs. motor learning models, are needed to corroborate this suggestion [146–148]. Similarly, to what extent the total trial numbers and/or longer intervention duration may affect motor learning advantages, requires further exploration in future studies.

Strengths and limitations

This study represents a pioneering effort to assess the acute effects of weightlifting training bouts on neurophysiological response, perceived exertion, and mental demand while controlling the possible influence of adopting different motor learning models. Nevertheless,

the study's design precludes generalization, and it is crucial to acknowledge several limitations that should be considered when interpreting the present findings. Epistemologically, the scope of application is determined by the boundary conditions of the study. Within these constraints, the study offers recommendations for further research that could expand the findings in specific areas and overcome certain limitations.

Previous reports have suggested different functional significance of the two alpha ranges, namely, the lower component ranging between 7.5 and 9 Hz and the upper component ranging between 9.5 and 12.5 Hz [149], as well as the two beta ranges (beta 1 ranging between 13 and 20 and beta 2 ranging between 21 and 30 [150]). For instance, Abhang et al. [118] indicated that low beta waves (beta 1) are mainly associated with focused concentration, while beta 2 waves are more linked to increases in energy and performance. Hence, computing the average alpha or beta power across all electrodes may yield imprecise results. Furthermore, in the present study, the EEG system and the Polar H10 sensor were not digitally synchronized. This limitation restricted our analysis to calculating correlations between averaged values recorded simultaneously during the 5-minute resting states and precluded the possibility of conducting cross-correlation analyses. Another limiting factor in the present study pertains to the number of electrodes employed for brain activity localization. A recent review, commissioned by the International Federation of Clinical Neurophysiology (IFCN), has suggested that a minimum of 48–64 electrodes may be necessary to achieve a high level of confidence in this localization. Consequently, future studies in the field of applied neurophysiology may benefit from employing a more rigorous methodology to confirm the precise anatomical localization of exercise effects on the brain.

CONCLUSIONS

Weightlifting training bouts show the potential to increase alpha, beta, and gamma frequencies in the majority of the tested brain regions, accompanied by an increase in HR and a reduction in RR-related parameters. While the impact of the adopted motor learning models on brain activity remains somewhat ambiguous, a more pronounced effect is observed in the DL model on both time and frequency domain HRV parameters. Especially when coupled with the lowest number of significant EEG changes, the DL approach once again confirms its extraordinary impact, not only on the brain but also on its interaction with the heart. These findings provide preliminary support for the acute neurophysiological benefits of strength-based exercise, especially when performed using a DL model. However, further studies encompassing a broader range of coordination-strength-based exercises are warranted to corroborate these suggestions. The use of stress-related EEG features combined with correlated HRV features appears to offer a more comprehensive understanding of neurophysiological responses to human movement under stress, potentially enhancing the detection and monitoring of related adaptations following acute and/or chronic interventions with different motor learning approaches.

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Conflict of interest declaration

The authors declare no conflict of interest.

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Tell me how much your opponent team runs and I will tell you how much you should run: A predictive model applied to Spanish high-level football

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ABSTRACT: The aim of this study was to predict a team's accumulated distance (TotDisTea) and accumulated distance at > 21 km/h (TotDis21Tea) in the Spanish Football First Division. 2,946 team physical performances (out of 3040 possible) during four seasons (from 2016–17 to 2019–20) were analysed. The outcome variables were the TotDisTea and TotDis21Tea when the ball was in play. Eight predictor variables were used: the distance accumulated and accumulated at > 21 km/h by the opponent (TotDisOpp and TotDis21Opp) were registered in km, the effective playing (EffPlaTim) and possession (BalPos) time were recorded in min, match location (MatLoc) had two levels (home and away), match outcome (MatOut) had three levels (lost, drawn, and won), and the teams were grouped in four levels (Champions League, Europa League, remained, and relegation) distinguishing the observed team (TeaLev) and the opponent team (OppLev) in the match. A total of 127 models were estimated from the all-possible regressions procedure for each outcome variable. The model with six predictor variables was selected as the best model to predict the TotDisTea ($R^2_{adj} = .82$). The predictor variables TotDisOpp, EffPlaTim, and BalPos had a greater contribution to the mean outcome value than the predictors OppLev, TeaLev, and MatLoc. All models estimated to predict TotDis21Tea had little predictive power ($R^2_{adj} < .38$). The findings of this study have both theoretical and practical implications for practitioners. The interaction between teams has a great effect on the conditional response. Before the match, teams could use this information to anticipate the physical demand expected in the next match, and after the match, be able to assess whether the physical response was similar to expected, and make decisions.

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INTRODUCTION

Effective playing time in football refers to the actual amount of time the ball is in play during a match, excluding stoppages for events such as fouls, injuries, substitutions, and other breaks in play. It represents the duration in which the game is actively progressing. More than a decade ago, it was proposed that effective playing time strongly conditions the physical response of professional football players in competition. Usually, the effective playing time accounts for a little over 50% of the total match time. Regarding external load, close to 70% of the running performance is accumulated in this period of play, being able to reach more than 95% when it comes to high-speed running [1]. In domestic league matches, only in effective game time can teams score or concede a goal. Above all, at this moment of the game, players are especially active. This does not prevent teams from making strategic use of breaks, trying to reduce stoppages when losing or lengthening them when the score is favourable [2].

Huge amounts of research have been carried out thanks to computerized tracking systems, applying the five properties under the concept of "the five Vs" [3]. One of these concepts is volume, that is, the magnitude of the data. Nowadays, due to the size of datasets in team sports, studies can be developed that span multiple leagues [4], categories [5, 6] or seasons [7]. The studies that gather thousands of individual and collective performances in their analyses address explanatory proposals based on the inclusion in the models of situational variables (e.g., match location, match outcome, level of the opponent, type of competition, etc.). Overall, nowadays, physical response is being better understood [8].

With respect to match location (e.g., home or away), the results are incomplete [9, 10], and probably influenced by other contextual variables (e.g., match outcome), which could explain the disparity of results [9]. Regarding match outcome, researchers reported that football players perform significantly less high intensity activity when

winning than when losing or drawing [1, 10]. However, the category of the league in which the teams participate (e.g., the second division compared to the Spanish first division of soccer) could increase the connection between physical response and success in the competition at the end of the season [6]. The results of one recent study [8] that studied the differences between both phases of play (attack and defence) show that the distance covered by teams when they have the ball is shorter than when they do not (relativizing the physical response to each minute of ball possession or non-possession). Finally, the results of the academic literature emphasize the importance of taking into account the opponent's level (e.g., high, medium, and low) during the assessment of the physical response of football performance [11, 12], briefly concluding that the higher the opponent's level, the higher will be the physical demands required. However, it is unknown whether the physical performance of the opponent affects the physical response of the other team.

Despite the constant use of descriptive and explicative analytical techniques in match analysis, there are still few available studies that have developed predictive models of sports performance [13]. These types of studies have the common purpose to determine the most effective ways of playing and using multidimensional qualitative data instead of unidimensional frequency data. The ability to describe football match play has improved [14]. Previously, a study proposed the implementation of predictive models of the physical response of running in professional football [10]. The authors attempted to predict the distance players would cover when the match status changed or the team played home/away against strong/weak opposition. It seems that in the dynamics of a match, the team performance is a combination of knowing what to do, wanting to do it, and what the opponent allows one to do. Furthermore, all this is conditioned by the independent and interactive effects of fixed (e.g., match location, type of championship) and changing (e.g., match status, substitutions, injuries) situational variables.

The aim of this study was to predict the total distance covered by a football team and the total distance covered above 21 km/h from different situational variables of the competition. The predictive variables involved in this study were: the match location (home or away), the final match outcome (lost, drawn or won), the level of the team and opponent team, the effective playing time (in min), the possession of the ball (in min), and the distance accumulated by the rivals in both total distance and at > 21 km/h. The starting hypothesis is that, especially, the physical performance of rival teams conditions the physical performance of the team and vice versa, in addition to other contextual variables. The results of the present study may allow football practitioners to predict the physical response in competition depending on possible scenarios in order to prepare players/teams during the training process.

MATERIALS AND METHODS

Approach to the problem

Data collection was carried out during four seasons of the Spanish Football First Division (LaLiga), from season 2016–17 to 2019–20. The computerized multi-camera tracking system TRACAB® was used for recording teams' physical performances. Several predictive linear regression models were proposed to estimate both the total distance covered and the distance running at more than 21 km/h in a match by teams considering predictor variables such as match location, distance covered by the opponent, quality of the team and opponent, time in possession, effective playing time, and match outcome.

Subjects

2,946 teams' performances (1,473 matches) were obtained from four seasons of the Spanish Football First Division (LaLiga), which authorised the use of the variables included in this study. The sample included 96% of the total possible performances (3,040 performances = 10 matches per match day × 2 performances of two teams × 38 match days × 4 seasons). Those matches where the information required was not available were excluded. In accordance with its ethical guidelines, this investigation does not include information that identifies football players. Data were treated in accordance with the Declaration of Helsinki and were approved by the Ethics Committee on Research on Human Beings (CEISH) of the university.

Variables

A total of eight predictive variables and two outcome variables were recorded (Table 1).

Procedures

The computerized multi-camera tracking system TRACAB® (ChyronHego, New York, USA) was used to record time-motion data. The ball-possession duration was obtained by OPTA® Sportsdata Company (Opta Sports, London, UK). Both TRACAB and OPTA are managed by Mediacoach® software. The reliability of the OPTA system has been previously proved [15] and the reliability of the TRACAB video-tracking system has also been recently tested for physical demand [16, 17], showing good quality data. Generated reports were exported into Microsoft Office Excel (Microsoft Corporation, Washington, USA).

Statistical analysis

Categorical variables were described by absolute and relative frequencies. Continuous variables were described by the mean (standard deviation) or median (interquartile interval) according to whether the assumption of normality was met or not. This assumption was verified using standardized normal probability plots (P-P plots) and histograms with normal-density plots. The descriptive analysis of the study variables was complemented with the estimation of the confidence intervals for a proportion (Wald method), mean (normal method), and median (exact binomial method).

The *all-possible regressions procedure* was used to select the best predictive linear regression model for both TotDisTea and TotDis21Tea. Nevertheless, no *stepwise regression procedure* was used

Table 1. Properties of the study variables.

Role	Name	Value	Description
Predictor	Match location (MatLoc)	0 = Away	The analysed team played away from home
		1 = Home	The analysed team played at home
	Match outcome (MatOut)	1 = Lost	The analysed team lost the match
		2 = Drawn	The analysed team drawn the match
		3 = Won	The analysed team won the match
	Team level (TeaLev)	1 = Relegation	The analysed team finished between the 18th and 20th position
		2 = Remained	The analysed team finished between the 8th and 17th position
		3 = Europa League	The analysed team finished between the 5th and 7th position
		4 = Champions League	The analysed team finished between the 1st to 4th position
	Opponent's level (OppLev)	1 = Relegation	The opposing team finished between 18th and 20th position
		2 = Remained	The opposing team finished between the 8th and 17th position
		3 = Europa League	The opposing team finished between the 5th and 7th position
		4 = Champions League	The opposing team finished between the 1st and 4th position
	Effective playing time (EffPlaTim)		Effective playing time in the match in minutes (min)
	Ball possession (BallPos)		Ball possession of the analysed team in minutes (min)
	Total distance covered by the opponent (TotDisOpp)		Total distance covered by the opposing team in kilometres (km)
	Total distance covered by the opponent at 21 km/h (TotDis21Opp)		Total distance covered at > 21 km/h by the opposing team in kilometres (km)
Outcome	Total distance covered by the team (TotDisTea)		Total distance covered by the analysed team in kilometres (km)
	Total distance covered by the team at 21 km/h (TotDis21Tea)		Total distance covered at > 21 km/h by the analysed team in kilometres (km)

Note. Within each variable, the category with the lowest numerical value (e.g., the category away in match location variable) was considered as the reference category in the multiple linear regression.

because these automatic predictive selection methods often are problematic [18]. The regression procedure consists of constructing all possible sub-models by combining the predictor variables of the maximum model and assessing the degree of compliance in each one with the established selection criteria [19]. For the outcome variable TotDisTea, the initial maximum model included seven predictive variables (MatLoc, MatOut, TeaLev, OppLev, EffPlaTim, BalPos, and TotDisOpp); and for the outcome variable TotDis21Tea, the initial maximum model also included seven predictive variables (MatLoc, MatOut, TeaLev, OppLev, EffPlaTim, BalPos, and TotDis21Opp). The selection criteria established were as follows: (a) the principle of parsimony; (b) a small value of Mallow's C_p; and (c) a large value of the adjusted coefficient of determination (R^2_{adj}).

Once the best model to predict the TotDisTea and the TotDis21Tea was chosen, the reliability of its predictions was evaluated by cross-validation. Next, it was determined whether this model met the following assumptions (the statistics and graphs used to test these assumptions are specified in parentheses): (a) absence of outliers and influential observations (internally and externally studentized residual, leverage, Cook's distance, DFIT statistic, and covariance ratio); (b) absence of collinearity (variance inflation factor); (c) normality of the distribution of the residuals (normal P-P plot of internally studentized residuals); and (d) linearity of the relationship and homogeneity of variances of the residuals (scatter plot between the externally studentized residuals and the predicted values or the values of the predictor variables). After checking the diagnostics of the selected model, its parameters (β_i) and its standardized regression coefficients (*beta*) were estimated. Finally, the model equation obtained was used to predict the outcome for certain value patterns of the predictive variables.

All statistical analyses were performed with Stata/IC version 17.0 software (StataCorp, College Station, TX, USA), considering a significance level of $p \leq .05$.

RESULTS

Table 2 shows the descriptive analysis (mean and standard deviation, median and interquartile interval, or absolute and relative frequencies) and inferential analysis (confidence intervals for a mean, median, or proportion) of the variables used to build the multiple linear regression model.

To predict TotDisTea or TotDis21Tea a total of 254 linear models (127 models for each outcome) were estimated from all the possible regression procedures. An attempt was also made to build a model for predicting TotDis21Tea, but it was ultimately not built because both the maximum model and the 126 derived sub-models had little predictive power ($R^2_{adj} < .38$).

Table 3 presents only 17 models (10 multiple and 7 single) of the 127 models estimated in total to predict TotDisTea, ordered from lowest to highest Mallow's C_p. Specifically, the model with the lowest C_p (9.47) and highest R^2_{adj} (.8237) was the model containing six predictor variables out of the initial seven and excluding MatOut. The second model with the lowest C_p (13.00) and highest R^2_{adj} (.8236) was the maximum model with seven predictor variables, which had a prediction loss of 0.01% with respect to the first model in the table. The rest of the models in the table had a prediction loss between 0.04 and 0.24% with respect to the first model. Thus, the model with all predictor variables except MatOut was selected as the best model to predict the TotDisTea for the following reasons: (a) model with two parameters less than the maximum model; (b) model with lower C_p and higher R^2_{adj}; and (c) only model with a C_p value lower than its number of parameters ($C_p < p+1$), which indicated that the selected model had a lower error variability than the maximum model.

In relation to the reliability of the selected model, $R^2_{mean} = .8229$ was obtained from cross-validation. This result indicated that the true predictive capacity of the model when executed with external samples was around 82%.

Regarding the diagnostics of the selected model, 13 observations with studentized residuals $> |3|$, with leverage values $> 2\times(p+1)/n$, with DFITs $> |\sqrt{((4\times(p+1))/n)}|$, or with covariance ratios outside the interval $1\pm3\times(p+1)$ were found. However, these outliers or influential observations were not removed from the sample because they were correctly recorded and because they were part of the reality of the game. The data did not present collinearity because the variance inflation factor was less than three in all the predictor variables. The normality assumption was met because all the internally studentized residuals were on the diagonal of the normal P-P plot; and the assumptions of linearity and homogeneity of variances were met because the externally studentized residuals did not present any defined pattern and were randomly distributed in the scatter plots.

The *F*-test of global significance revealed that the set of parameters of the selected model explained a significant part of the variability of the TotDisTea, $F_{(10,2935)} = 1376.84$, $p < .001$; and the *F*-tests of individual significance revealed that each predictor variable included in the model had a statistically significant contribution ($p < .005$) to the multiple linear regression equation (see Table 4). The *b* coefficients and their confidence intervals indicated that playing at home (relative to playing away), playing against a Champions League team (compared to playing against a relegation team), for each minute of increase in effective playing time, and for each kilometre of increase of the total distance covered by the opponent, the mean total distance, in kilometres, covered by the team increased significantly; in contrast, being a Champions League team (compared to being a relegation team) and for each minute of increased ball possession, the mean total distance covered by the team decreased significantly. For their part, the beta coefficients indicated that predictors TotDisOpp, EffPlaTim, and BalPos had a greater contribution to the TotDisTea outcome than the predictors OppLev, TeaLev, and MatLoc.

From the *b* coefficients in Table 4, the following equation was defined to predict the mean total distance covered by the team in kilometres:

Equation 1: TotDisTea (km) = 7.077 + 0.284 × MatLoc1 + (-0.079 × TeaLea2 + 0.486 × TeaLev3 – 0.633 × TeaLev4) + (0.153 × OppLev2 + 0.004 × OppLev3 + 0.751 × OppLev4) + 0.424 × EffPlaTim – 0.271 × BalPos + 0.712 × TotDisOpp

In Table 5, the previous equation was applied and the mean TotDisTea was predicted for different values of the predictor variables selected with practical criteria. This equation can be applied to other different values as long as they belong to the range of values observed in the sample used to estimate the model (i.e., TeaLev and OppLev between relegation and Champions League, EffPlaTim between 36.8 and 68.2 min, BalPos between 12.4 and 49.4 min, and TotDisOpp between 52.5 and 98.0 km). For example, to predict the mean total distance covered by a Champions League team that possesses the ball 41 min in a match with an effective playing time of 65 min and plays away from home against a team that is staying up, that covers a total distance of 55 km, the equation is applied as follows:

Equation 1 (example): TotDisTea = 7.077 + 0.284 × 0 + (-0.079 × 0 + 0.486 × 0 – 0.633 × 1) + (0.153 × 1 + 0.004 × 0 + 0.751 × 0) + 0.424 × 65 – 0.271 × 41 + 0.712 × 55 = 62.2 km

Without the TotDisOpp predictor variable, the equation was the following, but this model had $R^2_{adj} = .6429$ and a prediction loss of 18.08%:

Equation 2: TotDisTea (km) = 24.82 + 0.273 × MatLoc1 + (0.072 × TeaLea2 + 1.000 × TeaLev3 – 0.227 × TeaLev4) + (0.186 × OppLev2 + 0.722 × OppLev3 + 0.637 × OppLev4) + 1.081 × EffPlaTim – 0.159 × BalPos

DISCUSSION

The main aim of this study was to estimate two predictive linear regression models using the TotDisTea and TotDist21Tea by combining eight predictor variables. A total of 127 models were estimated from the all-possible regressions procedure for each outcome variable. The model with all predictor variables except MatOut was selected as the best model to predict the TotDisTea ($R^2_{adj} = .82$). The predictors

Table 2. Descriptive and inferential analysis of the study variables.

		95% CI	
Variable		***LL***	***UL***
Match location – *n* (%)			
Away	1534 (52.07)	50.27	53.87
Home	1412 (47.93)	46.13	49.73
Match outcome – *n* (%)			
Lost	1186 (40.26)	38.49	42.03
Drawn	602 (20.43)	18.98	21.89
Won	1158 (39.31)	37.54	41.07
Team/opponent's level – *n* (%)			
Relegation	442 (15.00)	13.71	16.29
Remained	1466 (49.76)	47.96	51.57
Europa League	444 (15.07)	13.78	16.36
Champions League	594 (20.16)	18.71	21.61
Effective playing time (min) – *M* (*SD*)	52.75 (4.95)	52.57	52.92
Ball possession (min) – *Mdn* [*IQI*]	25.80 [22.10, 30.10]	25.50	26.10
Total distance covered by the team/opponent (km) – *M* (*SD*)	78.28 (6.35)	78.05	78.50
Total distance covered at > 21 km/h by the team/opponent (km) – *M* (*SD*)	5.79 (0.84)	2.53	10.63

Note. *M* = mean; *SD* = standard deviation; *Mdn* = median; *IQI* = interquartile interval; *n* = number of observations; CI = confidence interval for a proportion, mean, or median; *LL* = lower limit; *UL* = upper limit.

Table 3. Comparison between the 10 multiple models with the lowest Mallow's C_p and the seven simple models (outcome: TotDisTea).

Model	**Predictors**	C_p	**p+1**	R^2_{adj}	***Shrinkage***
1	MatLoc TeaLev OppLev EffPlaTim BalPos TotDisOpp	9.47	11	.8237	base
2	MatLoc MatOut TeaLev OppLev EffPlaTim BalPos TotDisOpp	13.00	13	.8236	0.01%
3	TeaLev OppLev EffPlaTim BalPos TotDisOpp	15.46	10	.8233	0.04%
4	MatOut TeaLev OppLev EffPlaTim BalPos TotDisOpp	18.87	12	.8232	0.05%
5	MatLoc TeaLev EffPlaTim BalPos TotDisOpp	29.73	8	.8223	0.14%
6	MatLoc MatOut TeaLev EffPlaTim BalPos TotDisOpp	30.94	10	.8223	0.14%
7	TeaLev EffPlaTim BalPos TotDisOpp	37.14	7	.8218	0.19%
8	MatOut TeaLev EffPlaTim BalPos TotDisOpp	41.07	9	.8217	0.20%
9	MatLoc OppLev EffPlaTim BalPos TotDisOpp	46.45	8	.8213	0.24%
10	MatLoc MatOut OppLev EffPlaTim BalPos TotDisOpp	48.24	10	.8213	0.24%
…	…	…	…	…	…
64	TotDisOpp	1614.89	2	.7270	9.67%
95	EffPlaTim	3441.38	2	.6175	20.62%
111	OppLev	12469.64	4	.0762	74.75%
120	BalPos	13100.30	2	.0388	78.49%
123	TeaLev	13314.65	4	.0255	79.82%
125	MatOut	13701.66	2	-.0002	82.39%
127	MatLoc	13752.28	2	-.0003	82.40%

Note. C_p = Mallow's C_p; p+1 = number of parameters of the model (including the constant); R^2_{adj}= adjusted coefficient of determination; *shrinkage* = prediction loss.

Table 4. Parameters of the selected model to predict the total distance covered by the team.

		95% CI				
Predictors	*b*	*LL*	*UL*	$p_{t\text{-}test}$	$p_{F\text{-}test}$	*beta*
Match location					.005	
Away (0)	0 (base)					
Home (1)	0.284	0.087	0.480	.005		0.022
Team level					< .001	
Relegation (1)	0 (base)					
Remained (2)	-0.079	-0.364	0.206	.586		-0.006
Europa League (3)	0.486	0.131	0.842	.007		0.027
Champions League (4)	-0.633	-0.983	-0.284	< .001		-0.040
Opponent's level					< .001	
Relegation (1)	0 (base)					
Remained (2)	0.153	-0.132	0.438	.291		0.012
Europa League (3)	0.004	-0.352	0.360	.983		< 0.001
Champions League (4)	0.751	0.402	1.100	< .001		0.047
Effective playing time (min)	0.424	0.391	0.457	< .001	< .001	0.331
Ball possession (min)	-0.271	-0.292	-0.251	< .001	< .001	-0.254
Total distance covered by the opponent (km)	0.712	0.687	0.738	< .001	< .001	0.712
Constant	7.077	5.770	8.384	< .001		

Note. b = regression coefficient; CI = confidence interval for parameter β; *LL* = lower limit; *UL* = upper limit; $p_{t\text{-}test}$ = t-test of significance of regression coefficient; $p_{F\text{-}test}$ = F-test of significance of predictor; *beta* = standardized regression coefficient.

TotDisOpp, EffPlaTim, and BalPos had a greater contribution to the TotDisTea outcome than the predictors OppLev, TeaLev, and MatLoc. The model to predict TotDis21Tea was not built because both the maximum model and the 126 derived sub-models had little predictive power (R^2_{adj} < .38). With the results, it could be concluded that theoretically, the interaction of teams in the football matches has also been verified in the conditional dimension, with a close relationship between the physical responses of both teams. From a practical point of view, practitioners could have the possibility to estimate physical performances of teams in matches when it has not been possible to obtain that outcome (e.g., TotDisOpp).

Football is a sport of interaction and, therefore, the performance of a team is dependent on the performance of the opponent [20]. Multidimensional proposals to characterize playing styles are increasing [21]. The unique way teams play means that the distribution of roles among players is specific, so individual dimensions (e.g., emotional, cognitive, affective, behavioural, social, and conditional) will be unequally demanded. For this reason, the same positions in different teams carry with them different conditional responses, that is, adjusted to the way the team competes [22], greater movement of the ball or models that enhance the exploration of a more direct game. A revealing aspect of this study was the effect of TotDisOpp on TotDisTea when the rest of the model's predictors were held constant; the interpretation is as follows: for each km run by the opponent team, there is an expected increase in mean TotDisTea of 0.71 km, 95% CI [0.69, 0.74]. This would affect both positively (the need to run more) and negatively (forced to run less) whether the conditional profile of the opponent faced is higher or lower than the reference team. When the predictive model has been applied eliminating the variable TotDisOpp (equation 2) the model had R^2_{adj} = .64, and a level of prediction loss of 18.1% with respect to equation 1 (with TotDisOpp), which carries a great weight within the predictive model. For this reason, when one wants to assess the physical performance of a team, as can be deduced from this study, keeping in mind the physical performance of the opponent is essential.

The second predictive variable that most influenced the prediction of TotDisTea was EffPlaTim. More than a decade ago it was reported that in the effective playing time (EPT), the player accumulates the largest amount of physical demand, this percentage increasing as the running speed increases, and it can be close to 100% in the case of high-speed running [1]. On the other hand, nowadays, in professional football leagues, since the implementation of the Video Assistant Referee (VAR), there have been some changes, especially in total (TPT) and effective (EPT) playing time [23]. Although there was no significant effect in the technical-tactical dimension (e.g., passes, dribbles, crosses, shots, goals, corners, fouls, width, length, height, distance from the goalkeeper to their defence), physical performances in Spanish LaLiga teams had a slight decrease in the total distance covered (108.9 vs. 107.9 vs. 106.9 km) when VAR intervened (VAR0, VAR1 and VAR2, respectively). Probably, it is due to the decrease in EPT between VAR0 and VAR1 (52.5 vs. 51.5 min, respectively) and a slight increase in TPT in VAR2 compared to VAR1 and VAR0 (99.1 vs. 96.0 vs. 95.1 min, respectively).

TABLE 5. Prediction of the total distance covered by the team (km) for different values of match location, team level, opponent's level, effective playing time (min), ball possession (min), and total distance covered by the opponent (km).

				EffPlaTim and BalPos								
				40			50			60		
MatLoc	TeaLev	OppLev	TotDisOpp	15	25	35	15	25	35	15	25	35
Away	Relegation	Relegation	70	69.8	67.1	64.4	74.0	71.3	68.6	78.3	75.6	72.8
			80	76.9	74.2	71.5	81.2	78.4	75.7	85.4	82.7	80.0
			90	84.0	81.3	78.6	88.3	85.6	82.8	92.5	89.8	87.1
Away	Relegation	Champions	70	70.6	67.8	65.1	74.8	72.1	69.4	79.0	76.3	73.6
			80	77.7	75.0	72.2	81.9	79.2	76.5	86.1	83.4	80.7
			90	84.8	82.1	79.4	89.0	86.3	83.6	93.3	90.6	87.8
Away	Champions	Relegation	70	69.2	66.5	63.7	73.4	70.7	68.0	77.6	74.9	72.2
			80	76.3	73.6	70.9	80.5	77.8	75.1	84.8	82.0	79.3
			90	83.4	80.7	78.0	87.6	84.9	82.2	91.9	89.2	86.5
Away	Champions	Champions	70	69.9	67.2	64.5	74.2	71.4	68.7	78.4	75.7	73.0
			80	77.0	74.3	71.6	81.3	78.6	75.8	85.5	82.8	80.1
			90	84.2	81.4	78.7	88.4	85.7	83.0	92.6	89.9	87.2
Home	Relegation	Relegation	70	70.1	67.4	64.7	74.3	71.6	68.9	78.6	75.8	73.1
			80	77.2	74.5	71.8	81.4	78.7	76.0	85.7	83.0	80.3
			90	84.3	81.6	78.9	88.6	85.8	83.1	92.8	90.1	87.4
Home	Relegation	Champions	70	70.8	68.1	65.4	75.1	72.4	69.6	79.3	76.6	73.9
			80	78.0	75.2	72.5	82.2	79.5	76.8	86.4	83.7	81.0
			90	85.1	82.4	79.6	89.3	86.6	83.9	93.6	90.8	88.1
Home	Champions	Relegation	70	69.4	66.7	64.0	73.7	71.0	68.3	77.9	75.2	72.5
			80	76.6	73.9	71.1	80.8	78.1	75.4	85.0	82.3	79.6
			90	83.7	81.0	78.3	87.9	85.2	82.5	92.2	89.5	86.7
Home	Champions	Champions	70	70.2	67.5	64.8	74.4	71.7	69.0	78.7	76.0	73.2
			80	77.3	74.6	71.9	81.6	78.8	76.1	85.8	83.1	80.4
			90	84.4	81.7	79.0	88.7	86.0	83.3	92.9	90.2	87.5

Ball possession is one of the most studied variables in elite football, above all, in the attempt to associate it with success [5]. From the interpretation of the results, as shown in Table 4, the BalPos variable is the third of the variables in predictive importance of TotDisTea. For each minute of ball possession, a decrease in mean TotDisTea of -0.27 km, 95% CI [-0.29, -0.25] is expected. Usually, successful teams are those that have greater possession [5] and less accumulated distance [24, 25]; nevertheless, the methodology used in this type of research does not tell us whether the offensive phase is more or less physically demanding compared to the defensive phase. A recent study [8] tried to relate the effective time of the game (distinguishing the phases of possession and non-possession of the ball) with the locomotor response, from an intensity variable, meters per minute covered by the team. Two of the main conclusions of the study were that teams ran more per minute when players did not have the ball than when they did, and the distance accumulated per minute by the teams in ball possession does not correlate with the distance accumulated in the non-possession phase and vice versa [8]. Then, the physical response assessment needs information about the accumulated time of possession.

To assess the level of the teams, result indicators are usually used, such as the number of goals [5] or accumulated points [26], or the classification at the end of the championship [24], among others. In a previous study, carried out in the same Spanish league [5], some significant differences were observed (with a trivial effect size) in the physical performance of the teams in the upper half of the table compared to the last in the standings. Similarly, in the current study, the Europa League teams also had a greater physical response (increase of almost half a kilometre) with respect to the reference value taken from the relegation teams. By contrast, the groups of teams staying up and in the Champions League showed negative values with respect to the reference value; it can be interpreted that physical response was not a dimension that characterized them. It seems that each team tries to take advantage of its strengths (e.g., running or passing more than the opponent does) as their style of play. However, regarding the particularity of each match, the quality of the opponent shows a linear trend like that described in the literature [1, 10]; the greater the quality of the opponent (determined by their standing in the league) the greater is the locomotor activity demanded from the reference team. From the models proposed in the present

study, unlike what was observed in a previous study [27] where the time of possession of the ball was not taken into account, it can be predicted that the teams having to face teams that are in the Champions League will increase the TotDisTea between 0.4 and 1.1 km. In any case, caution must be exercised when interpreting the results because the small differences in the final standings depend not so much on the team's usual way of playing [21], but rather on the effectiveness of the team in shots on target [28]. Furthermore, exploring this study in other divisions (e.g., the Spanish second division of soccer) could be of interest [6].

The influence of match location on the physical response of players and teams is not new [1, 10]. The home teams usually covered a greater distance than away teams. A recent study found [8] that match location affected the distance covered at > 21 km/h but not the total distance accumulated per minute by the teams, running greater distance in matches played at home. In line with this, in the prediction model of our study, to play at home meant between 0.1 and 0.5 more km for teams. Several years ago, a review of the evidence for the hypothesized reasons for a home advantage was made [29]; crowd support, referee bias, psychological factors, travelling of away teams, familiarity with local conditions, territoriality and specific playing tactics were suggested as other possible influences. In any case, home teams seem to be forced to get a good result when they play at home.

MatOut was not included in the final predictive model. Nevertheless, it is known that match status has a big influence on the adopted strategy of the teams during a match, which accounts for the fact that players do not always use their maximal physical capacity for an entire match [2]. Changes in the match status create special needs within the team, being particularly affected the different positions in a playing system [30]. In line with this, Lago et al. observed that for every minute losing, players covered an extra metre of sprinting (> 19.1 km/h). However, while the team's overall physical performance might not be greatly affected by changes in the match status, it could alter the distribution of locomotor demands among teammates. Losing status could increase the total distance and the distance covered at 14–21, 21–24 and > 24 km/h by defenders, while attacking players could increase the distances accumulated in these ranges of velocity during winning status [30]. The difference between the studies could be explained by two reasons: methodologically, the fact that the physical performance evaluated in the present study is limited exclusively to effective playing time; and conceptually, because although the outcome of the match has been classified as win, draw or loss, it is known that during matches there may have been changes in the match outcome, being able to have a different temporal distribution (% of time winning, losing and drawing).

One of the methodological limitations of the present study was to determine the minimum sample size required to conduct a multiple linear regression analysis; the general rule-of-thumb of $N \geq 50 + 8 \times p$ was applied [31, 32], where p is the number of parameters of the maximum model. In our case, if the maximum model had 13 parameters, then this model had to be built with at least $50 + 8 \times 13 = 154$ observations. The reason for applying this rule was that no previous football studies were found that built a model to predict the total distance covered by teams. However, considering the results obtained in the present study ($R^2_{adj} = .82$; *M* and *SD* of TotDisTea = 78.3 and 6.3 km, respectively) and the four-step procedure proposed by Riley et al. [33, 34], future football studies will be able to more adequately calculate the minimum sample size required to build a linear regression model to predict the total distance covered by a team. A second limitation of the study concerns the levels set for some situational variables. Probably, distributing the match outcome according to minutes winning, losing or drawing could improve the degree of prediction of the model. In addition, the level of the team and its rivals, which has been established considering the ranking at the end of the season, does not take into account the variability of the position occupied by the teams on each match day of the championship. The third limitation is related to the idiosyncrasy of the players and teams in the way of task solving, that is, playing a football game. It would be interesting to know the weight carried by physical demand in each team performance, then apply this type of predictive model to each team in *LaLiga*.

CONCLUSIONS

Our results highlight a number of variables that could explain physical workload in football players, and combinations of these variables could be used to develop a model for predicting (from a probabilistic viewpoint) the physical activity profile in competition. The main conclusions of the present study focus on the importance of the opponents in the physical performance when a team tries to resolve the task of playing a match. Apart from the opponent, effective playing and possession times are placed in a secondary position. Finally, with less importance, match location and match-up quality must be considered when planning to predict the total distance that will be covered by the team. The findings of this study suggest again [8] that an effective assessment of football performance at a behavioural level needs to incorporate both the different contextual variables (and their interactions) where the match has developed, and the particular strategic variables that the teams have proposed in the match according to the needs at each moment of the match.

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Data availability

https://rhileno.shinyapps.io/TotDisTea_prediction/

Conflict of interest declaration

The authors declare no conflict of interest.

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