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ORIGINAL ARTICLE

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Effect of dynamic taping on pelvic movements in individuals with asymptomatic flexible flat-foot

ALI ZORLULAR[1], SERTAC BERKAN BOZYEL[2], NIHAN KAFA[1], NEVIN GUZEL[1]

Abstract
Introduction. The mechanical efficiency of one structure may be affected by the alignment of another segment. Inaccurate signals transmitted from distal to proximal segments result in altered biomechanics of the upper segments. Aim of Study. This study aimed to investigate the effect of dynamic taping on pelvic mobility in individuals with flat-foot. Material and Methods. Forty-two volunteers aged 20-32 years were recruited to this study. Participants were documented as normal-arched (n = 21) or flat-arched (n = 21). Participants' pelvic symmetry index was assessed using a G-Walk tri-axial accelerometer. Individuals with a flat-arched foot were taped with dynamic tape and the pelvic assessment process was repeated. Results. Pre-taping results showed that pelvic anterior-posterior (p = 0.029) and pelvic oblique symmetry indexes (p = 0.020) of the normal-arched group were significantly higher compared to the flat-foot participants. Application of dynamic tape increased symmetry indexes of the pelvis and navicular height with flat-arched foot. After taping, independent sample t-test showed no significant difference in symmetry indexes in the comparison of the control group and flat foot group in frontal (p = 0.734), sagittal (p = 0.120) and transverse (p = 0.127) planes. Conclusions. Dynamic taping can support the arch. In individuals with low arches, ensuring proper foot alignment after taping increases the biomechanical efficiency of the pelvis.

KEYWORDS: gait analysis, foot, taping, arch.

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Introduction

The medial longitudinal arch (MLA) is the inner arch of the foot. The arch is supported by the plantar fascia, plantar ligaments and muscles, especially tibialis posterior, which is the most important muscle in the maintenance of the arch and controlling the pronation movement [6].

Flat-foot is defined by the loss of the MLA of the foot. A decrease in medial arch height causes anatomic and biomechanic changes in foot structure [15]. Foot arch height alterations may lead to changes in load distribution. Foot arch structure is a recommended intrinsic risk factor for lower extremity injury [2].

Many compensations in lower extremity alignments, including excessive subtalar pronation, are associated with lower MLA [15]. Subtalar pronation is a normal biomechanical action occuring during the stance phase of the gait cycle. During the gait cycle the subtalar joint pronates to absorb the shock of heel strike. In the presence of low MLA height, subtalar pronation becomes excessive and overuse injuries such as sacroiliac pain, low back pain, and patellofemoral pain can occur [10, 11]. During pronation, the tibia internally rotates and the rotation of the tibia is accompanied by the rotation of the femur in the same direction, but of lesser amplitude. It has been showed that internal rotation at the femur causes the femoral head to put pressure on the posterior area of the acetabulum. This pressure on the posterior part of the pelvis would cause the pelvis to tilt anteriorly. Based on this interaction of the mechanical chain, excessive subtalar pronation has been shown to have an effect on the pelvis [5].

The foot is a component of the biokinetic chain that connects the lower extremity to the spine via the pelvis [5, 28]. Inappropriate biomechanics in the distal parts of the body may influence its proximal segments, mechanical efficiency of muscles and proprioceptive orientation [28]. Flat-foot causes inadequate gait due to decreased impact absorption of the foot. Patients with a low arch may develop lower extremity pain, abnormal gait pattern and difficulty walking.

Although flat-foot is a very common foot deformity, there is no consensus on its treatment. Patients with flat-foot commonly used foot arch supports such as taping and orthosis. Authors and clinicians have suggested that arch tapings are a common intervention strategy for patients with pain or injury due to overpronation [26]. Recent studies have shown that the use of the dynamic taping technique is increasing. Although the principles of use for dynamic tape and kinesio tape seem similar, their material and mechanical properties are different [22]. The viscoelastic structure of the tape provides 200% stretching. Thanks to its strong recoil and elastic resistance, dynamic tape helps joint movement by reducing the load on the tissue, thus enhancing movement patterns [16].

Aim of Study

While there are many different types of interventions for MLA, there are limited studies examining the biomechanical effectiveness of these interventions on the upper segments. The purpose of this study was to determine the effect of dynamic taping on pelvic mobility in flat-foot individuals during walking.

Material and Methods

Participants

This prospective, cross-sectional study received approval from the ethical committee of the university (Research code: 2017-405/19.08.2022) and followed the guidelines of the Declaration of Helsinki. In this study, 21 flat-footed individuals and 21 participants who had normal MLA height were selected. The age of participants ranged from 20 to 32 (mean 22.42 ± 1.82 years) years of age. Participants were excluded from the study if they had a history of a lower extremity injury or surgery in the previous 3 months, were unable to walk pain-free and suffered from known allergy to sports tape. Flat-footed participants met the inclusion criteria of having a navicular drop of more than 8 mm and all subjects were screened by one investigator [17]. The dominant extremity was taken into account in the evaluation for flat-foot. All participants provided written informed consent. The sample size was calculated based on the study of Wang et al. [27], who searched the effects of taping on the lower extremity in flat-footed individuals. The minimal number of participants required to attain a power of 0.8 and a bilateral α level of 0.05 was calculated to be 21 per group.

Procedures

The measurements were conducted by two clinicians. The navicular drop test and dynamic taping were conducted by one investigator and pelvic analysis by the other. Pelvic analyses were made by a blinded researcher. Each participant completed a self-report questionnaire on his/her demographic information. Each participant's measurements were performed during one visit at the laboratory.

Participants with a navicular drop of less than 8 mm were referred to as the control group. This group performed only pelvic analysis without taping during walking. Individuals with a navicular drop greater than 8 mm were initially subjected to gait analysis without tape. After a 15-minute break, the participants in the taping group were taped and the gait analysis was repeated.

Navicular-drop measurement

The subject was seated on an adjustable height chair with hips and knees at 90° and the foot resting on the floor with the ankle joint in a subtalar neutral position. The navicular tuberosity was palpated and marked with a marker. The distance from the floor to the navicular tuberosity was measured and recorded using height calipers. The subjects then stood in a natural stance on a flat surface, feet shoulder-width apart. The distance between the ground and the navicular protrusion was measured again. Navicular fall height was calculated by subtracting the standing and sitting measurements [1]. Navicular fall height was measured by the same investigator in all cases. The intra-rater reliability of the navicular drop test assessed using the intra-class correlation coefficient (ICC) is reportedly 0.73-0.96 [20]. The reliability and validity of the navicular drop test have been reported previously [1]. The navicular height was calculated by measuring the distance between the ground and the navicular tubercle while the patient was in the standing upright position [17].

Dynamic Tape® taping technique

In this study, the arch support technique was used with 5 cm × 5 m of Dynamic Tape®. The individuals were placed in the supine position. Forefoot adduction, calcaneal varus, and the big toe were placed in a flexion position. The taping was started from the proximal thumb, following the medial plantar surface of the foot, the tape was looped around the calcaneus, passed through the sole of the foot, pulled upwards to the navicular, and the navicular was raised. The dynamic taping technique is shown in Figure 1. After taping, the 1st MTP joint should be in flexion, the calcaneum inverted, and the metatarsals should be convex on the dorsum of the foot. If these changes are not present in non-weight bearing, the technique is unlikely to apply a genuine force into the system so it cannot affect foot biomechanics [16].

Figure 1. Dynamic tape arch support technique

Pelvic assessment

Pelvic mobility was evaluated while individuals walked freely wearing casual shoes along a 10-m walkway using a wireless tri-axial accelerometer. The analysis system was based on the center of mass using a wireless tri-axial accelerometer (G-Walk, BTS Bioengineering S.p.A., Italy) that was attached to the 5th lumbar vertebra and tightened with Velcro™. The accelerometer data were wirelessly transferred by a Bluetooth system and analyzed with the BTS G-Studio software (BTS Bioengineering S.p.A., Italy) of a computer [4]. The weight of the accelerometer was 37 g, with dimensions of 70 × 40 × 18 mm. The frequency of the accelerometer was from 4 to 1000 Hz, and sensor fusion was 200 Hz. The spatiotemporal parameters and pelvic oscillation including all the three planes were analyzed using a software program. The parameters evaluated using the G-Walk were the anterior-posterior tilt symmetry index of the pelvis, the oblique symmetry index of the pelvis, and the rotational symmetry index of the pelvis. The symmetry index is a ratio of mobility between the right and left pelvic structure during walking. The index ratio being close to 100% indicates that the right and left parts of the pelvis have equal mobility in the specified plane. Also, the index ratio being close to 100% is one of the indicators of the appropriate gait pattern [12]. An example of pelvic reporting is shown in Figure 2.

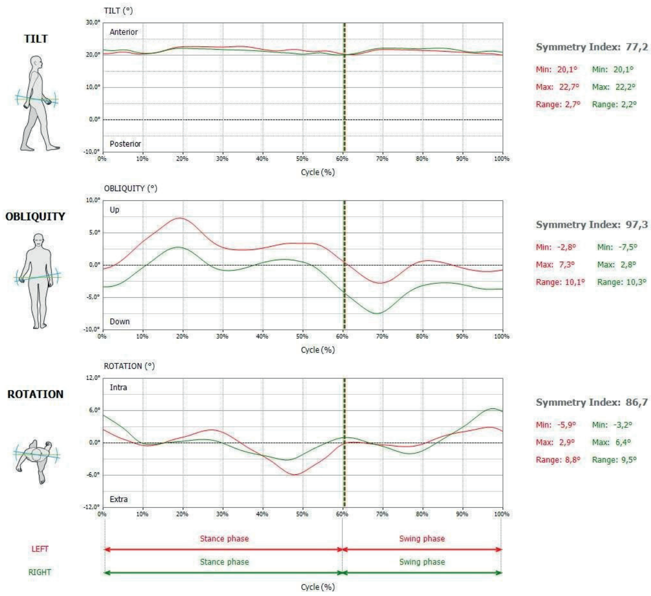


Figure 2. Pelvic report

Statistical analysis

The Statistical Package for Social Sciences (S.P.S.S.) Version 29.0 statistical software package was used in data analysis. Visual (histogram, probability graphs) and analytical methods (Kolomogrov–Smirnov/Shapiro–Wilk's test) were used to examine whether the variables showed normal distribution [7]. Independent groups t-test was used for the variables showing normal distribution in the comparison of the measurement results for flat-foot individuals and the control group. The effect size for independent sample t-tests were calculated using Cohen's d standards. Cohen's d results were interpreted using thresholds of 0.2, 0.5, and 0.8 for small, medium, and large effects, respectively. Repeated-Measure Analysis of Variance (ANOVA) was used to compare the effect of dynamic taping on the pelvic symmetry index at different time points. The effect size for Repeated-Measure ANOVA was computed according to partial eta-squared (η_p^2) standards.

Results

A total of 42 subjects, 16 male and 26 female, met the inclusion criteria and no difference was found between the age, body weight, height and body mass indexes (BMI) of the individuals with flat-foot and controls included in the study ($p > 0.05$). A statistically significant difference was observed between the two groups in navicular height ($p < 0.05$). Descriptive data of the individuals are summarized in Table 1.

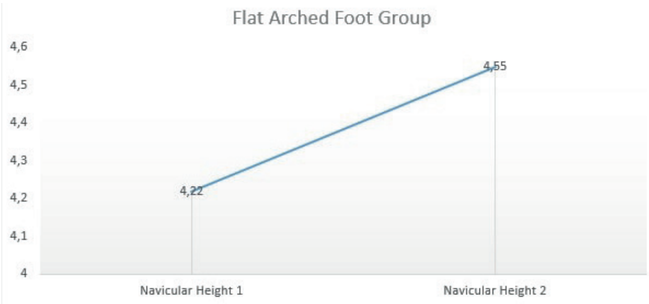
The pre-taping data set revealed that the pelvic anterior-posterior and pelvic oblique symmetry indexes of the control group were statistically significantly higher compared to the flat-foot participants ($p < 0.05$). The difference between the groups had a large effect size according to Cohen's d standards (0.699 and 0.750 for the pelvic anterior-posterior and pelvic oblique symmetry index, respectively). However, there was no statistically significant difference in the pelvic rotational symmetry index between the two groups ($p > 0.05$, Table 2). A more appropriate gait pattern was observed in the control group.

Figure 3 shows the change in mean navicular height after taping in individuals with flat arched foot and Table 3 indicates marked changes in the symmetry index of pelvis before and after taping for the flat-foot group.

Table 1. The characteristics of the subjects

	Flat-foot group (n = 21)	Control group (n = 21)	p significant (2-tailed)
	Mean ± Standard deviation	Mean ± Standard deviation	
Age (year)	22.57 ± 2.34	22.29 ± 1.31	0.628
Height (cm)	170.62 ± 7.56	167.48 ± 8.00	0.198
Body weight (kg)	62.24 ± 9.21	61.14 ± 11.80	0.739
BMI (kg/cm^2)	21.28 ± 1.83	21.71 ± 3.32	0.602
Navicular height (cm)	4.22 ± 0.32	4.53 ± 0.35	*0.041

* p < 0.05



Note: Navicular Height 1 – pre-taping height, Navicular Height 2 – post-taping height

Figure 3. The change in mean navicular height after taping in individuals with flat arched foot

Table 2. Pre-taping, a comparison of pelvic symmetry index variables between groups

	Flat-foot group (n = 21)	Control group (n = 21)	Effect size Cohen's d	p significant (2-tailed)
	Mean ± Standard deviation	Mean ± Standard deviation		
Anterior–posterior tilt symmetry index (%)	71.30 ± 14.61	80.29 ± 10.83	–0.699	***0.029**
Oblique symmetry index (%)	96.94 ± 2.74	98.46 ± 0.79	–0.750	***0.020**
Rotational symmetry index (%)	97.87 ± 2.39	97.71 ± 1.48	–0.084	0.788

* p < 0.05

Table 3. Effect of dynamic taping on pelvic symmetry index

	Before taping (n = 21)	After taping (n = 21)	Repeated measure ANOVA		
	Mean ± Standard deviation	Mean ± Standard deviation	F	p	η_p^2
Anterior-posterior tilt symmetry index (%)	71.30 ± 14.61	81.70 ± 15.42	18.577	***0.001**	0.482
Oblique symmetry index (%)	96.94 ± 2.74	97.46 ± 2.76	6.543	***0.019**	0.247
Rotational symmetry index (%)	97.87 ± 2.39	98.35 ± 1.19	1.683	0.209	0.078

* p < 0.05

Table 4. Post-taping, comparison of pelvic symmetry index variables between groups

	Flat-foot group (n = 21)	Control group (n = 21)	Effect size Cohen's d	p significant (2-tailed)
	Mean ± Standard deviation	Mean ± Standard deviation		
Anterior-posterior tilt symmetry index (%)	81.70 ± 15.42	80.29 ± 10.83	0.106	0.734
Oblique symmetry index (%)	97.46 ± 2.76	98.46 ± 0.79	–0.490	0.120
Rotational symmetry index (%)	98.35 ± 1.19	97.71 ± 1.48	–0.136	0.127

The paired t-test revealed that values of the anterior-posterior tilt symmetry index and the oblique symmetry index increased significantly after dynamic taping compared with their values before taping ($p < 0.05$). However, the rotational symmetry index increased slightly, although there was no significant difference compared with the baseline ($p > 0.05$).

The independent sample t-test showed no significant difference in symmetry indexes in the comparison of the control group and the flat-foot group after taping ($p > 0.05$, Table 4).

Discussion

This is the first study to examine the effects of Dynamic Tape® on the pelvis in participants with asymptomatic flexible flat-foot. We found significiant differences in the pelvic symmetry index between the control group and the flat-foot group. In the first measurements, the pelvic symmetry index was higher in the control group. However, the symmetry indices of the flat-foot group increased after dynamic taping and then no index difference was observed between the two groups.

Previous studies showed that low arched feet affect lower extremity biomechanical alignment. Individuals with low arched feet have been suggested to be at a greater risk of lower extremity injury [2]. Different attempts have been made towards MLA in order to eliminate these negative effects caused by the low arched feet. Twomey and McIntosh aimed to investigate the effects of low arched feet on lower limb gait kinematics in children. Results of their study showed that the low arched group was more externally hip rotated by 6-7° throughout the stance phase and the pelvis of participants in the low arched group was more internally rotated (1-2°) in the transverse plane [24]. As the low arched foot makes initial contact with the ground, the hip is forced into a more external position and this may also lead to a compensatory internal rotation of the pelvis. Also, changes in load distribution due to the loss of a natural foot arch may affect myofascial structures. Thus, the increased tension in the surrounding soft tissues will spread to the upper segments of the body. Incorrect tension in the long term may cause permanent biomechanical changes in the lower extremity. We also observed changes in pelvic mobility of flat-foot individuals in this study. There was asymmetrical pelvis mobility especially in the sagittal (anterior-posterior pelvic tilt) and frontal planes (oblique pelvic tilt). This is due to the compensatory mechanisms reaching from the foot to the pelvis as a result of a unilateral flat arched foot.

There are numerous studies aimed at re-raising low MLA [14, 25]. Ankle and foot taping is a common clinical intervention used to treat and prevent foot arch disorders [23]. Many different taping techniques have been introduced in the literature to limit excessive foot pronation. Low dye and reverse-6 methods are the most popular anti-pronation taping techniques [3]. Franettovich et al. announced that combining the low dye and reverse-6 taping methods resulted in a greater change of the MLA height and this taping technique was referred to as the "augmented low-dye." Their study showed that individuals with at least 10 mm of navicular drop had an average of 9.4 mm in the height of the arch after "augmented low-dye" taping [8]. In another study investigating the effectiveness of augmented low-dye taping and ankle brace in individuals with low arches, muscular activations were compared. In that study it was shown that the taping method only in the tibialis posterior muscle had a 15% decrease in the peak EMG value compared to bracing [9]. Franettovich et al. suggested that the taping technique used in this study could be useful in managing overuse and dysfunction of tibialis posterior, by reducing their level of activation during walking. Reducing the extra load on the tibialis posterior muscle will support the arch of the foot. In the systematic review compiled by Radford et al. researches showed that the low dye technique increased the arch height by an average of 5.9 mm [19]. The current study showed that the navicular height of individuals with flat-foot increased by 3.3 mm as a result of dynamic taping. The increase in foot arch height seen in the present study is smaller than in most previously published literature when using other taping techniques. The main factor that will cause this result may be connected with the use of elastic tape in this study instead of the rigid tape used in other studies. However, except for the current study none of those researches investigated the effect of increased arch height on pelvis biomechanics.

Abnormal biomechanical alignment originating from the distal parts may influence joint loads, mechanical efficiency of muscles, and the proprioceptive process [21]. Inaccurate signals transmitted from distal to proximal segments result in an altered neuromuscular function and control of the upper segments. The position of one segment may be affected by alignment deviations of an adjacent segment or result from compensatory changes toward a more efficient dynamic function. Misalignment can be seen in structural or functional characteristics [18]. Since individuals are structurally different, the positioning of adjacent segments in response to a particular alignment difference will also be unique to that individual.

Nguyen et al. examined relationships among lower extremity alignment characteristics. One of the subjects investigated in the Nguyen multi-factor analysis study is the relationship between the ankle joint and the knee joint. Their findigs showed that participants with a greater genu recurvatum also had an increased navicular drop [18]. Magnetic resonance imaging studies have shown that genu recurvatum is accompanied by a rotation of the tibiofemoral joint [13]. Genu recurvatum results in a medial rotation of the femur relative to the tibia and a medially rotated posture around the knee joint may increase medial rotational stress at the ankle, resulting in greater pronation and navicular drop. Also, their study showed that the position of the segments relative to each other changes with a kinetic interaction. Participants who had greater pelvic angles also had greater internal rotation at the hip. An increased hip internal rotation may also lead to compensatory external rotation of the tibia on the femur. It has been suggested that this compensatory mechanism, which starts from the pelvis and progresses to the tibia, would result in an increase in the quadriceps angle, and an excessive quadriceps angle increases the risk of lower extremity injury [18]. However, further studies are needed to confirm whether the mechanisms of action are from the proximal to distal plane or vice versa.

One of the strengths of the current study is connected with the fact that the effects of foot and ankle tape intervention on the pelvis have not been investigated before. Another strength is that the effectiveness of dynamic tape is evaluated with a 3D accelerometer, which provides the opportunity to analyze it in all 3 planes of the pelvis. The use of objective evaluation tools will increase the level of evidence of the research. One of the limitations of this study was that only immediate effects of the tape were evaluated and it is not known how long these effects would last after taping. The second potential limitation of this study was related to the collection and analysis of kinematic variables rather than kinetic data. Examination of muscular strength and muscular activation could also contribute to the interpretation of kinematic changes. In addition, the absence of a sham group in this research is another limitation of our study. This may limit the explanation of the actual effects of taping.

Conclusions

The results of the current study revealed that the application of dynamic tape can reinforce the medial longitudinal arch. The use of dynamic tape increased the height of the navicula and preserved the natural curve. After dynamic taping in individuals with low arched feet a more symmetrical movement of the pelvis is found during walking. The dynamic taping method improves the function of the upper segments by providing correct alignment at the foot.

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Conflict of Interest

The authors have no conflicts of interest to report.

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Correlation between performance in repeated sprints and performance in other laboratory and field fitness tests in female soccer athletes

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Abstract
Introduction. **The ability to perform repeated sprints is one of the most important physical abilities in soccer.** Aim of Study. **The aim of this study was to examine the relationship between the ability to perform repeated sprints (6 × 20 m in a 15-s cycle) in female soccer players from two different age groups, with aerobic capacity (YYIR1), jumping ability (single-leg hop test), isokinetic strength (60°/s, 180°/s, 300°/s), speed performance (S10-m and S30-m), and change of direction ability (505 COD test). The second objective of the study was to compare the performances of the two different groups in the tests.** Material and Methods. **Twenty-four female soccer players from Greece participated in this study and they were divided into two groups: (i) U-16 (division II) female soccer athletes (age: 15.8 ± 0.8 years, height: 160.5 ± 5.1 cm, body mass: 59.4 ± 7 kg), and (ii) adult female (division I) soccer athletes (age: 21.9 ± 4.1 years, height: 165.7 ± 6.1 cm, body mass: 62.2 ± 7.5 kg). The Pearson correlation coefficient and t-test for independent samples were used for statistical analysis.** Results. **The S10-m, S30-m and single-leg hop tests appeared to be the variables most associated with the total time in the RSA test in U-16 female soccer players. S10-m and the change of direction ability appeared to be the variables most associated with the total time in the RSA test in adult female players.** Conclusions. **It seems that U-16 Greek female soccer players do not differ from adults in most of the physical fitness tests conducted in the field and laboratory.**

KEYWORDS: women, soccer, physical fitness tests, repeated sprint ability.

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Introduction

Women's soccer has been developing rapidly in recent years, and the Fédération Internationale de Football Association (FIFA) has set a goal for the number of women soccer athletes worldwide to reach 60 million by 2026 [52]. During a women's soccer match, each player covers a total distance of 8.5-11 km, of which 1.5-1.8 km are high-intensity running and 14.9-460 m are sprints [11, 36, 37]. High-intensity actions are typically crucial for the outcome of a game, as they are associated with the attacking phase and goal-scoring opportunities [10, 16, 42]. Repeated high-speed actions consisting of two or more sprints with less than 20 s of recovery between efforts occur approximately 31-33 times during international match play [9, 19]. Repeated sprint ability (RSA) has been defined in the literature as short sprints, typically less than 10 s in duration with recovery periods of 60 s or less [3].

RSA can be influenced by both aerobic and anaerobic metabolism [5]. However, the relationship between aerobic and anaerobic variables and an athlete's ability to repeat sprints is uncertain, as it depends on the test used to assess RSA [4, 50]. From a physiological perspective, RSA is a complex quality that is correlated with motor unit activation and is essential to achieving maximal sprint speed and oxidate capacity for phosphocreatine (PCr) recovery and hydrogen (H+) buffering to provide the ability to repeat sprints [7]. Additionally, the power and strength of the lower limbs assist in acceleration and achieving maximum speed in the initial repetitions, while aerobic capacity helps sustain performance in the final sprints [34]. Performance in RSA could also depend on the player's agility, as agility has been correlated with straight-line sprinting [6]. It is important to understand which physical capacities impact RSA, as such knowledge may help coaches define better strategies for improving RSA [22].

In another recent study [22] conducted on female soccer players from Division I in Portugal, positive moderate correlations were observed between peak minimum RSA and adductor and abductor strength. Positive moderate correlations were also found between peak maximum RSA and adductor and abductor strength. Lastly, a moderate negative correlation was found between fatigue index in RSA and Yo-Yo intermittent recovery test level 1 (YYIR1 test) performance. In another study involving female collegiate soccer players in Division I, a correlation was found between RSA total time (RSATT) and time in the 10-m ($r = 0.50$) and 30-m $r = 0.71$) sprints, as well as the left leg in the agility test ($r = 0.57$) [32]. The percentage change in sprint time from the first to the last sprint in the RSA test was correlated only with the left leg in the agility test ($r = 0.53$) in the same study. Among Norwegian female soccer players from Division II, marked correlations were observed between various parameters of the RSA test and the total distance covered in an aerobic capacity assessment test. Specifically, there were significant negative correlations between the fastest time ($r = -0.483$, $p \leq 0.01$), total time ($r = -0.552$, $p \leq 0.01$), and mean time ($r = -0.552$, $p \leq 0.01$) recorded during the RSA test and the total distance covered in the aerobic capacity assessment test [46].

Furthermore, linear sprint over 40 m had a strong relationship with RSA fastest time, RSA mean time, and RSATT. In a study involving Brazilian female soccer athletes, negative correlations were observed between high-intensity exercise tolerance and the RSA performance decline, as well as oxygen uptake and overall RSA performance (e.g., RSAbest and RSAmean) [2]. Finally, in a group of 25 elite-level female athletes from Ireland, performance in an endurance test was negatively correlated with RSATT ($r = -0.58$, $P = 0.002$), and RSA first sprint time (RSAT1) was negatively correlated with endurance performance ($r = -0.58$, $P = 0.03$) [12]. The same study also found a correlation between 10-m sprint time ($r = 0.78$, $P = 0.0001$) and 20-m sprint time ($r = 0.89$, $P = 0.0001$) with RSATT, as well as a correlation between 30-m sprint time ($r = 0.92$, $P = 0.0001$) and RSAT1 ($r = 0.92$, $P = 0.0001$) with RSATT.

However, of these studies, only two [12, 32] used similar tests to assess RSA so that their results could be compared. Furthermore, regarding the evaluation of aerobic capacity, there is greater consensus among the studies, as three [12, 22, 32] used the same test. The same applies to speed, as most studies use similar distances [12, 22, 32, 46]. Additionally, studies that assess change of direction ability use different tests [1, 22, 32, 46], and those that study lower limb strength apply different methods and tools [22, 32, 46]. Furthermore, studies that examine vertical jump ability use bilateral jumps [22, 32, 46], and the available literature reports no study that has examined the correlations between variables from the RSA test and single-leg broad jump (SBJ) in female soccer players. In view of the above, it is not possible to make comparisons between the studies. In the present study on female soccer athletes, a potential correlation of RSA test performance with female athletes' performance on tests of speed, agility, strength, aerobic capacity and jumping ability was investigated. It was also verified whether age (adult female vs pubertal girls) affected these results. It needs to be mentioned that to the best of our knowledge there is no research in the literature that examines the existence of correlations between the RSA test and various laboratory and field fitness tests for U-16 female soccer players. The aim of this study was to examine the relationship between RSA and aerobic capacity, jump ability, isokinetic strength, speed performance, and change of direction (COD) ability in female soccer athletes from two different age groups. The second aim of the study was to compare the performance of the two different age groups in field and laboratory tests. It was hypothesized that athletes with better performance in the RSA test would also have better performance in the fitness tests in both field and laboratory settings. Specifically, it was hypothesized that stronger correlations would exist between aerobic capacity and all variables from the RSA test, as well as between sprint times at 10 and 30 m and the RSATT. Additionally, it was hypothesized that there would be differences in performance across all the tests between the two age groups.

Material and Methods

Design

The measurements were conducted over a two-day period during the competitive season. The measurements took place at the Laboratory of Evaluation of Human Biological Performance at the Department of Physical Education and Sport Science of the Aristotle University of Thessaloniki, as well as on the field on artificial turf. Before the assessment of muscle strength in the laboratory, the athletes performed a 5-min warm-up on a static bicycle (Monark 839, Vansbro, Sweden) at 60 W [15]. Subsequently, they performed dynamic stretches of the knee flexor and extensor muscles. Before the field measurements, the athletes followed a warm-up relevant to the tests to be performed for 10 min (a variation of FIFA 11+ and dynamic lower limb stretches). Additionally, the athletes had a familiarization session with the tests on a different day before participating in the field measurements. Finally, the athletes were advised to avoid alcohol and caffeine consumption in the last 24 hours [28] and to have their last meal at least 3 hours before their visit to the laboratory or the field.

- Day 1: In the laboratory, body weight and height were measured, body fat was assessed using the skinfold method, and subsequently, muscle strength was evaluated using an isokinetic dynamometer. Then, on the field, the RSA was assessed.
- Day 2: Single-leg horizontal jump ability, speed, agility, and aerobic capacity were measured.

Subjects

The study involved 24 female (semi-professional and youth) soccer athletes from divisions I and II of Greece, who were divided into two groups: (i) U-16 (division II) female soccer athletes (age: M = 15.8, SD = 0.8 years, height: M = 160.5, SD = 5.1 cm, body mass: M = 59.4, SD = 7 kg, % body fat: M = 27.6, SD = 4.7, training age: 8.2, SD = 1.7 years), and (ii) adult female (division I) soccer athletes (age: M = 21.9, SD = 4.1 years, height: M = 165.7, SD = 6.1 cm, body mass: M = 62.2, SD = 7.5 kg, % body fat: M = 28, SD = 0.04, training age: 10.8, SD = 2.6 years). All the athletes had at least three years of participation in competitions and followed strength training programs during the current competitive season, with a frequency of one to two times per week. In total, they participated in three to four training sessions and one match per week. The athletes had no injuries in the two months prior to their participation in the measurements. The participants in the study were informed about the benefits and possible risks. Before the data collection, all the participants were informed about the research, they signed their written consent, and were free to withdraw from the study at any time. For participants under 18 years of age, consent was obtained from their parent or guardian. The research ethics committee of the Department of Physical Education and Sport Science in Serres at the Aristotle University of Thessaloniki approved the conduct of the research.

Anthropometric measurements

The measurements of stature and body mass were taken using an electronic digital scale (Seca 220e, Hamburg, Germany) (ICC = 1) [38]. The percentage of body fat was calculated based on the sum of four skinfold measurements (biceps, triceps, subscapular, and suprailiac). Skinfold thickness was measured using a Lafayette skinfold caliper (Lafayette, Ins. Co., Indiana) on the right side of the body [48]. Body density was estimated according to the equations of Durnin and Rahaman [14], while the percentage of body fat was estimated using the Siri equation [47].

Repeated sprint ability

For the assessment of repeated sprint ability, the protocol designed by Gabbett [18] for elite-level female soccer players was used (Figure 1A). This test has been shown to be a valid and reliable assessment of RSA in elite female soccer players (ICC = 0.91, TE = 1.5%) [18]. Players performed 6 × 20-m maximal effort sprints on a 15-s cycle. Upon the completion of each timed 20-m sprint, players performed a 10-m deceleration followed by a 10-m active recovery jog back to the next start line before coming to a complete standstill prior to the next sprint [18]. Athletes began each sprint 0.5 m behind the photocell gate (Microgate, Bolzano, Italy), and sprint performance was recorded at a frequency of 0.01 s. The researcher recorded the active recovery period between 20-m sprints using a stopwatch (AMILA, Thessaloniki, Greece), and athletes were informed about this. The variables calculated in the RSA test were:

- RSATT = the sum of all six 20-m sprint times stated in seconds [18];
- FI = the decrease in sprint performance from the first to the last sprint was calculated and presented as a percentage. The following equation was used [18]:

$$Fatigue\ Index = 100 \times \frac{(Sprint_{best} - Sprint_{worst})}{Sprint_{best}}$$

- Sdec% = sprint decrement (%) calculates fatigue by comparing the actual performance against the

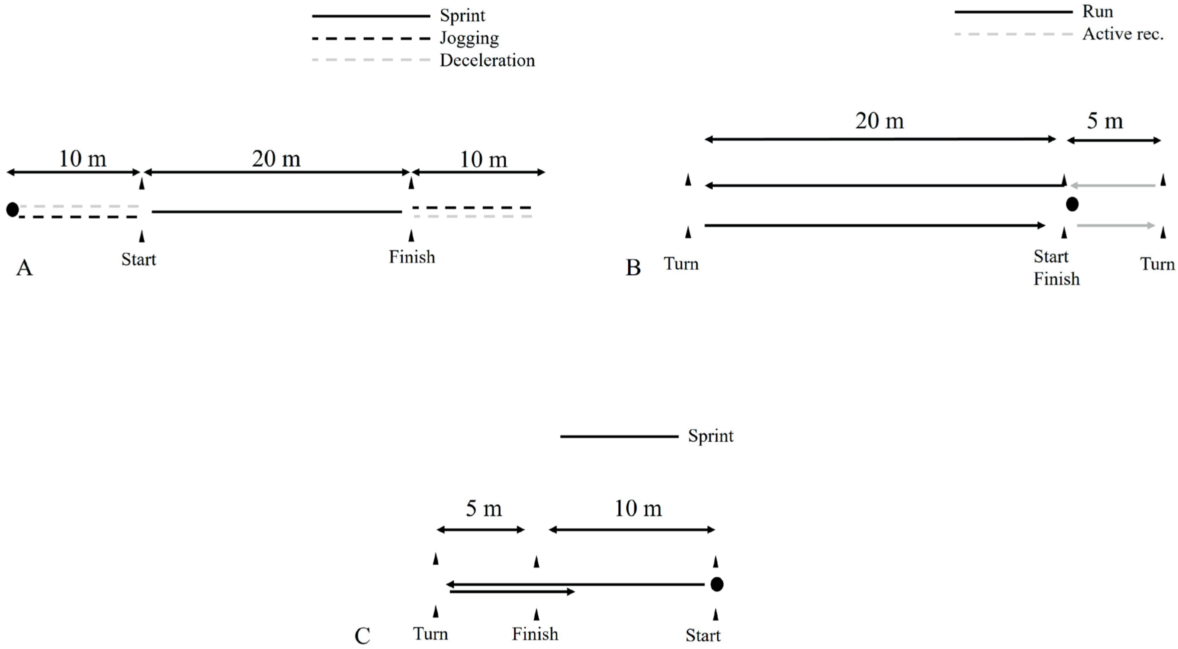


Figure 1. Descriptions of: A. RSA test; B. YYIR1 test; C. 505 COD test

ideal performance (i.e., should the best 20-m effort be replicated in each of the six repetitions). The following equation was used [21]:

$$\textit{Sprint decrement}\ (\%) = \{(S1 + S2 + S3 + S4 + S5 + S6 / S_{best} \times 6) - 1\} \times 100$$

Aerobic capacity
The subjects completed the YYIR1 test as described by Krustrup et al. [30], which has been deemed to be a reliable assessment of aerobic endurance [23]. The test was performed using an audio recording. Each athlete performed repeated 20-m shuttle runs at increasing velocities with 10 s of active recovery. During the active recovery, participants walked around a marker placed 5 m behind the finishing line (Figure 1B). An individual's test was terminated when the subject failed to reach the starting line within the allotted time period on two occasions or the participant felt unable to complete another shuttle at the assigned speed. The total distance covered by a subject was used as a performance measure.

Agility
For the assessment of agility, the 505 COD test (Figure 1C) was used, as described in various studies [33]. Subjects used a standing start with the same body position as per the sprints. They then sprinted through the photocell gate (Microgate, Bolzano, Italy), planted one foot at the point where they had to make a 180° turn, and sprinted again through the photocell gate. A trial attempt was performed for each leg, followed by two more attempts that were recorded. There was a 90-s break between the two attempts, and the fastest attempt was used for analysis. The ICC for the measurement was ICC = 0.88.

Isokinetic strength
The isokinetic strength of the knee flexor and extensor muscles in both legs was assessed using an isokinetic dynamometer (CSMI, Humac Norm Testing & Rehabilitation System, Stoughton, MA, USA). The maximum isokinetic strength was recorded as the torque of the knee flexor and extensor muscles for every 5° throughout the range of motion in the concentric phase. Participants were evaluated at angular velocities of 60°/s, 180°/s, and 300°/s [13]. Three trials were performed for each leg at each angular velocity, with a 40-s rest between the three trials at the different angular velocities. Prior to recording the maximum torque of the knee flexor and extensor muscles, participants performed three practice trials at each angular velocity for each leg.

Jumping ability
The single-leg broad jump test: the athletes, supported only on the jumping leg and with their hands on their hips, stood behind the starting line. They were instructed

to jump as far as they could and land on the same leg. Participants were asked to maintain their position for 2 to 3 s upon landing; otherwise, the attempt was considered invalid and had to be repeated [38]. The distance from the starting line to the athlete's heel was defined as the jumping distance and measured using a tape measure [39]. Before recording the jumps, the athletes performed a trial attempt with each leg. Two jumps were executed for each leg with a 30-s rest between them, and the best jump was used for analysis.

Linear speed

Linear speed was evaluated at 10 and 30 m using photocells (Microgate, Bolzano, Italy), which have been deemed reliable for assessing speed in female soccer athletes [35]. Participants started 0.5 m behind the initial timing gate in a 2-point split stance and were instructed to set off in their own time and run maximally to a marker placed 2 m beyond the 30-m timing gate. Each subject performed two sub-maximum effort sprints prior to three maximal effort sprints, separated with a minimum of 2 min of rest, but no longer than 3 min. Times were recorded to the nearest 0.01 with the fastest time of the three efforts at 10-m, 20-m, and 30-m used for analysis.

Statistics

The data were presented as mean ± standard deviation. The normality of the data was confirmed using the 1-sample Kolmogorov–Smirnov test. Based on the results it was determined that a non-parametric test was not necessary. Additionally, for the physical fitness variables their confidence intervals (95%) and the coefficient of variation were reported. To explore possible correlations between RSA (RSATT, FI, Sdec%) and other variables (strength, jump ability, speed, agility, aerobic endurance), the Pearson correlation coefficient was used. The strength of the correlation was determined based on the value of r: $r \leq 0.1$, trivial; $0.1 < r \leq 0.3$, small; $0.3 < r \leq 0.5$, medium; $0.5 < r \leq 0.7$, large; $0.7 < r \leq 0.9$, very large; and $r > 0.9$, almost perfect [26]. To compare the two age groups, an independent sample t-test was used. The significance level was set at $p \leq 0.05$. The SPSS software (version 25.0, SPSS Inc., Chicago, IL) was used for all the analyses.

Results

Comparison between the two age groups

The results of the statistical analysis for the strength of the knee flexor muscles of the right leg at 180°/s showed significant differences between the two groups ($t = –2.772$, $p = 0.012$, Cohen's d = –1.19, CI: –18.26-–2.58) (Figure 2E). Additionally, the results of the statistical analysis for the strength of the knee flexor muscles of the right leg at 300°/s indicated significant differences between the two groups ($t = –2.659$, $p = 0.015$, Cohen's d = –1.14, CI: –20.17-–2.44) (Figure 2F). For the remaining variables, no differences were found between the two groups (Figure 2). For the deficit between the two legs in the strength of the knee extensor muscles at 60°/s significant differences were also recorded between the groups ($t = –2.678$, $p = 0.014$, Cohen's d = –1.15, CI: –13.55-–1.68) (Figure 3A).

Correlations in the U-16 group of female soccer athletes

The results of the statistical analysis showed a large positive correlation ($r = 0.608$, $p = 0.036$) between RSATT and S10-m, a very large positive correlation ($r = 0.883$, $p < 0.001$) between RSATT and S30-m, a large negative correlation ($r = –0.631$, $p = 0.028$) between RSATT and SBJ right, a large negative correlation ($r = –0.694$, $p = 0.012$) between RSATT and SBJ left, a very large negative correlation ($r = –0.744$, $p = 0.006$) between FI and Flex def 60°/s, a large positive correlation ($r = 0.607$, $p = 0.036$) between FI and L Ratio F/E 60°/s, a large positive correlation ($r = 0.627$, $p = 0.029$) between Sdec% and Flex def 60°/s and a large positive correlation ($r = 0.584$, $p = 0.046$) between FI and L Flex 180°/s (Table 1).

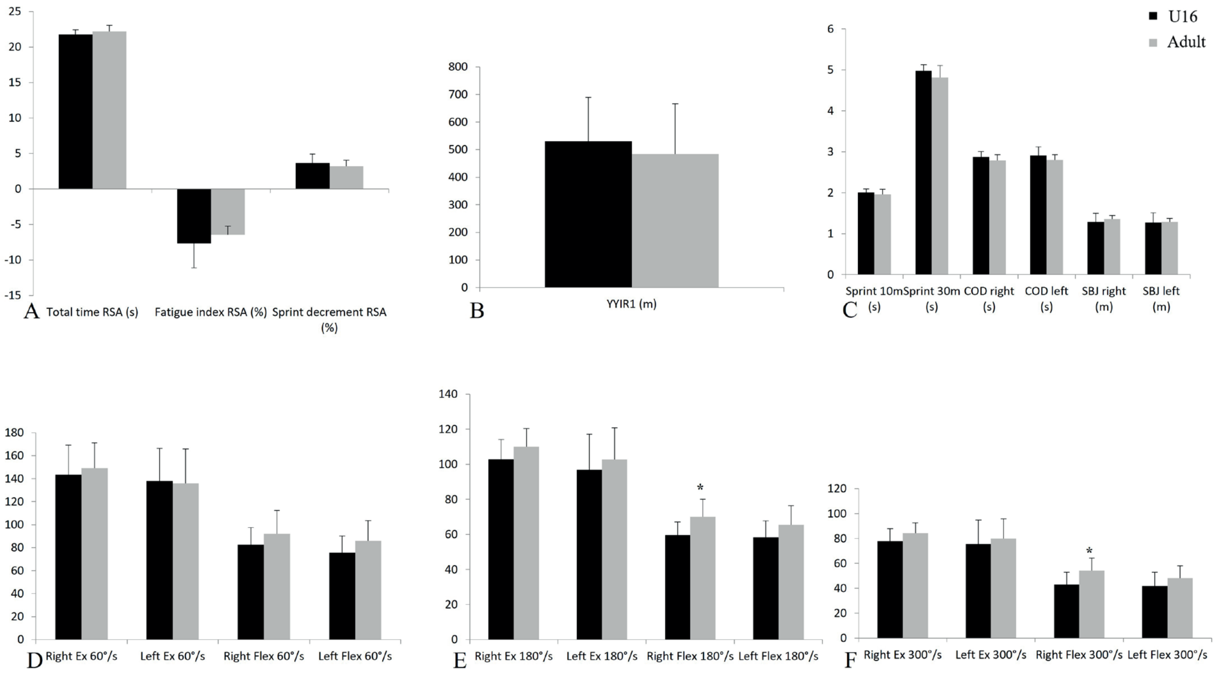
Correlations in the group of adult female soccer players

The results of the statistical analysis showed a large positive correlation ($r = 0.643$, $p = 0.024$) between RSATT and S10-m, a large positive correlation $r = 0.617$, $p = 0.032$) between RSATT and COD right, a very large positive correlation ($r = 0.749$, $p = 0.013$) between Sdec% and Ex def 60°/s, a large positive correlation ($r = 0.682$, $p = 0.030$) between Sdec% and L Ratio F/E 60°/s, a large negative correlation ($r = –0.699$, $p = 0.024$) between Sdec% and Flex def 300°/s (Table 2).

Discussion

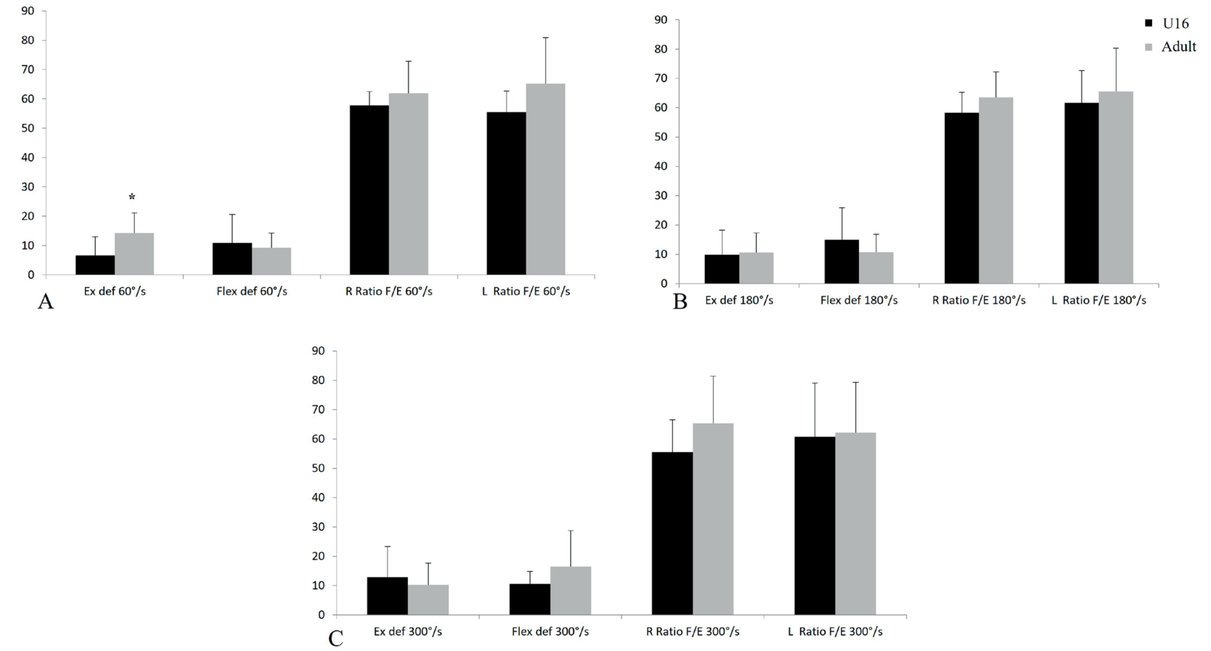
Comparison between the two age groups

As mentioned before, the aim of this study was to examine the relationship between the ability to perform repeated sprints (6 × 20 m in a 15-s cycle) in female soccer players from two different age groups, with aerobic capacity (YYIR1), jumping ability (single-leg jump test), isokinetic strength (60°/s, 180°/s, 300°/s),



Note: RSA – repeated sprint ability; YYIR1 – Yo-Yo intermittent recovery test level 1; COD – change of direction; SBJ – single-leg broad jump; Flex – flexor muscles; Ex – extensor muscles
* denotes significant difference at $p < 0.05$

Figure 2. Performance of the two groups in: A. the RSA test (total time, fatigue index, sprint decrement); B. the Yo-Yo intermittent recovery level 1 test; C. the sprint test (10 m and 30 m), COD test, jump test; D. isokinetic strength 60°/s; E. isokinetic strength 180°/s; F. isokinetic strength 300°/s



Note: Flex – flexor muscles; Ex – extensor muscles; def – deficit; R – right; L – left; F/E – ratio flexors/extensors
* denotes significant differences between the two groups

Figure 3. The deficit between the two legs in the strength of knee flexors and extensors and the ratio of knee flexor to extensor strength of both legs at: A. 60°/s; B. 180°/s; C. 300°/s

Table 1. Correlations of variables from the RSA test (RSATT, FI, Sdec%) with variables from physical fitness tests in the field and laboratory for the U-16 female soccer players

	RSATT	FI	Sdec%
YYIR1	r = –0.527, p = 0.078	r = 0.443, p = 0.150	r = –0.271, p = 0.394
Sprint 10 m	r = 0.608, p = 0.036*	r = –0.227, p = 0.477	r = 0.167, p = 0.604
Sprint 30 m	r = 0.883, p<0.001**	r = 0.050, p = 0.876	r = –0.091, p = 0.778
COD R	r = 0.285, p = 0.370	r = –0.202, p = 0.529	r = 0.165, p = 0.609
COD L	r = 0.296, p = 0.351	r = 0.088, p = 0.786	r = –0.033, p = 0.920
COD def	r = 0.164, p = 0.610	r = –0.180, p = 0.576	r = 0.155, p = 0.631
SBJ R	r = –0.631, p = 0.028*	r = 0.097, p = 0.764	r = 0.083, p = 0.797
SBJ L	r = –0.694, p = 0.012*	r = 0.301, p = 0.342	r = –0.017, p = 0.957
SBJ def	r = 0.326, p = 0.301	r = –0.458, p = 0.134	r = 0.297, p = 0.349
R Ex 60°/s	r = 0.109, p = 0.735	r = –0.109, p = 0.737	r = –0.001, p = 0.999
L Ex 60°/s	r = 0.057, p = 0.860	r = –0.194, p = 0.546	r = 0.024, p = 0.942
Ex def 60°/s	r = –0.130, p = 0.687	r = –0.075, p = 0.816	r = 0.237, p = 0.458
R Flex 60°/s	r = –0.014, p = 0.966	r = –0.257, p = 0.420	r = 0.144, p = 0.656
L Flex 60°/s	r = –0.092, p = 0.775	r = 0.217, p = 0.499	r = –0.209, p = 0.514
Flex def 60°/s	r = 0.002, p = 0.996	r = –0.744, p = 0.006*	r = 0.627, p = 0.029*
R Ratio F/E 60°/s	r = –0.251, p = 0.431	r = –0.336, p = 0.285	r = 0.310. p = 0.327
L Ratio F/E 60°/s	r = –0.235, p = 0.463	r = 0.607, p = 0.036*	r = –0.330, p = 0.294
R Ex 180°/s	r = –0.163, p = 0.613	r = –0.008, p = 0.981	r = –0.080, p = 0.805
L Ex 180°/s	r = –0.047, p = 0.884	r = –0.053, p = 0.870	r = –0.099, p = 0.760
Ex def 180°/s	r = –0.175, p = 0.586	r = –0.252, p = 0.429	r = 0.417, p = 0.177
R Flex 180°/s	r = –0.038, p = 0.907	r = –0.394, p = 0.205	r = 0.266, p = 0.403
L Flex 180°/s	r = –0.522, p = 0.082	r = 0.584, p = 0.046*	r = –0.528, p = 0.078
Flex def 180°/s	r = 0.170, p = 0.598	r = –0.528, p = 0.078	r = 0.273, p = 0.390
R Ratio F/E 180°/s	r = 0.090, p = 0.782	r = –0.442, p = 0.150	r = 0.387, p = 0.214
L Ratio F/E 180°/s	r = –0.374, p = 0.232	r = 0.495, p = 0.102	r = –0.278, p = 0.381
R Ex 300°/s	r = –0.274, p = 0.389	r = 0.102, p = 0.753	r = –0.128, p = 0.692
L Ex 300°/s	r = –0.072, p = 0.824	r = 0.007, p = 0.984	r = –0.170, p = 0.598
Ex def 300°/s	r = –0.255, p = 0.425	r = –0.466, p = 0.127	r = 0.514, p = 0.087
R Flex 300°/s	r = –0.458, p = 0.134	r = –0.089, p = 0.784	r = 0.077, p = 0.813
L Flex 300°/s	r = –0.441, p = 0.151	r = –0.043, p = 0.895	r = 0.105, p = 0.747
Flex def 300°/s	r = –0.154, p = 0.632	r = 0.092, p = 0.776	r = –0.186, p = 0.563
R Ratio F/E 300°/s	r = –0.353, p = 0.260	r = –0.174, p = 0.588	r = 0.190. p = 0.555
L Ratio F/E 300°/s	r = –0.465, p = 0.128	r = –0.004, p = 0.991	r = 0.177, p = 0.581

Note: RSA – repeated sprint ability; RSATT – RSA total time; FI – decrease in sprint performance from the first to the last sprint; Sdec% – sprint decrement (%); YYIR1 – Yo-Yo intermittent recovery test level 1; COD – change of direction; SBJ – single-leg broad jump; Flex – flexor muscles; Ex – extensor muscles; def – deficit; R – right; L – left; F/E – ratio flexors/extensors
* denotes significant difference at p < 0.05; ** denotes significant difference at p < 0.001

Table 2. Correlations of variables from the RSA test (RSATT, FI, Sdec%) with variables from physical fitness tests on the field and in the laboratory for adult female soccer players

	RSATT	FI	Sdec%
YYIR1	r = –0.484, p = 0.111	r = –0.336, p = 0.286	r = 0.226, p = 0.480
Sprint 10m	r = 0.643, p = 0.024*	r = 0.302, p = 0.339	r = –0.301, p = 0.341
Sprint 30m	r = 0.447, p = 0.145	r = 0.319, p = 0.312	r = –0.035, p = 0.913
COD R	r = 0.617, p = 0.032*	r = 0.238, p = 0.456	r = –0.270, p = 0.396
COD L	r = 0.140, p = 0.664	r = 0.136, p = 0.673	r = –0.244, p = 0.444
COD def	r = –0.239, p = 0.454	r = –0.188, r = 0.558	r = 0.102, p = 0.752
SBJ R	r = –0.439, p = 0.153	r = –0.210, p = 0.513	r = 0.084, p = 0.794
SBJ L	r = –0.400, p = 0.198	r = –0.041, p = 0.899	r = 0.067, p = 0.837
SBJ def	r = 0.443, p = 0.149	r = –0.031, p = 0.924	r = 0.049, p = 0.881
R Ex 60°/s	r = –0.492, p = 0.148	r = –0.056, p = 0.878	r = 0.205, p = 0.571
L Ex 60°/s	r = –0.398, p = 0.255	r = 0.121, p = 0.740	r = –0.183, p = 0.614
Ex def 60°/s	r = 0.126, p = 0.729	r = –0.450, p = 0.192	r = 0.749, p = 0.013*
R Flex 60°/s	r = –0.565, p = 0.089	r = –0.550, p = 0.099	r = 0.532, p = 0.114
L Flex 60°/s	r = –0.398, p = 0.254	r = –0.481, p = 0.160	r = 0.509, p = 0.133
Flex def 60°/s	r = 0.192, p = 0.596	r = 0.338, p = 0.339	r = 0.051, p = 0.888
R Ratio F/E 60°/s	r = –0.307, p = 0.387	r = –0.607, p = 0.063	r = 0.472, p = 0.169
L Ratio F/E 60°/s	r = 0.008, p = 0.984	r = –0.582, p = 0.078	r = 0.682, p = 0.030*
R Ex 180°/s	r = –0.426, p = 0.219	r = –0.184, p = 0.611	r = 0.179, p = 0.620
L Ex 180°/s	r = –0.254, p = 0.479	r = –0.024, p = 0.947	r = –0.118, p = 0.744
Ex def 180°/s	r = –0.069, p = 0.849	r = –0.266, p = 0.458	r = 0.552, p = 0.098
R Flex 180°/s	r = –0.274, p = 0.443	r = –0.578, p = 0.080	r = 0.422, p = 0.224
L Flex 180°/s	r = –0.038, p = 0.918	r = –0.386, p = 0.270	r = 0.511, p = 0.131
Flex def 180°/s	r = 0.468, p = 0.172	r = 0.533, p = 0.112	r = –0.590, p = 0.072
R Ratio F/E 180°/s	r = –0.038, p = 0.918	r = –0.525, p = 0.119	r = 0.379, p = 0.280
L Ratio F/E 180°/s	r = 0.133, p = 0.715	r = –0,311, p = 0.381	r = 0.526, p = 0.119
R Ex 300°/s	r = –0.430, p = 0.215	r = –0.106, p = 0.770	r = 0.076, p = 0.835
L Ex 300°/s	r = –0.151, p = 0.677	r = 0.037, p = 0.920	r = –0.096, p = 0.792
Ex def 300°/s	r = –0.041, p = 0.910	r = –0.286, p = 0.422	r = 0.407, p = 0.243
R Flex 300°/s	r = 0.006, p = 0.986	r = –0.208, p = 0.564	r = –0.016, p = 0.966
L Flex 300°/s	r = 0.187, p = 0.605	r = –0.368, p = 0.295	r = 0.499, p = 0.142
Flex def 300°/s	r = –0.007, p = 0.985	r = 0.550, p = 0.100	r = –0.699, p = 0.024*
R Ratio F/E 300°/s	r = 0.134, p = 0.712	r = –0.125, p = 0.732	r = –0.024, p = 0.948
L Ratio F/E 300°/s	r = 0.118, p = 0.745	r = –0.426, p = 0.220	r = 0.568, p = 0.087

Note: RSA – repeated sprint ability; RSATT – RSA total time; FI – decrease in sprint performance from the first to the last sprint; Sdec% – sprint decrement (%); YYIR1 – Yo-Yo intermittent recovery test level 1; COD – change of direction; SBJ – single-leg broad jump; Flex – flexor muscles; Ex – extensor muscles; def – deficit; R – right; L – left; F/E – ratio flexors/extensors
* denotes a significant difference at $p < 0.05$

speed performance (S10-m and S30-m), and COD ability (505 COD test). The second objective of the study was to compare the performances of the two different groups in the tests. The initial assumption that the two groups would differ in all the measurements was not confirmed. As mentioned above, the two groups differed only in the isokinetic strength of the right leg at 180°/s and 300°/s and at the deficit between the two legs in the strength of the knee extensor muscles at 60°/s. More specifically, the older players exhibited a greater deficit between the two legs in the strength of the knee extensor muscles at 60°/s, as well as higher values of strength in the knee flexor muscles of the right leg at 180°/s and 300°/s.

Regarding the strength of the knee extensor and flexor muscles of both legs, Eustace et al. [15] also found differences in isokinetic muscle strength between different age groups. Specifically, older female soccer athletes showed higher values of strength during eccentric contractions of the knee flexor muscles of both the dominant and non-dominant leg and during concentric contractions of the knee extensor muscles of both the dominant and non-dominant leg at all examined velocities (60°/s, 180°/s, and 270°/s). However, in our study the athletes were evaluated only for concentric contractions, unlike the previous research mentioned. In the study by Manson et al. [36], differences in isokinetic strength were also found between three different age groups. More specifically, U-17 female soccer players exhibited lower strength values during concentric and eccentric knee flexion at 60°/s compared to U-20 and adult female soccer players. Additionally, the U-17 female soccer players had lower strength values during eccentric knee extension at 60°/s compared to the U-20 female players. However, the study by Manson et al. [36] did not examine the other two angular velocities (180°/s and 300°/s) used in our current research. On the other hand, Hannon et al. [24] did not find differences in muscle strength between the two age groups they studied at 60°/s and 180°/s. However, these researchers studied different age groups (10-14 years old and 15-18 years old) compared to our current work and used a different protocol to assess the isokinetic muscle strength of the knee extensors and flexors. In another study by Holmes and Alderink [25], no difference was found in the isokinetic strength of the quadriceps and hamstring muscles at 60°/s and 180°/s between two different age groups (15-16 years old and 17-18 years old). However, also in that case the researchers used a different isokinetic dynamometer protocol and the sport of the participants is unknown. Additionally, the age groups compared in the study by Holmes and Alderink [25] had fewer years between them compared to the age groups in our study. For all the remaining variables (RSATT, FI, Sdec%, S10-m and S30-m, YYIR1, COD, and SBJ), there were no differences between the two age groups.

Due to the lack of research on females, references are made to studies conducted on males. Previous research analyzing this topic [20] found differences in the fastest sprint and total time of the RSA test between male soccer players from two different age groups (U-17, U-19), while they found no differences between the two groups in the percentage decrease during the six sprints performed in the test. Another study [49] reported no differences in 5 and 10-m sprints among male soccer players aged 14-18, but differences were found in the 20-m sprint, YYIR1, and COD with the dominant and non-dominant leg. Also, another study [31] recorded no differences between two different age groups (19.29 ± 1.1 and 21.20 ± 1.32) of male soccer players in 5, 10, and 30-m sprints, SBJ, COD, RSATT, percentage decrement in the RSA test, and YYIR2. In a study by Fiorilli et al. [17], differences were found in COD in U-12, U-14, U-16, and U-18. The above studies conducted on male soccer players indicate that it is particularly difficult to compare and explain the results, as they differ in the tests used, the age of the participants and their level and equipment. However, we can observe that the wider the range of age groups, the more likely differences are to be observed. In the current study, differences were observed only in some strength variables between the two age groups and in no other physical ability tests.

It becomes evident that there is a significant lack of research in the literature concerning the comparison of female soccer players of different ages in fitness tests. It has been observed that in male soccer, differences emerge with age in various fitness tests, and it would be worthwhile to examine whether the same occurs in women's soccer. The results of this study did not reveal many differences between the two age groups studied. This is interesting, because in the U-16 group athletes are still developing physically and may lag in their performance in fitness tests compared to adult athletes [29, 45]. Additionally, female athletes differ in training experience by approximately three years. The fact that the female athletes did not differ significantly from one another in many of the fitness tests used may be influenced by training frequency and methods. It might also suggest that the difference in the level, at which they compete is due to technical and tactical characteristics rather than fitness. It would be interesting to investigate in the future whether the absence of differences between the two age groups in almost all the variables examined in this study translates into a lack of differences in variables related to their running profile during the match (e.g., total distance covered, meters in different speed zones, the number of sprints, etc.).

Correlation of the repeated sprints performance with the performance in fitness tests of soccer female athletes on the field and in the laboratory

The hypothesis that strong correlations will be observed between the YYIR1 test, the parameters of the RSA test and the speed tests was partially confirmed. More specifically for U-16 group, large positive correlations were observed between RSATT and S10-m and S30-m, respectively, along with a large negative correlation between RSATT and SBJ left and right, a very large negative correlation, a large positive correlation, and a large positive correlation between FI and the deficit between the two legs in the strength of the knee flexor muscles at 60°/s, the ratio of the strength of the knee flexor and extensor muscles of the left leg at 60°/s, and the strength of the knee flexor muscles of the left leg at 180°/s, respectively. Lastly, there was a large positive correlation between Sdec% and the deficit between the two legs in the strength of the knee flexor muscles at 60°/s. The results for the group of female athletes over the age of 18 showed a large positive correlation between RSATT and S10-m and COD right, respectively, a very large positive correlation, a large positive correlation, and a large negative correlation between the Sdec% and the deficit between the two legs in the strength of the knee extensor muscles at 60°/s, the ratio of the strength of the knee flexor and extensor muscles of the left leg at 60°/s, and the deficit between the two legs in the strength of the knee flexor muscles at 300°/s, respectively.

Initially, it should be noted that there is no research in the literature that examines the existence of correlations between RSA test and various laboratory and field fitness tests for U-16 female soccer players.

In the present study, no correlation between the variables of the RSA test and the YYIR1 was found for both age groups. Similarly to our findings, Lockie et al. [32] also found no correlation between the variables of the RSA test and the YYIR1 in women soccer players around the age of 20. However, other studies have found correlations between RSA test variables and the YYIR1 [12, 22] or other tests related to aerobic capacity [2, 46]. Still, a comparison with our research can only be made with those that used the same RSA test and the YYIR1 [12, 32]. Therefore, based on the results of the present study and those of Lockie et al. [32] no correlation is evident between total time, fatigue index, Sdec%, and the percentage decrement with YYIR1 in U-16 and adult female soccer players. The findings of the present study are consistent with those of Lockie et al., possibly because the same tests were used to assess RSA and because the sample shared some common characteristics. Certainly, as mentioned above, Doyle et al. [12] found a correlation between RSATT and the time of the first sprint in the RSA test with the YYIR1 test. It is worth noting that the athletes who participated in the above-mentioned study by Doyle et al. [12] were elite-level, unlike the athletes in our study. It might have been expected that there would be some correlation between RSA test variables and a test assessing aerobic capacity, such as YYIR1, since high aerobic capacity accelerates recovery after high-intensity efforts like sprints [44]. Additionally, since an RSA test includes intermittent maximal efforts, it would have been possible for a fatigue variable from it (e.g., a fatigue index) to be correlated with another intermittent running test like YYIR1. This could be because fatigue affects performance in the RSA test [18, 31] and YYIR1 [30]. However, as mentioned in the present research, for both age groups no correlation was evident between the RSA test variables and the YYIR1. Furthermore, Rampinini et al. [43] argued that although aerobic capacity contributes to it, other physiological parameters may be more important for improving the ability to perform repeated sprints in trained soccer players.

Furthermore, in the present study there appeared to be a correlation between RSATT and S10-m for both groups. However, only for the group of younger female athletes there seemed to be a correlation between RSATT and S30-m. In these two variables (S10-m and S30-m), Lockie et al. [32] reported data partially consistent with the findings of the present study, as they found a correlation between RSATT and S10-m and S30-m in the adult female athletes they studied. Similarly, Doyle et al. [12] found a very large positive correlation between RSATT and S10-m and S20-m in adult female athletes. Also, they found an almost perfect correlation between RSATT and S30-m. Moreover, Shalfawi et al. [46] also reported a correlation between the total, mean, and fastest time in the RSA test (different from ours) and S40-m in adult female athletes. Gonçalves et al. [22], using a different RSA test than ours and studying female soccer athletes aged around 23 years, found no correlation between different variables (minimum and maximum peak power, fatigue index – a different equation from the one used in the present study) from the RSA test and S10-m and S30-m. The result of the correlation between RSATT and sprint over 10 and 30 m was somewhat expected, as both tests involve achieving maximum speed in a straight line, supporting the results of studies conducted in male soccer [8, 27, 33]. From the results of the present study and existing literature it appears that maximum linear speed is the variable that most affects RSA.

Additionally, the results of the study showed a correlation between RSATT and COD in the group of adult female soccer athletes. In female soccer players of similar age to those in our study, Lockie et al. [32], using the same RSA test and the same agility test as in those in our study, also found a correlation between RSATT and percentage decrement and COD left. In other studies that applied different RSA tests and agility tests, significant correlations were not found between variables from the RSA test and those from the agility test [22, 46]. Although the 505 is a COD speed test, it still features two 5-m linear sprints performed around the direction change [40]. As a result, an individual's linear speed capabilities can still have a positive influence on 505 performance [40]. As mentioned earlier, in the group of adults RSATT correlated with S10-m, which measures athletes' acceleration [31, 33], and is necessary for performing the 505 COD test.

Furthermore, a correlation was found between RSATT and SBJ right and left in the group of U-16 female athletes. So far, there has been no study to examine potential correlations between variables from the RSA test and SBJ in female U-16 soccer players, or in adult female soccer athletes. Lockie et al. [32] found no correlation between RSATT and percentage decrement with a bilateral jump, while other researchers used vertical jumps and not horizontal ones in their studies [22, 46]. Lower limb strength has been correlated with sprint time in female athletes [41]. To run faster, an athlete needs to have the ability to utilize their overall strength and power during the sprint, which requires good neuromuscular control [51].

This is the first study to investigate correlations between variables from the RSA test and strength variables from the isokinetic dynamometer. Previous researchers have attempted to correlate variables from the RSA test with isometric hip adductor and abductor strength [22] and with maximal squat strength [32]. More research is needed to explain the correlation between variables from the isokinetic dynamometer and those from the RSA tests. Nevertheless, it appears that imbalances between the lower limbs and the ratio of knee flexor to extensor muscle strength in the lower limbs affect fatigue variables from the RSA test, as several correlations emerged between fatigue variables from the RSA test and variables related to lower limb imbalances and the ratio of knee flexor and extensor muscle strength, as assessed by the isokinetic dynamometer.

There are some limitations in the current research that need to be mentioned. The sample size of the study was limited. Future research should study groups of different ages and levels. Additionally, in this specific research a particular test designed for female soccer athletes was used [18]. Another RSA test with more sprint meters or more repetitions could alter the correlations of total time, fatigue index, and sprint decrement from the RSA test with the fitness tests used. Lastly, differences between the two age groups and the correlations mentioned could be influenced not only by a different RSA test, but also by the timing of measurements (preparation period, transitional period).

Conclusions

In conclusion, it appears that Greek female soccer players under the age of 16 do not significantly differ from adults in most of the fitness tests conducted, both in the field and laboratory settings. These tests include the RSA test, YYIR1, S10-m, S30-m, 505 COD test, SBJ, and isokinetic evaluation of strength at 60°/s, 180°/s, and 300°/s. However, notable differences were found in the deficit between the two legs in knee extensor strength at 60°/s and the knee flexor strength of the right leg at 180°/s and 300°/s. Practically, this means that U-16 female athletes are physically capable of participating and competing with adult female soccer players. S10-m, S30-m, and SBJ appear to be the variables most correlated with the total time in the RSA test for U-16 female soccer players. S10-m and COD, which require rapid acceleration, seem to be the variables most correlated with the total time in the RSA test for adult female athletes. In practice, this suggests that conditioning coaches can improve performance in RSA by enhancing speed at 10 and 30 m, SBJ, and COD ability in these two age groups.

Conflict of Interest

The authors declare no conflict of interest.

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The effect of high-intensity interval training on hematological variables and lipid profiles in team game athletes

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Abstract
Introduction. High-intensity interval training (HIIT) has gained popularity as a quick and effective way to exercise, and the training consists of a short burst of intense exercise that precedes a period of rest or low intensity exercise. Aim of Study. The present study aimed to investigate the effect of an 8-week HIIT program on the lipid profiles and hematological variables of young players. Material and Methods. A training program was introduced to 40 male players [football (n = 20) and field hockey (n = 20)]. The training set includes 2-min intense sprint interval workout (at 90-95% of HR_{max}, work:rest = 1 : 1) followed by a minute each of active recovery and complete rest continued for 8 weeks with 3 days/week alterations. The lipid profiles, hematological variables, maximum oxygen uptake capacity (VO_{2max}), and anaerobic power (W_{peak}) of the participants were assessed following standard procedures. Results. A significant ($p < 0.001$) decrease was recorded in body fat% (7.6%), white blood cell (9.2%), red blood cell (2.3%), ferritin (21.5%), hemoglobin (2.5%), hematocrit (2.3%), total cholesterol (7.5%), triglycerides (8.7%), total cholesterol/high-density lipoprotein cholesterol (14.2%) and very-low-density lipoprotein cholesterol (6.1%, $p < 0.05$) after the introduction of the HIIT protocol. In turn, body weight (1.1%), body mass index (1.1%), high-density lipoprotein cholesterol (7.9%), platelet (5.9%), mean corpuscular volume (3.5%), platelet-to-leukocyte (16.7%), VO_{2max} (13.6%) and W_{peak} (11.6%) were found to be significantly ($p < 0.001$) increased after the training. Conclusions. The 8-week sprint HIIT protocol resulted in an improved endurance capacity and anaerobic power with an overall improvement in the lipid profiles of young athletes. The HIIT also contributed to a reduction in oxygen-carrying capacity with increased erythrocytic hemolysis.

KEYWORDS: sprint interval training, high-intensity training response, lipid profile measure, blood hematological parameters, endurance capacity.

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Introduction

High-intensity interval training (HIIT) improves both aerobic and anaerobic energy systems over a short period by involving alternating bouts of intensive sprinting exercise with low-intensity recovery periods [18, 29]. HIIT is even defined for longer (30 sec-4 min) intervals with submaximal oxygen consumption levels (approx. 85-90% VO_{2max}) [9]. Whyte et al. [29] reported the impact of HIIT on fat and body weight loss and concluded that HIIT can increase fat oxidation (even up to 18%), especially on post-training days. Besides, Sarkar, et al. [27] reported that low lactate accumulation and reduction in glycogen utilization by improving the efficiency of muscular buffering capacity resulted from high-intensity sprint interval training (HISIT) compared to conventional endurance training.
The lipid profile is an indicator for predicting cardiovascular disease (CVD) development in adults,

with an increase in low-density lipoprotein-cholesterol (LDL-C) and a decrease in high-density lipoprotein cholesterol (HDL-C) serum levels also indicating the onset of coronary heart disease (CHD) in adults [23]. During physical activity, 30-80% of energy comes from fats and muscles use free fatty acids and triglycerides (TG), either circulating or stored, for energy production [23]. Earlier Musa et al. [24] reported that the 8-week HIIT protocol significantly elevated the HDL-C and reduced the total cholesterol (TC) of healthy adults.

Exercise has a pronounced physiologic impact on hematological variables depending on the type, intensity, and duration of the exercise [6]. During HIIT, an increasing number of erythrocytes was reported, which may compensate for the elevated demand for oxygen [13]. Earlier studies reported that endurance training likely has an impact on blood volume changes mainly due to an exercise-induced plasma volume (PV) expansion, which results in lower hemoglobin (Hb) and hematocrit (HCT) levels in athletes. Leukocytes and circulating platelet (PLT) counts have been found to increase during the post-exercise phase in healthy adults [8]. Intense exercise can increase the plasma viscosity and erythrocyte rigidity, but can decrease the sedimentation rate [1]. Studies such as e.g. Belviranli et al. [2] and Heidari et al. [15] confirmed HIIT-induced acute alterations in hematological variables of immediate post-exercise conditions, but the 12-24 hr recovery conditions were only reported by Jamurtas et al. [16].

Except for the study of Jastrzebska et al. [17], previous studies of Belviranli et al. [2], Jamurtas et al. [16], etc. only focused on the effect of high-intensity interval exercise (acute/short-term effect) on various indices. Musa et al. [24] reported unpredictable results of high-intensity exercise-induced changes in the lipid profile and hematological variables mainly due to various work:rest intervals. The present study aimed to investigate the effect of an 8-week sprint HIIT protocol on lipid profile variables, and hematological [complete blood count (CBC)] indices as a long-term adaptive response in young Indian male team game athletes.

Material and Methods

Subject Selection

Forty (n = 40) young Indian male football and field hockey players were recruited as subjects of the present study. The subjects were divided into two age-matched groups: (i) the control group (n = 20; mean age = 16.7 ± 1.43 yrs), and (ii) the HIIT group (n = 20; mean age = 16.4 ± 1.34 yrs), respectively. All the participants had a minimum 5 years of formal training experience and they were only included after clinical examination. During the clinical examination prior to the commencement of the study, the athletes were examined for any serious health issues, hereditary diseases, and injury history. All the subjects were residential players and thus they were considered to form a homogeneous group, characterised by similar dietary habits and socioeconomic status. They underwent training in identical environmental/climatic conditions. The study protocol followed the guidelines of the Declaration of Helsinki and a signed informed consent was obtained from each subject. Proper ethical clearance (Ref No. IHEC/AB/P82/2019) was also obtained from the Institutional Human Ethical Committee (IHEC), the Department of Physiology, the University of Calcutta.

Detailed training program

Three hours of HIIT (sprint intervals) were introduced for 3 days/week (alternative days) for 8 weeks. Daily 3-hr HIIT was divided into two sessions [both morning and evening sessions of 1:30 hr each]. Each training session started with a warm-up session and ended with a cool-down session (both sessions consisted of 15-20 min of slow running at an intensity around 50% of HR_{max}). During HIIT the subjects were asked to perform a total 4 sets/sessions (morning or evening) of all-out training, which was divided into 2 phases of 2 sets each, 2 minutes of intense sprint workout (at 90-95% of HR_{max}) followed by an active recovery and complete rest each with a 1-min interval. The whole training workload was maintained at work:rest = 1 : 1. Each interval training set comprised max. 3 repetitions. Sets of repetition were modified by increasing their number throughout the 8 weeks, i.e., 5 sets in the 1st–2nd week, 6 sets in the 3rd–4th week, 7 sets in the 5th–6th week, and 8 sets in the 7th–8th week, respectively.

The control group players continued systematic low-volume physical training, which included low-intensity physical activity (i.e., stretching, jogging, low-intensity running, etc.) for 6 days/week for 2 hours/day. Players of both groups performed game-specific skill training (i.e., dribbling, passing, tackles, movement techniques, etc.) activity on a regular basis.

Anthropometric variables

Physical characteristics, i.e., standing height (cm) and body weight (kg), were measured using a Seca Alpha stadiometer (model 213, Seca Deutschland, Germany) and a Seca Alpha weighing scale (model 770, Seca Deutschland, Germany), respectively. The body mass index (BMI) was calculated by the standard formulae [26].

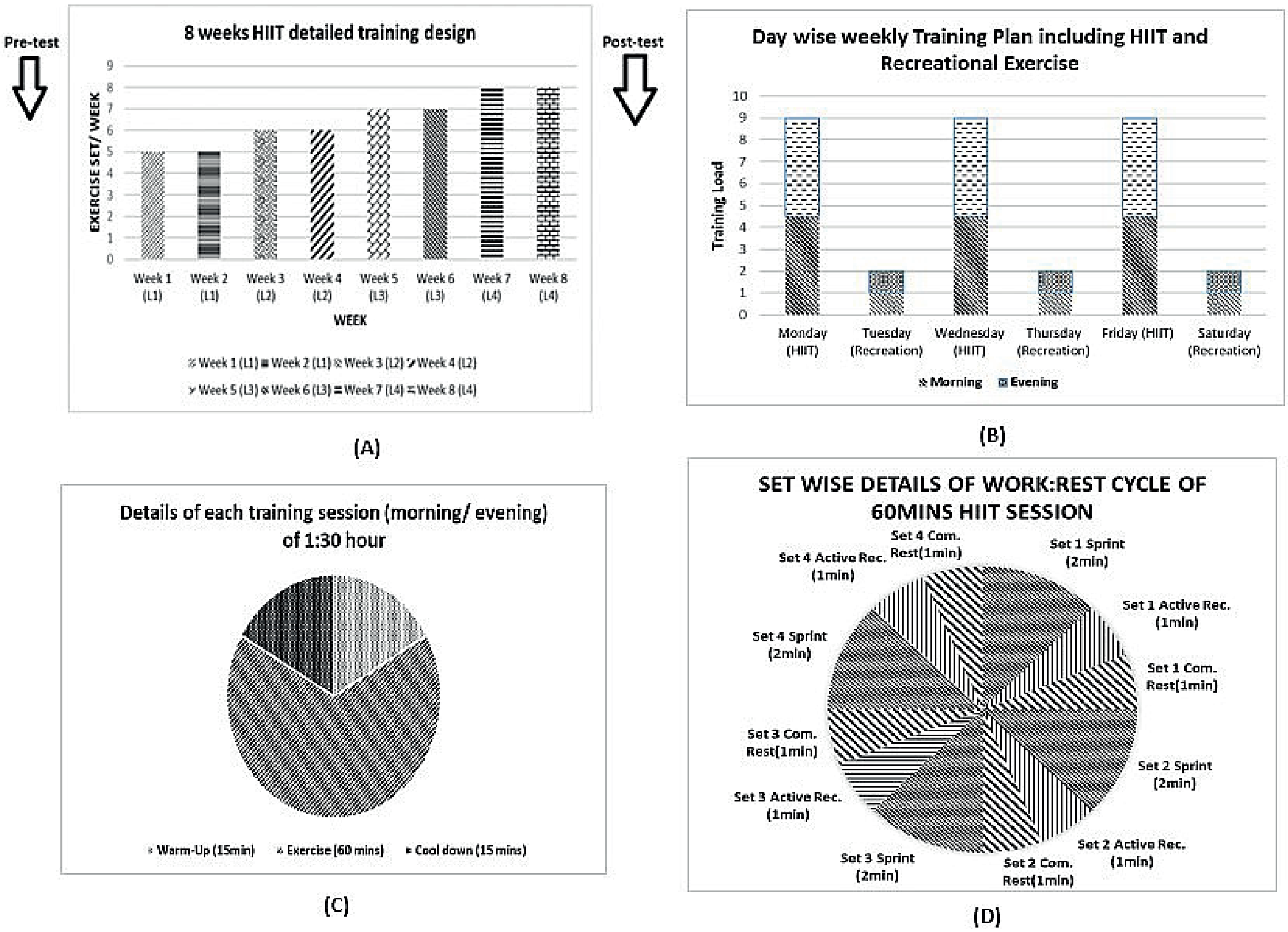

Figure 1. (A) 8-weeks HIIT detailed training design, (B) day-wise weekly training plan including HIIT and recreational exercise, (C) details of each training session (morning/ evening) of 1:30 hour, (D) set-wise details of work:rest cycle of 60-min HIIT session

Body fat percentage (BF%) was calculated by measuring the skinfold thickness at the biceps, triceps, subscapular, and supra-iliac muscles [12].

Blood collection and plasma sample preparation

Blood samples were collected from the antecubital vein into centrifuge tubes for serum preparation (without anticoagulant) between 6:00 AM-8:00 AM in the pre-prandial state (after 10 hrs of fasting) to avoid possible differences due to diurnal variation. Blood samples were centrifuged (REMI centrifuge, R-8C) at 3000 rpm for 15 minutes to ensure complete separation of serum. All laboratory tests were performed at room temperature of 23-25 ºC with 50-60% relative humidity.

Assay for lipid profile

The serum lipid profile included TC, TG, HDL-C, LDL-C, and very-low-density lipoprotein cholesterol (VLDL-C). Complete lipid profile measurements were made using the reagents of Span Diagnostics Ltd., India. The TC and the HDL-C fractions were estimated by the method of Manna and colleagues [22] at 560 nm. TG was estimated at 500 nm on a spectrophotometer and LDL-C was indirectly measured using the following standard procedure. All values of TC, TG, HDL-C, LDL-C, and VLDL-C were expressed in ‘mg/ dl’ [22].

$$\text{LDL-C} = \text{TC} - (\text{HDL-C} + \text{TG}/5)$$

Determination of CBC

White blood cell (WBC), PLT, red blood cell (RBC), Hb, HCT, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were the inclusive parameters under the CBC profile. The CBC was analyzed using a Beckman Coulter ACT 5 DIFF CP analyzer

(Bera, California, US) [11]. MCV was calculated using the formula Ht/RBC × 10. MCH was calculated by Hb/RBC × 10. MCHC was calculated by Hb/Ht × 100 [11].

Physical fitness variables

A breath by breath automated pre-calibrated metabolic gas analyzer (MetaMax 3B, CORTEX Biophysik GmbH, Leipzig, Germany) was used to determine the maximal oxygen consumption (VO_{2max}) by maintaining the following criteria: (i) a plateau in VO_2 (2 ml/kg/min) despite the increasing workload, (ii) respiratory exchange ratio (RER) ≥ 1.1, (iii) > 90% of age-predicted HR_{max} ± 5%, and (iv) voluntary exhaustion [27]. Relative anaerobic peak power output (W_{peak}) was measured by dividing absolute peak anaerobic power (A_{peak}) by body weight, whereas A_{peak} was predicted by using the running-based anaerobic sprint test (RAST). The participants were asked to perform six consecutive sprints at maximal speed over the distance of 35m with a 10-second rest period between each sprint. The timing of each sprint was recorded using a Brower timing gate system [26].

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) version 18.0 (SPSS Inc., Chicago, Il, USA, 2009) was used for the statistical analysis of the data. All the data were expressed as means ± standard deviation (SD). Shapiro-Wilk's test was conducted to check the normality of distribution. The two-tailed paired sample t-test was carried out to calculate the mean differences between pre- and post-phase variables within the same group. ANOVA was also performed to calculate the mean differences between the post-phase variables of the two different groups. A 95% confidence interval was considered as the level of significance. The linear regression prediction models were tested at the $p < 0.05$ level of significance.

Results

The effect of HIIT intervention on the anthropometric variables is presented in Table 1. Weight and BMI were significantly increased in the post-training phase of both the HIIT ($p < 0.001$) and control ($p < 0.01$) groups when compared with their pre-training data. In turn, fat mass percentage (FM%) of the post-training phase was significantly decreased in the HIIT group ($p < 0.001$) and increased in the control group ($p < 0.001$) in comparison to their pre-training values. On the other hand, body height was found to be significantly ($p < 0.01$) increased in the post-training phase of both the HIIT and control groups in comparison to their pre-training counterparts. The effect of HIIT intervention on lipid profile variables is presented in Table 2. Among the lipid profile variables, TC, TG, VLDL-C, and the TC/HDL-C ratio were found to be significantly ($p < 0.001$) lower in the post-intervention phase of the HIIT group when compared with the baseline data except for VLDL-C at $p < 0.05$. In turn, only HDL-C was found to be significantly ($p < 0.001$) increased after the training intervention in the HIIT group. On the other hand, the lipid profile of the control group players was altered in a statistically significant manner.

The effect of HIIT intervention on hematological variables is presented in Table 3. In the HIIT group, WBC, RBC, ferritin, Hb, and HCT were found to be decreased significantly ($p < 0.001$) in the post-intervention phase in comparison to the PT1 phase data. On the other hand, PLT, MCV, and platelet-to-leukocyte

Table 1. Comparison of anthropometric parameters between pre- and post-HIIT intervention

Parameters	Groups	Pre-training (n = 20)	Post-training (n = 20)	Level of significance	P value	% change	ANOVA
Height (cm)	Control	168.20 ± 5.22	168.24 ± 5.21	–3.559**	0.002	0.02(~)	0.953^{NS} (p = 0.335)
	HIIT	169.65 ± 4.17	169.70 ± 4.16	–2.932**	0.009	0.03(~)	
Weight (kg)	Control	59.19 ± 5.37	60.08 ± 4.73	–3.192**	0.005	1.5(↑)	0.036^{NS} (p = 0.853)
	HIIT	59.13 ± 4.78	59.80 ± 4.74	–10.642***	<0.001	1.1(↑)	
BMI (kg/m^2)	Control	20.92 ± 1.59	21.23 ± 1.46	–3.164**	0.005	1.5(↑)	1.082^{NS} (p = 0.305)
	HIIT	20.54 ± 1.43	20.76 ± 1.40	–10.157***	<0.001	1.1(↑)	
FM%	Control	11.90 ± 2.92	12.24 ± 2.85	–8.643***	<0.001	2.9(↑)	1.269^{NS} (p = 0.267)
	HIIT	12.08 ± 3.04	11.16 ± 3.17	11.942***	<0.001	7.6(↓)	

Note: Values are means ± SD, **p < 0.01, ***p < 0.001, NS – non-significant, HIIT – high-intensity interval training, BMI – body mass index, FM% – fat mass percentage, ANOVA – ANOVA between the post-training phase of HIIT and the control group

Table 2. Comparison of plasma lipid profiles between pre- and post-HIIT intervention

Parameters	Groups	Pre-training (n = 20)	Post-training (n = 20)	Level of significance	P Value	% change	ANOVA
TC (mg/dl)	Control	150.0 ± 30.71	150.5 ± 29.29	–5.185***	<0.001	0.3(↑)	10.079** (p = 0.003)
	HIIT	147.60 ± 22.70	136.50 ± 18.73	4.888***	<0.001	7.5(↓)	
TG (mg/dl)	Control	63.7 ± 9.30	63.9 ± 8.85	–3.018**	0.007	0.3(↑)	8.649** (p = 0.006)
	HIIT	61.15 ± 12.23	55.85 ± 10.34	7.491***	<0.001	8.7(↓)	
HDL-C (mg/dl)	Control	43.1 ± 8.49	43.0 ± 7.73	0.076(NS)	0.940	0.2(↓)	3.812^{NS} (p = 0.058)
	HIIT	44.20 ± 7.42	47.70 ± 7.49	–20.571***	<0.001	7.9(↑)	
LDL-C (mg/dl)	Control	87.3 ± 21.14	87.9 ± 19.64	–2.196*	0.041	0.7(↑)	0.533^{NS} (p = 0.470)
	HIIT	86.20 ± 18.01	84.60 ± 17.09	0.406(NS)	0.689	1.9(↓)	
VLDL-C (mg/dl)	Control	16.5 ± 5.48	16.7 ± 5.03	–2.436*	0.025	1.2(↑)	1.068^{NS} (p = 0.308)
	HIIT	16.80 ± 2.93	15.78 ± 3.25	2.765*	0.012	6.1(↓)	
TC/HDL-C	Control	3.5 ± 0.66	3.6 ± 0.51	–2.526*	0.021	2.9(↑)	36.910*** (p < 0.001)
	HIIT	3.37 ± 0.45	2.89 ± 0.36	9.147***	<0.001	14.2(↓)	

Note: Values are means ± SD, *p < 0.05, **p < 0.01, ***p < 0.001, NS – non-significant, HIIT – high-intensity interval training, TC – total cholesterol, TG – triglycerides, HDL-C – high-density lipoprotein-cholesterol, LDL-C – low-density lipoprotein-cholesterol, VLDL-C – very high-density lipoprotein-cholesterol, ANOVA – ANOVA between the post-training phase of HIIT and the control group

Table 3. Comparison of hematological variables between pre- and post-HIIT intervention

Parameters	Groups	Pre-training (n = 20)	Post-training (n = 20)	Level of significance	P Value	% change	ANOVA
WBC (*10^3/μl)	Control	6.84 ± 1.20	6.71 ± 1.67	0.326(NS)	0.748	1.9(↓)	2.083^{NS} (p = 0.157)
	HIIT	6.63 ± 1.45	6.02 ± 1.34	13.038***	<0.001	9.2(↓)	
RBC (*10^6/μl)	Control	5.42 ± 0.49	5.35 ± 0.50	1.000(NS)	0.330	1.3(↓)	3.371^{NS} (p = 0.074)
	HIIT	5.21 ± 0.27	5.09 ± 0.30	10.450***	<0.001	2.3(↓)	
PLT (*10^3/μl)	Control	247.40 ± 58.93	244.60 ± 57.32	0.836(NS)	0.413	1.1(↓)	0.029^{NS} (p = 0.866)
	HIIT	228.20 ± 52.37	241.65 ± 52.15	–12.543***	<0.001	5.9(↑)	
Ferritin (ng/ml)	Control	61.10 ± 21.72	59.78 ± 21.87	0.719(NS)	0.481	2.2(↓)	3.572^{NS} (p = 0.066)
	HIIT	60.60 ± 20.37	47.58 ± 18.99	23.936***	<0.001	21.5(↓)	
Hb (gm/dl)	Control	14.76 ± 0.93	14.59 ± 0.72	1.398(NS)	0.178	1.2(↓)	1.940^{NS} (p = 0.172)
	HIIT	14.66 ± 0.68	14.29 ± 0.61	9.797***	<0.001	2.5(↓)	
HCT	Control	43.86 ± 2.53	43.37 ± 2.86	0.573(NS)	0.574	1.1(↓)	1.083^{NS} (p = 0.305)
	HIIT	43.45 ± 3.07	42.44 ± 2.81	5.992***	<0.001	2.3(↓)	
MCV (fl)	Control	82.43 ± 6.33	82.65 ± 4.98	–0.162(NS)	0.873	0.3(↑)	3.639^{NS} (p = 0.064)
	HIIT	82.45 ± 4.13	85.38 ± 4.03	–36.254***	<0.001	3.5(↑)	
MCH (pg)	Control	27.74 ± 2.63	28.25 ± 2.00	–0.842(NS)	0.410	1.8(↑)	0.091^{NS} (p = 0.764)
	HIIT	28.57 ± 1.30	28.43 ± 1.76	0.344(NS)	0.734	0.5(↓)	
MCHC (gm/dl)	Control	33.65 ± 1.09	34.19 ± 1.08	–1.499(NS)	0.150	1.6(↑)	2.513^{NS} (p = 0.121)
	HIIT	33.79 ± 0.95	33.72 ± 0.74	0.260(NS)	0.798	0.2(↓)	
PLR	Control	36.83 ± 9.22	37.71 ± 9.79	–0.478(NS)	0.638	2.4(↑)	1.265^{NS} (p = 0.268)
	HIIT	34.97 ± 7.17	40.80 ± 7.37	–21.029***	<0.001	16.7(↑)	

Note: Values are means ± SD, ***p < 0.001, NS – non-significant, HIIT – high-intensity interval training, WBC – white blood cell, RBC – red blood cell, PLT – platet, Hb – hemoglobin, HCT – hematocrit, MCV – mean corpuscular volume, MCH – mean corpuscular hemoglobin, MCHC – mean corpuscular hemoglobin concentration, PLR – platelet-to-leukocyte ratio, ANOVA – ANOVA between the post-training phase of HIIT and the control group

Table 4. Comparison of endurance capacity and anaerobic power output between pre- and post-HIIT intervention

Parameters	Groups	Pre-training (n = 20)	Post-training (n = 20)	Level of significance	P Value	% change	ANOVA
VO_{2max} (ml/kg/min)	Control	50.34 ± 3.66	50.95 ± 3.51	–5.719***	<0.001	1.2(~)	31.007*** (p < 0.001)
	HIIT	51.23 ± 4.78	58.19 ± 4.64	–31.830***	<0.001	13.6(↑)	
W_{peak} (watt/kg)	Control	7.51 ± 1.05	7.44 ± 0.95	1.313(NS)	0.205	0.9(↓)	9.578** (p = 0.004)
	HIIT	7.60 ± 1.35	8.48 ± 1.35	–16.474***	<0.001	11.6(↑)	

Note: Values are means ± SD, *p < 0.05, **p < 0.01, ***p < 0.001, NS – non-significant, HIIT – high-intensity interval training, VO_{2max} – maximum oxygen consumption, W_{peak} – relative anaerobic peak power output, ANOVA – ANOVA between the post-training phase of HIIT and the control group

Table 5. Pearson's product-moment correlation coefficient of selected lipid profile and hematological variables with endurance capacity and anaerobic power output

Variables	VO_{2max}	W_{peak}
Cholesterol	–0.126	–0.322*
HDL	0.330*	–0.005
TC/HDL ratio	–0.448**	–0.291
WBC	–0.144	0.064
RBC	–0.402*	–0.171
PLT	–0.164	–0.086
Ferritin	–0.388*	–0.485**
Hb	–0.212	0.214
HCT	–0.267	0.112
PLR	–0.067	–0.126

Note: *p < 0.05, **p < 0.01, NS – non-significant, VO_{2max} – maximum oxygen consumption, W_{peak} – relative anaerobic peak power, TC – total cholesterol, HDL – high-density lipoprotein, WBC – white blood cell, RBC – red blood cell, PLT – platet, Hb – hemoglobin, HCT – hematocrit, PLR – platelet-to-leukocyte ratio

Table 6. Prediction of regression coefficient based on the linear regression model of young players

Variables	VO_{2max}					W_{peak}				
	R^2 0.333	Adj R^2 0.211	β	t value	Sig.	R^2 0.420	Adj R^2 0.315	β	t value	Sig.
TG			0.028	0.164	0.871			–0.102	–0.638	0.528
TC/HDL ratio			–0.354	–2.349	0.025			–0.221	–1.571	0.126
RBC			–0.223	–0.963	0.343			0.093	0.430	0.670
Ferritin			–0.183	–1.065	0.295			–0.580	–3.626	0.001
Hb			–0.156	–0.865	0.393			0.228	1.355	0.185
HCT			0.049	0.226	0.822			0.199	0.983	0.333

Note: Adj R^2 – adjusted R^2, VO_{2max} – maximum oxygen consumption, W_{peak} – relative anaerobic peak power, TG – triglycerides, TC/HDL ratio – total cholesterol to high-density lipoprotein ratio, Hb – hemoglobin, HCT – hematocrit

(PLR) ratios were found to be increased significantly ($p < 0.001$) in the post-intervention phase of the HIIT group when compared with the baseline data. Only the MCH and MCHC variables of the HIIT group were altered in a statistically insignificant manner. Among the control group data, all the hematological variables of CBC were found to be altered in a statistically non-significant manner.

The effect of HIIT intervention on some selected physical fitness parameters is presented in Table 4 VO_{2max} and W_{peak} were found to be significantly increased in the post-training HIIT group in comparison to pre-training HIIT ($p < 0.001$) and post-training control ($p < 0.001$ for VO_{2max}; $p < 0.01$ for W_{peak}) counterparts. In turn, the changes in VO_{2max} and W_{peak} were statistically non-significant when comparing the post-training with pre-training periods of the control counterparts.

Table 5 presents Pearson's correlation coefficient between the selected lipid profile and hematological parameters with physical fitness variables (i.e., VO_{2max}, and W_{peak}). VO_{2max} was found to be negatively and significantly correlated with the TC/HDL ratio, RBC, and ferritin. In turn, VO_{2max} was only positively and significantly correlated with the HDL level. On the other hand, W_{peak} was found to be negatively and significantly correlated with cholesterol and ferritin levels.

Table 6 presents the prediction regression model for VO_{2max} and W_{peak}. The first regression prediction model represents F = 2.741, Sig = 0.028 for the dependent variable – VO_{2max} (predictors: TG, HDL/cholesterol ratio, RBC, ferritin, Hb, and HCT). In turn, the second model represents F = 3.989, Sig = 0.004 for the dependent variable – W_{peak} (predictors: TG, HDL/cholesterol ratio, RBC, ferritin, Hb, and HCT).

Discussion

The present study investigated the adaptive training effect of 8-week sprint HIIT on the lipid profile and hematological parameters in trained male athletes. The results of the present study showed an overall improvement in lipid profile variables with a reduction in body weight. The study also showed a reduction in the oxygen-carrying ability of blood cells, which may be the effect of HIIT.

In the present study, HIIT intervention was suggested to improve body composition by significantly ($p < 0.001$) reducing BF% (7.6%) and increasing body weight (1.1%) and BMI (1.1%). This observation is in agreement with the study report of Musa et al. [24]. High-intensity training generally leads to a reduction in body weight and fat% by a suggested increase in the lipolysis rate (fat oxidation) induced by an increase in certain hormonal profiles (i.e., catecholamine) controling the β-adrenergic receptors in the adipose tissue [19]. Skeletal muscle fat oxidation is a highly regulated process and may be limited by several long-chain fatty acid membrane transporters, among which fatty acid-binding protein ($FABP_{pm}$) and fatty acid translocase CD36 are the most important [3]. Previously high-intensity exercise/ HIIT was suggested to increase the lipolysis rate/fat oxidation triggered by an increase in certain hormonal profiles, i.e., catecholamine, which controls the β-adrenergic receptors in the adipose tissue and increased $FABP_{pm}$ content in skeletal muscle [28]. On the other hand, the increase in skeletal muscle fat oxidation likely results from several adaptations, including an increase in mitochondrial volume [7], and altering several regulatory steps; adipose tissue lipolysis of TG to fatty acids, transport of fatty acid into the cell, intramuscular lipolysis of TG to fatty acids and ultimately fatty acid transport into the mitochondria [3].

In the present study, TC, TG, TC/HDL-C, and VLDL-C ($p < 0.05$) were found to be decreased significantly ($p < 0.001$) by 7.4%, 9.2%, 14.7%, and 5.9%, respectively, after the 8-week HIIT program. In turn, only HDL-C was found to be increased significantly ($p < 0.001$) by 7.9% after the same exercise protocol. The present study corroborates the research data of Musa et al. [24] and Ouerghi et al. [25]. Musa et al. [24] reported an 18% increase in HDL-C and an 18% reduction in the atherogenic index (TC/HDL-C ratio) followed by an 8-week HIIT (work:rest = 1 : 1) training with 4-5 min interval length. Finally, the longer interval training and/or a higher work:rest ratio were found to be more efficient in raising HDL-C and lowering the TG response. The HIIT-induced increase in HDL-C and reduction in the atherogenic index could potentially lead to a reduction in heart disease risk by 18-27%. It has been hypothesized that the risk of CHD can even be reduced by 53% with every unit drop in the TC/HDL-C ratio [20]. The study of Manna et al. [22] showed that the aerobic part of high-intensity training felicitates beneficial changes in HDL-C compared to low-intensity exercises. Furthermore, Ouerghi et al. [25] reported that training can induce significant changes in the lipid profile both in athletes and in sedentary untrained subjects not only for high-intensity training, but also for continuous or intermittent aerobic training. Such discrepancies among previous findings may be due to variations in several factors i.e., training intensity, training type, the timing of blood draw, the subject's ethnicity, study methodology, etc. [25].

In their study Jamurtas et al. [16] reported a significant increase in MCV, MCHC, WBC, and lymphocyte counts among all the hematological variables in immediate post-exercise conditions. However, the altered hematological profile will get back to its resting level after 3-6 hrs of the recovery phase [2]. In turn, Halson et al. [14] reported a consistent change in some hematological parameters at even resting-state conditions. In the present study, a decrease in WBC, RBC, ferritin, Hb, and HCT and an increase in PLT, MCV, and PLR counts were observed even after 8 weeks of the HIIT protocol, which corroborates the findings of Jastrzebska et al. [17] and Chou et al. [5]. The HISIT-induced decrease in RBC (2.3%), Hb (2.5%), and HCT (2.4%) might be due to the exercise-induced PV expansion and increased hemolysis of RBC, which confers the consequence of exhaustive exercise-induced muscular fatigue [8, 30]. The strenuous exercise-induced RBC hemolysis may be caused by oxidative stress, and/or physical trauma in circulation, and the damaged erythrocyte membrane can lead to hemolysis due to the reduced cellular deforming capability and increased membrane rigidity [5]. The presently studied decrease in HCT (2.4%) may indicate the mechanism of blood dilution and movements of transmission fluids inside the blood vessels, which helps to compensate for the immediate effect of exercise-induced blood volume loss and to restore cellular homeostasis [14]. Similarly, Jastrzebska et al. [17] and Wilkinson et al. [30] found a 25-30% reduction in ferritin levels after 6-8 weeks of HIIT, indicating latent iron deficiency which may be explained by the dilution effect of blood volume expansion. However, a reduction in serum ferritin was reciprocally related to the TIBC, which maintains the storage of iron in the blood by improving the iron-binding capacity [30]. The presently studied non-significant increase in PLT count (5.9%) may be due to the variation in releasing fresh platelets from the spleen and bone marrow and/or may be due to the increased secretion of epinephrine during exercise [14]. Further, an increase in MCV count (3.6%) in the present study may reflect the increasing number of erythrocytes deformed (structural damage) by high-intensity exercise resulting in hemolysis [11].

The present study showed a significant ($p < 0.001$) rise in VO_{2max} (13.6%), and W_{peak} (11.6%), thus corroborating the study of MacPherson et al. [21] where VO_{2max} and W_{peak} improvements were reported for the HIIT protocols varying in durations, training sets, work:rest cycle gaps, athletic groups, and genders. Significantly increased VO_{2max} was mainly due to improved mitochondrial enzyme activities, i.e., citrate synthase [7], cytochrome c oxidase (COX), COX subunits II, IV protein content [10], and β–hydroxy acyl-CoA dehydrogenase [7, 4]. In turn, MacPherson et al. [21] and Gibala and McGee [10] demonstrated that post-training enhancements in stroke volume and maximal cardiac output (Q_{max}) led to a 15-35% improvement in VO_{2max} following HIIT. Considerably, the improved W_{peak} corresponds to an improved anaerobic capacity index, which might be due to the improved lactate tolerance and developed sprinting ability within the anaerobic zone with a higher glycolytic activity level [27]. Similarly, Sarkar et al. [27] showed that the HIT response comprises increased glycolytic enzymatic activities (e.g., hexokinase, glycogen phosphorylase, phosphofructokinase), increased muscle buffering capacity, and ionic adaptations including increased $Na^+ – K^+$ –ATPase content and function as the adaptive response to compensate for the increased energy demand during an anaerobic HIIT. In other studies, Burgomaster et al. [4] and Sarkar et al. [27] reported an improved anaerobic capacity that may be due to muscular adaptations [reduced phosphocreatine degradation, enhanced glycogen content] along with an increased type IIA ratio and a reduced IIB ratio, which has been repeatedly demonstrated in response to low-volume high-intensity sprint interval training.

In this study Pearson's product-moment correlation for VO_{2max} not only showed the oxygen-carrying dependency (a direct significant relation), but also revealed a novel finding indicating lipid profile variance for training adaptations in VO_{2max}. Furthermore, in the correlation study ferritin was identified to be the most definite variable for predicting and varying physical fitness levels (both VO_{2max}, and W_{peak}) as the individual capacity of training adaptation. In the present study, two separate linear regression models were drawn from phase II data to predict endurance capacity (VO_{2max}) and relative anaerobic power (W_{peak}). Here, TG, the HDL/cholesterol ratio, RBC, ferritin, Hb, and HCT were able to significantly predict the linear regression model for VO_{2max} with $t = 4.762$ (significance at $p < 0.001$) and F value = 2.741 (significance at $p = 0.028$). However, those variables were not able to predict the linear regression model for W_{peak} with $t = 0.038$ (significance at $p = 0.970$) and F value = 3.989 (significance at $p = 0.004$).

Strengths and limitations of the study

This is one of the pioneering studies to assess the effect of sprint HIIT on the blood hematological parameters and lipid profile among the young athlete population of the Indian subcontinent. The study results will not only help athletes and coaches to establish guidelines, but also help to maintain the balance between increasing thetraining load and blood hematological balance within the physiologic fatigue limit to reach optimal performance. The present study was concerned only with a specific type of intense sprint interval training (2 min intense sprinting at 90-95% of HR_{max} with work:rest = 1 : 1) as HIIT, which is a limitation of the study, as there are many more types of HIIT available, which may modify training-induced alterations in the hematological profile. On the other hand, the study included only male players as the subjects because of their easy availability, so it can add another limitation to the study. Another limitation of the study is the ethnicity of the study sample, which may only be found in south-east Asia.

Conclusions

The present study suggested an 8-week sprint HIIT protocol as an effective and time-efficient strategy to significantly induce the enhancement in fat oxidation [Fat% (↓7.6%)] with an improved lipid profile [↑7.9% HDL-C and ↓14.2% atherogenic index (TC/HDL-C)] along with enhanced endurance capacity (↑13.6%) and anaerobic power output [W_{peak}(↑11.6%)]. However, the present findings also indicate that the HIIT protocol may limit the oxygen-carrying capacity of blood cells with an increased risk of erythrocytic hemolysis (↓2.3% RBC) supported by a significant decrease in ferritin (21.5%), Hb (2.5%) and HCT (2.3%) values. The present training mode is also likely to have some damaging effect on the immune system, as it significantly decreases the WBC (9.2%) count with increased PLT (5.9%) and PLR (16.7%) values after HIIT intervention. So, the present high-intensity training intervention may be observed to improve performance variables for endurance capacity, anaerobic power, and explosive strength with enhanced fat oxidation and better lipid profiling, but in terms of inducing the hemolytic prone condition. The present observations may also help athletes and coaches in planning/formulating a systematic HIIT program under impairing exercising intensity with proper work:rest intervals, which might not induce any damage and help to maintain the athletes' physiologic harmony under the fatigue limit. However, the present study results may also be helpful for future research in the sprint HIIT program based on the limitations and expected outcomes.

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Conflict of Interest

The authors declare no conflict of interest.

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The development of electric herbal pad treatment for delayed onset muscle soreness after exercise training

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Abstract
Introduction. **Intense exercise causes muscle pain or delayed onset muscle soreness. The most common symptoms are decreased muscle strength, muscle tenderness, and swelling. These symptoms are limited to physical activity in daily life. Treatment for muscle soreness after exercise is essential and easy to administer for example by applying superficial heat. The herbal compress is a traditional treatment in Thailand that has been used for relieving pain.** Aim of Study. **This study was to develop and determine the effect of an electric herbal hot compress pad on perceived muscle soreness sensation, muscle strength, range of motion after intense exercise induced muscle soreness.** Material and Methods. **There were 24 participants, aged 18-24 years old. The participants randomly received: (i) an herbal hot compress ball; or (ii) an electric herbal hot compress pad. Both groups received the respective treatments 24, 48, and 72 h after exercise for 40 min once a day. Muscle soreness was induced by Winged cycling using weight of 0.076 kg for 30 min. The perceived muscle soreness, muscle strength, and range of motion were measured immediately at the baseline, after intense exercise, and after treatment at 24, 48, and 72 h.** Results. **The results showed that all the variables were increased compared to baseline measurements after 30-min intense exercise. This indicates muscle damage. Furthermore, they showed that muscle soreness sensations decreased 72 h after treatment, with slight changes in muscle strength and range of motion after treatment. However, in the immediate application of heat in both groups there were no significantly different changes.** Conclusions. **This pilot study indicated that pain relief efficacy with the use of the electric herbal compress pad for delayed onset muscle soreness was not different from that of an herbal compress ball. However, a large long-term follow-up study on the therapy is needed.**

KEYWORDS: electric herbal hot pad, Thai herb.

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Introduction

Intense exercise, particularly eccentric exercise, which exceeds your original performance or beyond what you previously performed, can cause delayed onset muscle soreness [3, 9]. It is a condition that requires a break from using muscle. The common symptoms include decreased strength, pain, muscle tenderness, stiffness, and swelling [16, 19, 27]. These symptoms are limited in physical activity, causing effects on various body systems, such as the musculoskeletal system. However, the severity of muscle pain depends on the intensity and duration of exercise [4, 5, 8, 26].
Muscular pain or muscle soreness after exercise is caused by morphological changes inside muscle fibers. The damage affects sarcomeres, causing an inflammatory response process by secreting prostaglandin E_2, in response to the inflammatory process causing pain, swelling, redness, the heat of the tissues, and inflammatory substances secreted by white blood cells (leukotriene synthesis) [14]. In the excitation-contraction interaction mechanism of the muscles, the onset of muscle discomfort occurs 8-12 h after activity, peaking 24-72 h after exercise. The pain gradually subsides in 5-7 days

[18]. According to the study of Eston et al. [7], muscle soreness after the downhill run causes muscle loss of strength and an increased level of the enzyme creatine kinase. Muscle pain after exercise is the leading cause of reduced muscle strength and angle of motion. Increased pain and inflammation of the muscles inhibit muscle recovery, which is the main reason for the reduction in exercise performance or athletic training, also having mental and psychological effects [2, 17, 25].
Treatment for muscle soreness after exercise is essential and may be administered e.g. by applying superficial heat, which requires the practice of 20-30 min per session. When a herbal compress (herbal ball) is applied, the heat and scent of the essential oils from the herbal compress help relieve muscle ache and tension [23]. Thailand has traditionally used herbs in the treatment or care of people's health using herbal compress ball products. The herbal compress ball is a cloth wrapped in herbs that has properties helping reduce muscle pain. The effects of using herbal hot compress balls include increased skin temperature and heart rate, drop in blood pressure because they can effectively transfer heat to the treated skin area, but they require steaming for 20 min before use [10, 21]. Lehmann et al. stated that the muscle temperature should be 40 °C to increase tissue temperature, blood circulation in the skin, and the body's metabolic rate [11]. However, local heat therapy relieves muscle fatigue during exercise, increases flexibility, and inhibits muscle pain [20, 22]. It involves herbal extracts with anti-inflammatory effects and the aromatic scent to help induce relaxation, such as carotenoids and polyphenols. Herbal extracts have a stimulating effect on the central nervous system through the stimulation of the hormones catecholamines. They increase alertness and decrease reaction time to reduce oxidative stress caused by exercise in athletes. The herbal hot compress ball has the advantages of increasing the tissue temperature and blood circulation, applying herbal extracts with pain-relieving effects, which better penetrate skin tissue into the bloodstream, carrying protein nutrients and oxygen into the injured cells and muscle tissue for faster healing, by reducing pain and inflammation, creatine kinase and prostaglandin E_2 in the blood. The heat causes an increase in the elasticity of the collagen tissue and the motion angle [12]. Steam in the hot compress ball for 20-30 min allows the medicines or essential oils to spread into the treatment area. In addition, constant pressing of the massage transfers heat to the untreated skin; however, the limitation of the use of herbal hot compress ball is that it cannot penetrate the skin. Therefore, the researchers aimed to develop the electric herbal hot compress pad treatment of delayed onset muscle soreness after exercise.

Material and Methods

Participants
Twenty-four university students participated in this study and signed an informed consent document approved by the Ethics Committee in Human Research, Mahasarakham University. The certification number was 038-019/2564. The participants were 18-24 years old. Inclusion criteria included being healthy, aged 18-24 years, exercising on an irregular basis max. three times a week, having no muscle injuries before joining the study, and having a normal body mass index (18.5-24.9 kg/m^2). The exclusion criteria included having no muscle soreness after exercise, unwillingness to participate in the study, and lack of attendance.

Study design
This study was controlled trial human experimental research. It was designed to develop and determine the effect of the electric herbal hot compress pad on perceived muscle soreness sensation, muscle strength, and range of motion after intense exercise. Muscle soreness of knee extensors was stimulated by indoor cycling using a weight of 0.076 kg for 30 min. Participants were determined all outcomes and received either treatment for 40 min. After treatments, perceived muscle soreness, muscle strength, and range of motion were measured at the baseline, at 30 min, 24, 48, and 72 h. The perceived muscle soreness, muscle strength, and range of motion were measured at the baseline, at 30 min, 24, 48, and 72 h after intense exercise.

Treatment procedures
The participants were randomly assigned to two groups of the same size, i.e. 12 participants each. Participants with eligibility criteria were randomized by drawing lots into two treatment groups: the herbal compress ball group and the electric herbal compress pad group. Participants received treatment for 40 min after intense exercise.

Herbal compress ball group
Participants in this group received an application of a herbal compress ball on both front thighs (Figure 1). Each herbal compress ball weighed 231 g and contained dried herbs including Zingiber cassumunar Roxb (100 g), Curcuma longa L. (50 g), Citrus hysteric D.C (36 g), Cymbopogon nardus (20 g), Dryobalanops aromatica Gaertn. (10 g), Cinnamomum camphora J.Presl (10 g),

and Sodium chloride (5 g). However, before being used in the treatment the herbal balls were steamed for 20 min. Afterward, the ball was wrapped in a towel to protect the participants' skin from being burnt due to excessive heat, and then the herbal ball was gently pressed and rolled on the treated areas for approximately 15 sec for each area. In this study, the herbal balls were used for three treatment visits before being replaced with the new balls when the next round of treatment started.

Figure 1. Treatment with the herbal compress ball

Electric herbal compress pad group
Participants in this group received the treatment of an electric herbal hot pad on both front thighs (Figure 2). The electric herbal hot compress pad consisted of two parts: an electrical device consisting of a temperature setting device with a temperature setting of 0-100 °C and a heat-generating device with a width of 10 cm by 12 cm in length. It was light and flexible with good heat transfer properties. The material used was laminated silicone fused with a rubber sheet. It transmitted heat and high pressure on both sides of the coil embedded inside the silicone. It could withstand the heat of 200 °C and the AC voltage of 12 V with no harm to the body. The size of the bag was 10 cm in width by 12 cm, containing herbal medicines. Inside the bag there was an herbal gel pad that had properties to relieve muscle pain. The herbal gel pad contained Plai oil (15 g), Bergamot oil

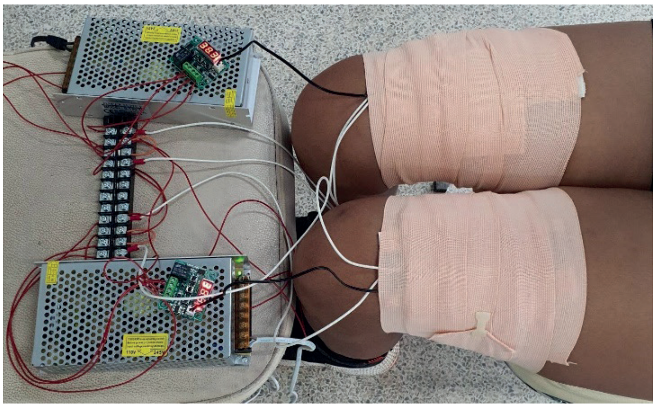

Figure 2. Treatment with the electric herbal compress pad

(5 g), Ginger oil (5 g), salt (5 g), Carbopol 934 (5 g), Cremophor RH 40 (0.5 g), and Triethanolamine (0.5 g). The treatment temperature was 40-45 °C.

Exercise induced delayed onset muscle soreness
Delayed onset muscle soreness was induced in all the participants by standardized repetitive quadriceps muscle exercise using indoor cycling with a weight of 0.076 kg/body weight. The test required maximum effort for 30 min. Participants received instructions on the test. Weights were loaded onto the pan and suspended so that participants could begin pedaling with only the resistance of the flywheel; they were instructed to increase pedal cadence to 60-65 rpm during countdown and maintain this cadence until the signal to begin pedaling maximally was given. At "Go" the weight pan was lowered, and the participants began to pedal maximally against the resistance. Verbal encouragement was given to participants throughout the test to ensure maximal effort. The participants spun up to 100^{+} rpm on the "Go" signal. They received a final countdown near the end of the test. The weight pan was lifted to allow the participants to pedal without additional resistance. Participants were directed to the warm-up bike for 5 min to allow recovery.

Measurement
The perceived muscle soreness, muscle strength, and range of motion were measured immediately at the baseline, after intense exercise, after treatment, at 24 h, 48 h, and 72 h.

Perceived muscle soreness sensation
Perceived muscle soreness sensation was assessed on a numeric rating scale for pain (used because of its simplicity). The numeric rating scale consists of a horizontal bar marked with whole numbers from 0 to 10, with 0 being no pain and 10 being the worst pain. The participants were evaluated while flexing and extending the knee joint. Then they were asked to rate their pain level on the scale. The number of pain levels was recorded during the session. The pain was measured before the induction of muscle soreness at 24, 48, and 72 h subsequently. Participants rated their pain at the exact time when the measurement was taken, rather than as an average period of over 24 h.

Muscle strength
Participants' muscle strength was evaluated for their dominant quadriceps using leg dynamometry. The participants stood on the foot of the machine, bent their

knees at 90°. Both hands held the puller in a prone position, and then arranged the cable accordingly. The participant exerted a full leg stretch and held it for 10-15 sec. The unit of measure in kilograms and the results were divided depending on the body weight. The average of the two closest trials was recorded. Muscle strength was measured immediately before exercise and then again at the same time after 30 min of intense exercise, then measured again at 24, 48, and 72 h after treatment.

Range of motion
The research assistant used a goniometer to measure the degree of knee motion in a supine position from full extension to full knee flexion. The knee joint angle was between the lateral epicondyle of the femur, lateral malleolus of the fibula, and greater trochanter. The goniometer was placed with the goniometer pivot point upright the epicondyle of the femur, the stationary arm being positioned between the greater trochanter and middle of the femur, and the other between the femur. The moving arm was between the lateral malleolus and the middle of the fibular bone. The range of motion of the tested knee joint was measured to evaluate the degree of joint stiffness from muscle soreness.

Data analysis
The study results are presented by descriptive statistics including means, standard deviation, with statistical significance set at $p < 0.05$. They were used to compare the values of age, body mass, height, body mass index, heart rate, systolic and diastolic blood pressure at the baseline, and an independent sample t-test was also used. Overall effects and differences in perceived muscle soreness sensation, muscle strength, and range of motion between the two groups during the baseline and treatment periods were analyzed using two-way repeated-measures ANOVA and Tukey's test. The statistical analysis was conducted using the IBM SPSS statistics package (version 27.0).

Results
The physical characteristics of the participant groups are presented in Table 1 (n = 24; 12 male, and 12 female). There were no significant differences in all the variables found between the two groups in the treatment. There were no participants who dropped out of this study.

Response symptoms of muscle damage
An indirect symptom response indicating muscle damage after intense exercise includes e.g. the perceived muscle soreness sensation, muscle strength, range of motion as shown in Table 2. The intense exercise protocol was successful in producing perceived muscle soreness sensation. In the within-group analysis, the mean values of perceived muscle soreness sensation showed a significant increase ($p < 0.05$). However, after intense exercise in both groups muscle strength, and range of motion in all the groups were not significantly improved compared with their baselines.

Table 1. Baseline characteristics of participants

Characteristics	Treatment groups		p
	Herbal compress ball	Electric herbal compress pad	
n (male : female)	12 (6 : 6)	12 (6 : 6)	
Age (y)	19.33 ± 0.49	19.08 ± 0.65	0.31
Body weight (kg)	61.42 ± 11.62	64.83 ± 13.43	0.51
Height (cm)	165.08 ± 7.09	167.75 ± 10.67	0.48
BMI (kg/m^2)	21.94 ± 2.45	22.85 ± 2.71	0.40
HR (bpm)	76.08 ± 7.96	80.83 ± 14.07	0.32
Systolic (mmHg)	115.83 ± 9.09	117.17 ± 8.10	0.71
Diastolic (mmHg)	70.67 ± 7.88	73.58 ± 9.91	0.43

Note: There were no significant differences between both groups (all $p < 0.05$).

Treatment study
In the within-group analysis the mean values of perceived muscle soreness sensation showed a significant increase at the following periods ($p < 0.05$), after 30-min intense exercise, at 24, 48, and 72 h after treatment. The perceived muscle soreness sensation was greater in 48 h and decreased slowly after 72 h after treatment in both groups. There was no difference in muscle strength and range of motion in both groups after intense exercise and 24, 48, and 72 h after treatment compared to after 30 min. The muscle strength decreased within 48 h and gradually improved at 72 h after treatment. The slight changes in the range of motion were observed throughout the experiment. However, 72 h after treatment a greater range of motion was observed in the herbal hot compress ball group compared to the electric herbal hot compress pad group. Statistical tests showed that there was no significant difference in perceived muscle soreness sensation, muscle strength, and range of motion between the treatment groups (Table 2).

Table 2. Perceived muscle soreness, muscle strength, and range of motion of the two groups at baseline, after intense exercise, and after treatment

Groups	Baseline	After 30-min intense exercise	After treatment (hours)			p
			24	48	72	
Herbal compress ball						
Perceived muscle soreness sensation	0.00 ± 0.00	1.67 ± 0.78	5.17 ± 0.72	7.25 ± 0.87	5.17 ± 0.84	0.00*
Muscle strength	1.43 ± 0.27	1.63 ± 0.29	1.57 ± 0.34	1.38 ± 0.18	1.68 ± 0.32	0.10
Range of motion	130.33 ± 8.31	127.08 ± 8.94	127.00 ± 7.58	127.42 ± 7.48	131.00 ± 7.42	0.37
Electric herbal compress pad						
Perceived muscle soreness sensation	0.00 ± 0.00	1.92 ± 1.00	5.75 ± 0.75	6.75 ± 0.75	5.00 ± 0.74	0.00*
Muscle strength	1.43 ± 0.24	1.70 ± 0.29	1.60 ± 0.30	1.51 ± 0.34	1.79 ± 0.43	0.70
Range of motion	129.25 ± 6.20	130.50 ± 5.54	131.42 ± 3.92	132.42 ± 5.89	129.33 ± 5.82	0.58

* significant difference compared to baseline, after 30-min intense exercise, and after treatment ($p < 0.05$)

Discussion

This study shows that vigorous exercise causes delayed onset muscle soreness by significantly increasing the perceived muscle soreness sensation compared to the baseline in each group. The treatment of delayed onset muscle soreness with a herbal hot compress ball and an electric herbal compress pad lasted 40 min. Effects after compress application at 24, 48, and 72 h after intense exercise showed that there was no statistically significant difference at the 0.05 level due to the following factors; 1) The effects of heat on the nervous system and muscles reduce pain and inflammation, since the prolonged use of heat increases the temperature inside the tissue and affected the expansion of blood vessels, providing nutrients and oxygen into the cells. As substances and wastes were eliminated from the cells this led to a reduction in pain levels. Nadler et al. [20] found that an increase in body tissue temperature leads to blood circulation increasing in the skin, resulting in fluid movement in the body's tissues and increasing the metabolic rate of the body. Topical heat therapy improved flexibility and suppressed muscle pain. The increased heat expanded blood vessels, promoted blood flow, and released residual pain-causing substances. It resulted in increased cellular metabolism and enzyme activity. In addition, the heat was a stimulant of pain. According to Melzack and Wall's gate control theory, heat increases the pain threshold and reduces muscle spasms [1]. The heat from the herbal hot compress ball and the electric herbal hot compress pad stimulated large nerves to send signals to Substantia Gelatinosa (S.G.) and inhibited the conduction of nerve signals sent to the forwarding cells. Therefore, no nerve signals were sent to the brain, resulting in decreased muscle pain. According to the gate control theory, the detection of pain and pain control is executed by nerve signals, stimulated by different parts of the body, going through large and small nerve fibers. The nerve signals that pass through the two nerve fibers enter the spinal cord through the dorsal gate (Dorsal Horn) and then split in two ways. One part is forwarded to the Transmission Cell or T to carry the signal forward to the brain, and another part was to S.G., a tight group of neurons in the spinal cord where large and small nerve fibers meet. There is also an inhibitor neuron for receiving nerve signals that pass through the spinal cord, by secreting neurotransmitters to the forward cells. The large nerve fibers have more nerve signal power than the small ones that simulate S.G., causing no nerve signal to be sent to the transmission cells. Therefore, no further nerve signal is sent to the brain, resulting in the situation called "The door will close," and pain will not occur. However, if there is an increase in the neurotransmitter power in the tiny nerve fibers, it will cause the inhibition of S.G. as "The door will open." The nerve signals that pass through the gate to the cells to the brain cause the perception of pain. The nerve signals pass through the door to the cell, and then to the brain, causing the perception of pain. The nerve signals controlling the pain mechanism of the spinal cord are transmitted to the brain and the body's motion system. The application of a herbal hot compress and an electric herbal hot compress pad created superficial heat applied to the area to reduce pain, based on conduction and convection, in which the heat source contacted with the treated area directly and the heat penetrated the dermis layer. As a result, heat reduced pain and muscle tension, increased the rate of blood circulation, caused the expansion of arteries and capillaries, reducing inflammation and swelling, and reducing symptoms of joint stiffness.

2) The active substances in the herbal hot compress and electric herbal hot compress pad have anti-inflammatory effects. Cassumunarins A, B, and C are compounds in the group of Complex Curcuminoids that have anti-inflammatory activity. In addition, Plai essential oils contain terpenoid groups such as α-pinene, sabinene, α-terpinene, terpinen-4-ol and phenylbutanoid substances such as (E) -1- (3,4-dimethoxyphenyl) butadiene (DMPBD), (E) -4 (3'-4'-dimethylphenyl) but-3-en-2-ol [24]. Previous studies revealed that various phytochemical extracts from Plai can reduce pain and inflammation. The heat caused the essence in the herbal hot compress ball and the electric herbal hot compress pad to evaporate from the herbal matter, thus it could well penetrate the skin because under the skin there is a fat layer, allowing easy penetration of important substances from Plai. For 20-60 min of therapy vital substances from Plai penetrate the circulatory system [6]. The heat source from the used herbal hot compress ball and electric herbal hot compress pad increased the temperature in the tissue area and blood circulation. It enhanced the penetration of substances from herbs with pain-relieving propertiesthrough the skin tissue into the bloodstream. With the introduction of protein nutrients, oxygen enters the injured area cells for faster healing of muscle tissue, reducing pain, soreness and inflammation, decreasing the levels of creatine kinase and prostaglandin E_2 in the bloodstream. Moreover, the heat also changed the elastic properties of collagen tissue and increased the angle of motion [1, 12]. The administration of a finished product containing 14% of essential oils from Plai rhizomes healed ankle injuries in male athletes by reducing swelling and pain, so that they could move their ankles downwards more [13, 15]. However, eccentric contraction exercises result in such high levels of muscle stiffness that tearing of the muscle fibers and connective tissue in the sarcolemma leads to the proliferation of calcium and enzymes such as myoglobin and cytosolic out of the cells, causing muscle strength to decline 24 h after exercise, while the amount of neutrophils and histamine increased due to the inflammatory process. After 48 h of exercise, the amount of macrophages was maximized, while the level of PGE_2, a stimulant for pain receptors, increased in the bloodstream [4, 5]. Using a herbal hot compress ball and an electric herbal compress pad provided superficial heat that was applied to treat the muscle soreness. When the heat source directly contacted the treated area, it carried the vital substances contained in Plai herb to penetrate the skin to the dermis layer, the circulatory system, and the lymphatic system. Therefore, the muscle area treated with a herbal hot compress ball and an electric herbal hot compress pad was affected by the active substances in Plai herb that had no significantly different properties to reduce pain and inflammation. However, for the treatment of muscular pain after exercise a product should provide constant heat for the herbal compress ball, and the time of use should be longer. In this way sore muscle treatment after exercise would be more effective according to the individual needs. In addition, the area treated using an electric herbal heat compress pad was heated throughout the treatment period and received the essential substances in the herbs evenly, unlike using an herbal hot compress ball that needed steaming to provide heat. After 20 min of use, the treatment was inconsistent and time consuming. Some limitations should be mentioned concerning this study. Further studies should be conducted with longer study periods. In addition, post-treatment follow-up is required to evaluate the carryover effect produced.

Conclusions

In this small study, these investigations reveal that superficial heat (using a herbal hot compress ball and an electric herbal hot compress pad) provided some significant benefits in the reduction of muscle soreness sensation and improved muscle strength following intense exercise. However, an electric herbal hot compress pad has the advantage of being able to distribute heat more evenly than the herbal compress ball. The use of the herbal compress ball required lifting to change the compress position, which resulted in uneven heat contribution. In summary, in the treatment of muscle soreness after exercise both methods help relieve pain. The herbal hot compress can increase the muscle tissue temperature and blood circulation, which provides protein nutrients. Oxygen enters the injured cells and muscle tissue for faster healing. This reduces pain and inflammation, reduces plasma creatine kinase and prostaglandin E_2 in the blood. Heat causes changes in the elasticity of collagen tissue and increases the angle of motion. This results in better performance in the activities of daily living.

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Conflict of Interest
The authors declare no conflict of interest.

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The effect of pitch dimensions and players' format on heart load and external load in semi-professional soccer players

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Abstract
Introduction. **Soccer players need to acquire high level of technical, tactical, and physical skills to be able to play at professional level. Changing the pitch size (while keeping the same format of play) causes variations in the relative area per player (calculated as the area of the pitch divided by the number of outpitch players involved in the game). This manipulation is one of the main concerns while using small-sided games (SSG), since different relative areas per player for the same format change the players' responses.** Aim of Study. **The present study is part of a doctoral thesis, the purpose of which was to investigate the internal and external load in semi-professional soccer players during SSGs with different numerical ratios and pitch dimensions.** Material and Methods. **The study sample included 16 semi-professional male soccer players, who played random 1v1, 2v2, 3v3 and 4v4 SSG without goalkeepers and with small goals on individual pitches of 1 : 150 m², 1 : 100 m², and 1 : 75 m² areas, respectively, for 8 weeks until the end of the season. GPS monitoring was used to record the elements of the internal and external loads during the whole training session. The level of significance was fixed at p 0.05.** Results. **The results indicate that training in different dimensions of pitches and with different players' format has a different effect on the internal and external load of soccer players.** Conclusions. **Numerical relationships and the player/space ratio affect the physiological responses of soccer players and should be taken into consideration by coaches for the best coaching design of technical, tactical, and physiological elements. The research findings will help coaches to choose the best training methods based on their training goals. Moreover, coaches in semi-professional teams now have consistent information to design and optimize their training time in mixing the technical and physical aspects.**

KEYWORDS: heart rate, soccer, physiological responses, GPS devices, small-sided games, internal and external load.

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Introduction

Soccer is classified as an intermittent exercise, in which the effort exerted depends on the dynamic of the game [3, 22, 26]. Small-sided games (SSG) are typically described as smaller versions of the formal game [10], with adjustments in the number of players (format) and the size of the pitch [25]. These games have been very popular and used in the last decade to improve the physiology of training sessions affecting game performance in team sports [10]. The potential of such modality for sports training has been of interest to many coaches as it simultaneously develops the players' physical, technical, and tactical performance. SSGs improve physical fitness while also increasing levels of enjoyment and competence [27]. Physical trainers prefer SSG and conditioned games [i.e., match-play with a reduced number of players [6] since: a) they enhance work on technical and tactical parameters [10], and b) they elicit high heart rate (HR) intensities (i.e. > 90% HR_{max}) [11] in the range of those reported to be functional in improving aerobic fitness in soccer players (i.e. 90–95% of HR_{max}) [10]. During the last decade, researchers have attempted to estimate the resulting training load. To a large extent, technological advancement has contributed to this progress. There are two types of training load evaluation methods: those that monitor the internal load and those that monitor the external load [4].

The predominant method, by which the external load is measured involves the use of GPS systems (Global Positioning System). GPS systems operate by tracking the position of soccer players using the data they receive from satellites. It has been proven that the higher the frequency (Hz), the more accurate the measurements. This particular recording system is used to measure the total distance travelled, the speed at which the distance is covered, as well as accelerations and decelerations during training.

Internal load, on the other hand, refers to the athletes' physiological response to the training stress. It is clear that internal load affects player adaptations [17]. Internal load is most accurately quantified by monitoring the HR, measuring lactate concentration, and using the rate of perceived exertion (RPE, CR-10, 6-20) [4].

In relation to physical conditioning, it has been suggested that SSGs may be a good alternative to classical physical conditioning in young soccer players to maintain or improve aerobic fitness, after pre-season and during the season. Hill-Hass et al. [8] showed that SSGs and generic training are equally effective in improving pre-season YYIRTL1 (i.e., Yo-Yo Intermittent Recovery Test Level 1) performance. Impellizeri et al. [13] observed that SSGs and aerobic intermittent training (IT) were also equally effective in aerobic fitness after pre-season and after 8 additional weeks of training. In turn, Reilly and White [26] reported that after a 6-week program during the competitive period the effects on aerobic capacity between SSG and IT were similar. Radzimiński et al. [23] found that in young soccer players SSG training was more effective in improving VO_{2max} than an IT protocol. The main findings of the studies indicate a greater internal load of soccer players (higher HR, RPE, lactate concentration) during small numerical relations (1v1, 3v3). Small numerical relations lead to a higher pace of play, but with a smaller overall distance travelled [20]. Nevertheless, Hoff et al. [12] reported that players with higher VO_{2max} tend to exercise at a lower percentage of VO_{2max} when participating in SSGs. Smaller formats have been found to increase HR responses, blood lactate concentration, and perceived exertion [10], as well as the frequency of ball contacts per player [19]. As far as the pitch dimension is concerned, the literature suggests that larger pitch dimensions increase the physiological responses and distance covered by players [1]. The effects of these task constraints have already been extensively studied [7, 10].

Furthermore, part of the research led to contradictory results. Köklü and Alemdarolu [15] reported a higher percentage of HR_{max} values in the 3v3 and 4v4 configurations compared to 2v2. However, Köklü [14] observed greater HR values during 3v3 compared to 2v2 and 4v4. Despite this observation, most studies revealed an inverse association between the number of players and internal load [5]. Extensive research has been conducted to investigate the effect of pitch size on external load. A study conducted on collegiate students found that larger relative pitch sizes (120 m^2/player and 200 m^2/player) resulted in increased distance covered as well as a higher number of decelerations and accelerations, when compared to smaller relative pitch sizes [11]. Furthermore, in a study conducted in youth under 17 (U-17) soccer players researchers observed considerably greater values of total distance and high intensity running with a relative pitch size of 175 and 273 m^2/player [2].

In addition, studies examining the external load of adults and young soccer players during the SSGs displayed that the comparison was challenging, as there are differences in the methodology and no relative values were reported that made the comparison between the variables possible [16,28]. However, where relative values were reported and comparisons were possible, researchers observed greater distance travelled per minute (m/min) as the number of players increased [5], while others noted greater distance travelled per minute (m/min) as the number of players decreased [20].

The purpose of this study was to investigate the internal and external load in semi-professional male soccer players during small-sided games with a different number of players (4v4, 3v3, 2v2, 1v1) and pitch size (150 m^2/player, 100 m^2/player, 75 m^2/player).

Material and Methods

Experimental design

The study was conducted over an 8-week period (April-May 2023); throughout the duration of the study there were no significant changes in environmental conditions, with average temperature of 19.9 °C and 63% humidity.

The training sessions were held on natural grass. Participants were instructed to abstain from any training stimulus for two days before the initial measurements. During the research period the participants did not engage in any other physical activity. For the next eight weeks the soccer players completed SSGs (4v4, 3v3, 2v2, 1v1 without goalkeepers and with only small goals) in a random sequence with relative pitch sizes of 150 m^2/player, 100 m^2/player, and 75 m^2/player. During SSGs the participants wore portable GPS (Polar Team Pro) tracking sensors to capture their internal and exterior loads. SSGs were conducted following a 15-minute standardized warm-up consisting of slow jogging, strolling locomotion, active stretching, progressive sprints, and accelerations. SSGs were followed by 5′ of recovery. Training sessions were performed at the same time in order to avoid the possible effects of the circadian rhythm on the variables. SSGs were followed by a 5-minute passive recovery period.

Participants

The study sample included 16 semi-professional male soccer players from the municipality of Katerini in Central Macedonia, Greece (mean ± SD: age = 18.36 ± 2.3 years and training age = 10.06 ± 2.2 years, height = 177.00 ± 4.2 cm, body mass = 69.75 ± 8.1 kg, body fat (%) = 22.64 ± 4.5%, 10 m sprint = 1.74 ± 0.08 s, sprint 40 m = 5.69 ± 0.43 s, VO_{2max} = 48.86 ± 4.21 $ml \cdot kg^{-1} \cdot min^{-1}$), who competed in the championship of E.P.S. Pieria, Greece and participated voluntarily in the study. Table 1 shows the participants' anthropometric and physical fitness parameters.

Inclusion criteria for participating in the study were the following: a) no musculoskeletal injuries over the last 4 months, b) abstention from any ergogenic supplement or medication for ≥4 months, c) 95% training and match compliance, and d) participation in all SSGs. All the players had played federation soccer for an average of 8 years before the study. Their training involved 4 sessions per week (each lasting 90 minutes) in addition to a competitive match. All the participants were notified of the research design and its requirements, as well as the potential benefits and risks, and they each gave their informed consent before participation. They were informed that they may withdraw from the study at any moment. The study was carried out in accordance with the rules of the Aristotle University of Thessaloniki's Ethical Committee (323/2023) and the revised Declaration of Helsinki.

Table 1. Anthropometric and performance characteristics

Variable	Mean (±SD)
Age (yrs)	18.36 (2.3)
Height (cm)	177 (4.2)
Weight (kg)	69.75 (8.1)
Body fat (%)	22.64 (4.5)
Sprint 10 m (s)	1.74 (0.08)
Sprint 40 m (s)	5.69 (0.43)
VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	48.86 (4.21)
Playing experience (yrs)	10.06 (2.2)

Procedure

Anthropometric measurements were carried out at the initial appointment. The participants then completed a 15-second warm-up followed by a 40-meter Maximal Sprint Test to define speed zones. Subsequently they underwent a Yo-Yo intermittent recovery test level 1 to determine their VO_{2max}. All the measurements were taken on natural grass at least 48 hours following a game. Players' movements throughout the following training sessions were tracked using portable GPS trackers. In this experimental design SSGs were conducted at 48-hour intervals. The equipment was thoroughly tested two weeks before the initial readings.

Anthropometric measurements

The participants' body mass and height were measured using an electronic digital weight scale and a height scale (Seca 220e, Seca, Hamburg, Germany). In the relevant evaluations these two measurements were accurate to 0.1 kg and 0.1 cm. The individuals were barefoot and wore only underpants throughout the measurements. A Lafayette skinfold caliber (Lafayette Instrument, Indiana, USA) was used to measure the thickness of the soccer players' hypodermic fat in four of their skinfolds (biceps, triceps, suprailiac, and subscapular) to estimate body fat. All skinfold measurements were taken on the right side of the body, and body fat (%) was estimated using Siri's algorithm [29].

Yo-Yo Intermitted Recovery Test Level 1

The YYIR1 consisted of two 20-meter intervals of running separated by regular 10-second rest breaks. Furthermore, a CD-ROM provided signals to control the speed. The player ran 20 meters forward, adjusting his speed to arrive at the 20-meter marker precisely at the moment of the signal. A turn was also made at the 20 m marker and the player sprinted back to the beginning

marker, which was to be reached at the next signal. The athlete then paused for 10 seconds to run slowly around the third marker, which was placed 5 meters behind him. He had to wait for the next signal at the marker.
The course was repeated until the player failed for two consecutive shuttle runs. When the start marker was not reached for the first time, a warning ("yellow card") was sent, and the test was cancelled at the second failure ("red card"). The last running interval completed by a player before being removed from the test was recorded, and the test result was reported as the overall running distance travelled in the test. The YYIR1 also began at a speed of 10 km/h. Afterwards, the speed was increased by 2 and 1 km/h in the next two speed levels, respectively. Following that, the speed was increased by 0.5 km/h at each speed level. The YYIR1 was maintained during the last 40 meters. The following equation was used to forecast the players' VO_{2max} based on their distance covered in the YYIR1: Prediction of VO_{2max} (ml/kg/min) = YYIR1 distance (m) 0.0084 + 36.4.

Speed evaluation
The sprint test was conducted using three pairs of photocells (Witty, Microgate, Bolzano, Italy) placed at three different points: the starting point at 10 m, and at the finish line (at 0 m, 10 m, and 40 m). Each pair of photocells served as a gate, through which the soccer players passed. The soccer players began their attempt from a standing stance, 0.3 meter behind the first gate. The photocells were placed around the height of the hip joint to detect torso movement rather than a false signal caused by upper limb movement. As the two efforts were completed in a circular format, recuperation time of more than 3 minutes was provided. The measurement–re-measurement tests had a coefficient of variation of 3.6%.

Internal load
Internal load was measured in real time using a Polar Team Pro (Kempele, Finland). The variables recorded during SSGs included HRmax, HR_{max}% and Cardio load. It needs to be stressed that in the present study only the variables that resulted from the calculation of the GPS were evaluated as internal load.

External load
External load was measured using the Global Positioning System (GPS, 10 Hz Polar Team Pro, Kempele, Finland). The variables recorded were total distance (TD), distance/min (m/min), number of sprints (>25 km/h), distance covered in five speed zones (Distance Speed: z1: 0.10-6.99 km/h; z2: 7.00-10.99 km/h; z3: 11.00-14.99 km/h; z4: 15.00-18.99 km/h; z5: >25.00 km/h), the total number of decelerations (NoDec –5.00-3.00,–2.99-–2.00, –1.99-–1.00 m/s²) and the total number of accelerations (NoAcc 1.00-1.99, 2.00-2.99, 3.00-5.00 m/s²).

The structure of SSGs
Table 2 shows the number of SSGs, their duration, interval rest durations, relative pitch size, and pitch dimensions. Furthermore, there was excess of reserved soccer balls throughout the pitch to replace the ball, assuring the necessary playing time. The soccer players were free to drink water during their breaks.

Statistical analysis
The IBM SPSS software (Statistics for Windows, version 25.0 Armonk, NY: IBM Corp.) was used to analyze the data. The data was presented as the means and standard deviations using descriptive statistics. The Shapiro-Wilk test was applied to determine the normality of the distributions. When normality was discovered, repeated measures of variance analysis (GLM Repeated Measures ANOVA) were used, followed by the post-hoc Bonferroni test when a statistically significant difference was discovered. A non-parametric Friedman test was carried out in the case of a non-normal distribution. The Wilcoxon signed-rank test was applied if there was a statistically significant difference between the samples. The statistical significance level was set at $p < 0.05$.

Table 2. Pitch sizes used for small-sided games

SSG	W	R	Small (S)	Medium (M)	Large (L)
1v1	4 × 1′	1′	1/75 m² 10 × 15 m	1/100 m² 20 × 10 m	1/150 m² 20 × 15 m
2v2	4 × 2′	2′	1/75 m² 20 × 15 m	1/100 m² 27 × 15 m	1/150 m² 30 × 20 m
3v3	4 × 3′	3′	1/75 m² 25 × 18 m	1/100 m² 30 × 20 m	1/150 m² 36 × 25 m
4v4	4 × 4′	4′	1/75 m² 30 × 20 m	1/100 m² 35 × 25 m	1/150 m² 40 × 30 m

Note: W – work; R – rest

Additionally, mediation analysis declares how a prognostic variable is related to an outcome variable, indicating that the relationship between two variables is affected by a third variable called the mediator. Direct and indirect effects emerge from mediation analysis. A direct effect is defined as the relation between the predictor variable and the outcome variable, while an indirect effect is considered the effect of the predictor on the outcome through the mediator.

Mediation analysis was applied in this study (JASP 16) to examine whether the dimensions of the pitch (the predictor) directly and/or indirectly affect performance in dependent variables. The ratio of players was considered as a mediator. The bootstrapping procedure was used in order to examine the significance of the indirect effect. Indirect effects were computed for each of the 1000 bootstrapped samples. Correlation analysis was conducted at the initial stage to examine prerequisites for mediation analysis; only highly significantly correlated variables were included into the mediation analysis.

Results

Anthropometric characteristics and the results from the fitness tests of the 16 participants are presented in Table 1 above. The mean scores and standard deviations of the examined variables for all the pitch dimensions are presented in Table 3.

Table 3. Means and standard deviations of all dependent variables in all pitch dimensions in all players' formats

	75 m²			
	1v1	2v2	3v3	4v4
HR_{max}	187.17 (9.5)	178.4 (41.8)	163.2 (41.6)	179.4 (12.5)
HR_{max}%	94.2 (4.6)	89.6 (21)	81.8 (20)	90 (6.2)
Total distance	98.5 (17.3)	188.8 (52.9)	225.8 (85.3)	341.6 (89.6)
Distance/minute	94.1 (16.6)	93.8 (26.3)	74.8 (28.2)	85 (22.2)
Distance speed zone 1	39.6 (6.5)	71.4 (20.5)	115.2 (38.1)	160.2 (20.9)
Distance speed zone 2	30.5 (9.7)	60.9 (20.7)	58.6 (28.2)	96 (42.7)
Distance speed zone 3	17.6 (10.4)	39.5 (17.8)	37.8 (27.8)	60.5 (34.5)
Distance speed zone 4	10.7 (7.8)	16.9 (13)	13.9 (16)	24.9 (20.8)
Sprints	1.2 (1)	1.2 (1.1)	0.95 (0.94)	1.7 (1.5)
Number of decelerations (–5.00-–3.00)	0.64 (0.7)	0.91 (1.1)	0.38 (0.64)	1.15 (1.2)
Number of decelerations (–2.99-–2.00)	2.28 (1.5)	4.11 (2.2)	3.25 (2)	5.47 (3.1)
Number of decelerations (–1.99-–1.00)	5.56 (2.3)	11.25 (4.3)	12.7 (4.7)	19.4 (5.8)
Number of decelerations (–0.99-–0.50)	6 (2.2)	11.1 (4.6)	16.87 (5.7)	23.8 (6.1)
Number of accelerations (0.50-0.99)	5.75 (2.4)	10 (4.1)	16 (5.7)	20.52 (5.2)
Number of accelerations (1.00-1.99)	4.97 (1.7)	10.41 (3.9)	11.63 (4.8)	18.57 (6)
Number of accelerations (2.00-2.99)	2.19 (1.3)	4.53 (2.5)	3.83 (2.4)	6.83 (3.1)
Number of accelerations (3.00-5.00)	0.94 (0.88)	0.80 (0.9)	0.60 (0.7)	1.20 (1.1)
Training load	4.97 (1.3)	7.41 (2.6)	7.37 (2.5)	11.57 (2.5)
Cardio load	2.25 (0.9)	4.67 (1.8)	4.53 (1.8)	7.95 (2.3)
	100 m²			
HR_{max}	184.6 (14.5)	182.6 (14.6)	162.8 (45.6)	164.4 (46.8)
HR_{max}%	92.65 (7.9)	91.65 (7.2)	81.82 (22.9)	82.60 (23.4)
Total distance	87.88 (25.4)	190.7 (55)	218.7 (84)	303.1 (119)

Distance/minute	86.37 (24.6)	92.6 (26.5)	72.67 (27.9)	75.30 (29.7)
Distance speed zone 1	38.14 (8.7)	74.06 (16)	113.7 (37.5)	154.7 (54.5)
Distance speed zone 2	28.75 (13.2)	51.19 (19.6)	55.35 (30.4)	79.47 (40.6)
Distance speed zone 3	14.46 (12.1)	37.77 (17.5)	35.25 (24.6)	50.63 (36.5)
Distance speed zone 4	6.60 (8.2)	27.62 (20.1)	14.15 (15.8)	18.25 (20.1)
Sprints	0.66 (0.7)	1.48 (1.2)	0.87 (1)	1.38 (1.1)
Number of accelerations (–5.00-–3.00)	0.40 (0.55)	0.88 (0.90)	0.45 (0.62)	0.82 (0.89)
Number of accelerations (–2.99-–2.00)	2.03 (1.5)	3.58 (2)	3.37 (2.5)	4.33 (2.8)
Number of accelerations (–1.99-–1.00)	5.09 (2.2)	9.44 (3.6)	11.70 (4.6)	17.03 (7)
Number of accelerations (–0.99-–0.50)	5.91 (2.4)	10.96 (4.5)	16.88 (6)	22.83 (8.2)
Number of decelerations (0.50-0.99)	6.23 (2.6)	11.12 (4.1)	15.73 (5.9)	20.42 (6.8)
Number of decelerations (1.00-1.99)	5.25 (2.3)	8.25 (3.2)	11.05 (4.8)	16.35 (7.2)
Number of decelerations (2.00-2.99)	1.62 (1.3)	3.67 (1.7)	3.33 (2.2)	4.92 (2.9)
Number of decelerations (3.00-5.00)	0.46 (0.63)	1.06 (0.97)	0.50 (0.74)	0.95 (1)
Training load	4.37 (1.2)	6.37 (1.9)	7.45 (2.6)	10.23 (3.9)
Cardio load	2.02 (0.93)	3.92 (1.5)	4.98 (2.1)	7.05 (3.2)
150 m^2				
HR_{max}	186.2 (8.4)	188.1 (11.1)	185.7 (17.3)	189.5 (13.4)
HR_{max}%	93.52 (4)	94.45 (5.3)	93.28 (8.8)	95.17 (6.9)
Total distance	118.4 (20)	232.5 (37.7)	329.9 (71.4)	441.2 (82.3)
Distance/minute	116.4 (19.9)	114.1 (18.6)	104.5 (23.4)	109.9 (20.5)
Distance speed zone 1	35.17 (8.1)	72.92 (13)	126.45 (22.5)	158.3 (21.8)
Distance speed zone 2	38.08 (11.4)	67.62 (20.1)	91.28 (33.5)	125.70 (36.2)
Distance speed zone 3	25.07 (11.1)	54.80 (19.9)	68.70 (32.1)	98.98 (39.9)
Distance speed zone 4	20.02 (13.4)	36.63 (26.2)	40.02 (27)	57.66 (36.1)
Sprints	0.95 (0.85)	1.30 (1.1)	1.12 (1.1)	1.52 (1.2)
Number of accelerations (–5.00-–3.00)	0.77 (0.85)	0.90 (1.1)	1.08 (0.8)	1.42 (1.2)
Number of accelerations (–2.99-–2.00)	1.87 (1.2)	3.67 (1.7)	4.66 (2.6)	6.34 (2.8)
Number of accelerations (–1.99-–1.00)	5.02 (2.1)	10.88 (3.4)	14.50 (3.6)	18.73 (4.6)
Number of accelerations (–0.99-–0.50)	5.92 (2.1)	10.13 (2.8)	13.75 (4.3)	18.80 (4.6)
Number of decelerations (0.50-0.99)	5.12 (2.3)	9.85 (2.8)	14.67 (4.1)	19.6 (4.3)
Number of decelerations (1.00-1.99)	4.52 (2.1)	9.82 (3.2)	14 (4.3)	18.73 (5)
Number of decelerations (2.00-2.99)	2.05 (1.3)	4.38 (2)	4.77 (2.4)	6.44 (2.5)
Number of decelerations (3.00-5.00)	0.73 (0.07)	0.75 (0.08)	0.63 (0.08)	0.97 (0.09)
Training load	3.98 (1)	8.17 (1.6)	9.98 (3.3)	13.61 (3.9)
Cardio load	2.13 (0.05)	4.98 (1.4)	7.23 (2.7)	10.22 (3.1)

The above Table shows means and standard deviations of all variables assessed in the present study across all conditions – players' ratio and pitch dimensions.
Reviewing the results in detail, a significant difference emerged in the HR_{max} (F = 8,198, p < 0.001) and HR_{max}% (F = 8,222, p < 0.001) variables, with the dimensions of the pitch significantly affecting the maximum HR. The post-hoc Bonferroni test showed differences between the dimensions of the pitches. In particular, the 3v3 format at 75 m^2 resulted in significantly lower HR_{max} compared to 3v3 at 150 m^2 (I-J = –22,485, p < 0.001). Also, the 4v4 at 100 m^2 produced a significantly lower HR_{max}, compared to the 4v4 format at 150 m^2 (I-J = –25,144, p < 0.001).
For the HR_{max} and HR_{max}% variables no mediation analysis was performed, as the variables were not related to the independent variable, i.e. pitch dimensions (r = –0.029, p = 0.432; r = 0.028, p = 0.444 respectively).
The results showed differences for the total distance and distance/minute in each player variant (4v4, 3v3, 2v2, 1v1) between three different relative pitch sizes (150 m^2/player, 100 m^2/player, 75 m^2/ player). Also, differences were observed between SSG formats in the same pitch size. These two variables were correlated highly significantly (p < 0.001) with the independent variable defined as the predictor, i.e. pitch dimensions. The mediation analysis revealed significant effects both directly (for total distance: z = 9,794, p < 0.001; for distance/minute: z = 3,436, p < 0.001) – the dimensions of the pitch have a significant effect on the total distance travelled but also on the distance per minute – and indirectly (for total distance z = 11,538, p < 0.001; for distance/minute z = –4,954, p < 0.001) the ratio of players mediated in the dimensions of the pitches. It is noted that for the last zone (5-25 km/h) no significant difference was found, as probably very few players reached the said speed. Post-hoc Bonferroni analyses shed light on significant differences in the ratio of players within the same dimensions of the pitch. In particular, the 4v4 format resulted in a longer distance travelled compared to the other three ratios in all the examined pitch dimensions (p < 0.001).
Closely reviewing the comparisons within the Zones, it appeared that in Speed Zone 1 (0.10-6.99 km/h) a significant difference (F = 204,711, p < 0.001) was found between the ratio of players and within the same dimensions. Typically, the 4v4 ratio always had a significantly greater distribution distance compared to the other three player ratios. Significant differences (F = 67,444, p < 0.001) were also observed in Zone 2 (7.00-10.99 km/h), where the proportion of players on the pitch appear to significantly differ statistically. In the small pitch of 75 m^2 the ratio 1v1 had a shorter distance travelled compared to the ratio 2v2 (I-J = –31.812, p < 0.001), which had a shorter distance travelled than the ratio 3v3 (I-J = –43.794, p < 0.001), with the ratio 4v4 having a greater distance travelled compared to the 3v3 ratio (I-J = 45.067, p < 0.001). The previous result was confirmed for the other two dimensions of the pitches. Regarding the comparison based on pitch dimensions, significant differences were recorded only in the 3v3 and 4v4 ratios in 150 m^2, which resulted in a greater distance travelled than the corresponding ratios in 75 m^2 (I-J = 32,615, p < 0.001 and I-J = 29,703, p < 0.001, respectively). For the middle pitch (100 m^2) no significant differences were observed.
In Zone 3 (11.00-14.99 km/h) a difference was also observed between the ratio of players (F = 53.403, p < 0.001). In the 75 m^2 pitch the 1v1 variant had a shorter distance travelled compared to 2v2 (I-J = –21.891, p < 0.001), 3v3 (I-J = –20.225, p < 0.001) and 4v4 (I-J = –42.908, p < 0.001). Regarding pitch size comparisons there were significant differences in 3v3 ratios at 75 m^2 and 150 m^2 (I-J = –30.853, p < 0.001), while (I-J = –33.453, p < 0.001) for 100 m^2 compared to 150 m^2. For 4v4, it was compared to the smaller and large pitch (I-J = –38,451, p < 0.001) and (I-J = –48,35, p < 0.001) for medium and larger pitches. The same proportions of players in larger pitches recorded longer distances travelled.
For Zone 4 (15.00-24.99 km/h) significant differences (F = 32.494, p < 0.001) were recorded in the largest pitch, where the longest distances travelled for all the proportions of players were recorded; namely, 1v1 in the 150 m^2 pitch had a longer distance travelled compared to the 100 m^2 pitch, while the 2v2, 3v3 and 4v4 ratios in 150 m^2 had longer distances travelled compared to the same ratios in the 75 m^2 pitch. Regarding the in-pitch comparison, all the ratios differed significantly on the larger pitch, with 4v4 recording the longest distance travelled and 1v1 recording the shortest.
For the next dependent variable, i.e. sprints, it was observed that the independent variables – pitch dimensions and players' format – differentiate the mean scores of the measurements. However, due to the fact that the variable was not correlated with the independent variable of pitch dimensions (r = 0.048, p = 0.190) no mediation test could be performed.
In multiple post-hoc comparisons marginal differences were observed between the 3v3 and 4v4 ratios in the small pitch; namely, 4v4 displayed a higher number of sprints (I-J = 0.783, p = 0.008). In the middle pitch

(100 m^2), differences were recorded between 1v1 and 2v2 where the second ratio recorded more sprints (I-J = 0.819, $p = 0.006$). Additionally, there were differences between 1v1 compared to 4v4 (I-J = 0.722, $p = 0.021$) on the same pitch, with the latter displaying a higher number of sprints. It is emphasized that no significant difference was observed in the large pitch.

Regarding the dependent variable Decelerations, significant differences were observed ($p < 0.001$). In the mediation analysis that followed in order to investigate direct and indirect effects from the independent variables, significant direct effects of pitch dimensions were observed only at the two medium speeds (–2.99-–2.00, –1.99-–1.00 m/s^2) ($z = 2.138$, $p = 0.033$ and $z = 2.478$, $p = 0.013$, respectively), while indirect effects of pitch dimensions on decelerations were observed at all the four examined speeds (z = 3,021, $p = 0.003$ for –5.00-–3.00 m/s^2; z = 8,362, $p < 0.001$ for –2.99-–2.00 m/s^2; z = 11,507, $p < 0.001$ for –1.99-–1.00 m/s^2; z = 11,640, $p < 0.001$ for –0.99-–0.50 m/s^2).

In the Bonferroni multiple comparison test, speeds of –5.00-–3.00 m/s^2 resulted in more decelerations in the larger pitch compared to the other two, and only for the 3v3 and 4v4 ratios. In the next two –2.99-–2.00 m/s^2 and –1.99-–1.00 m/s^2, significant differences were observed within the same dimensions of the pitch between the proportions of the players. More specifically, 4v4 always displayed more decelerations compared to the other three proportions of players in all the pitch sizes. In the last zone –0.99-–0.50 m/s^2, there was also an increase in the number of decelerations as the number of players increased, with the 1v1 ratio recording the lowest number of decelerations in all the three pitch dimensions. It is worth noting at this point that comparing the dimensions of the pitches was also important. In detail, 3v3 and 4v4 on a 75 m^2 pitch displayed significantly more decelerations compared to 3v3 and 4v4 at 150 m^2 ($p < 0.001$).

To conclude, it seems that decelerations increase as the number of players increases and as the dimensions of the space decrease.

Regarding Accelerations, direct effects of space were observed in the first two speed zones (z = 4,293, $p < 0.001$ for 0.50-0.99 km/s^2 and z = 3,260, $p = 0.001$ for 1.00-1.99 m/s^2), while indirect effects of pitch dimensions occurred through the ratio of players in all the four speed zones.

Bonferroni post-hoc tests shed light mainly on differences between player ratios; the 1v1 variant resulted in fewer decelerations on all the three pitches compared to the other ratios, with 4v4 displaying the highest number of decelerations. The finding was confirmed in the other two speed zones (1.00-1.99 m/s^2, 2.00-2.99 m/s^2). However, specific variations were observed in the last speed zone (3.00-5.00 m/s^2). In particular, in the smaller pitch the ratio of 4v4 differed from the ratio of 3v3, while the medium (100 m^2) displayed the greatest differences. 4v4 differed from all other player ratios. In the smaller pitch 4v4 differed only from 3v3, while no differentiation occurred in the larger pitch.

The dependent variables training load and cardio load appeared to differ in the various measurements ($F = 80.088$, $F = 91.161$, respectively, with statistical significance $p < 0.001$). The mediation analysis showed direct significant effects of the predictor on both variables ($p < 0.001$) and indirect effects in the case of the player ratio ($p < 0.001$). In the Bonferroni post-hoc test significant differences were recorded in comparisons between the pitches as well as between the player ratios.

In detail, in the training load variable differences were observed for the player ratios within the pitch variant. The 4v4 ratio displayed the greatest load in all the pitch dimensions compared to the other proportions, with a lower load for the 1v1 ratio. In the proportions with more players, 3v3 and 4v4, differences were observed relative to the pitch dimensions. The 3v3 and 4v4 format in the larger 150 m^2 pitch had a higher load compared to the other two pitches. No significant differences were observed for the smaller proportions.

In the cardio load variable, differences also emerged between the pitches and the proportions of the players. In particular, the highest proportion of players recorded a higher cardio load compared to the other three ratios in all the pitches, with the 1v1 ratio recording the lowest heart load in all the pitch dimension variants. The proportions with a larger number of players, 3v3 and 4v4 on the

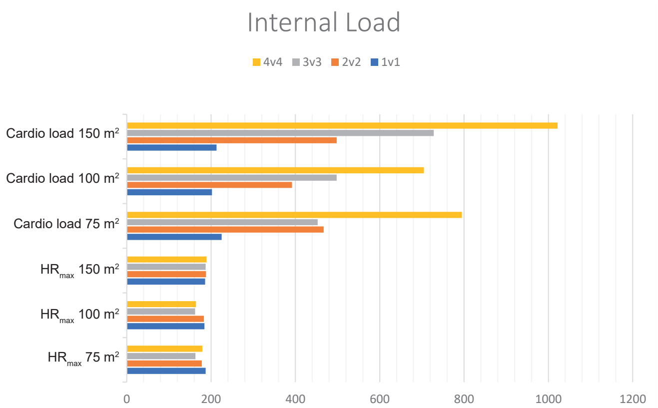

Figure 1. Internal load – cardio load, heart rate – on all pitch dimension variants for all players' formats

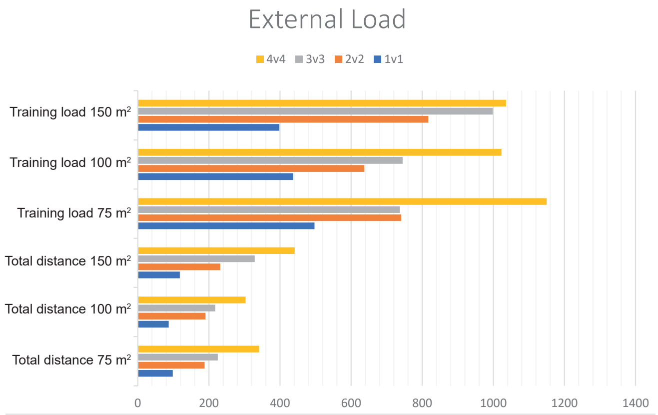

Figure 2. External load – training load, total distance – on all pitch dimension variants in all players' formats

150 m^2 pitch displayed a higher heart load compared to the two smaller pitches. Figure 1 displays the internal load (heart rate and cardio load) as recorded on all the pitches and in all the player ratios, while Figure 2 shows the external load (total distance and training load) in all the dimensions and in all the player proportions.

Discussion

The aim of the present study was to investigate the internal and external load on semi-professional soccer players during training in different pitch dimensions (75 m^2, 100 m^2, 150 m^2) and different player ratios of 1v1, 2v2, 3v3, 4v4. For the internal load the variables calculated were the HR(specifically HR_{max} and HR_{max}%) and the heart load (cardio load). It is noted that the RPE was not evaluated in this study, as only objective assessments were used from the application of GPS. On the other hand, for the external load total distance, distance/minute, accelerations, decelerations, sprints and training load were calculated. The analyses shed light on different relations between the size of the pitches and the proportion of players having a different effect on the load experienced by soccer players. Important results are discussed in aggregate for internal and external load, respectively.

Internal load

The analyses conducted for the internal load showed that it is significantly affected by the dimensions of the pitch. The 150 m^2 pitch contributed to a greater internal load. In fact, in the cardio load variable the effect of the pitch dimension is immediate. This finding was not confirmed for the maximum HR. Regarding the player ratios, heart load appeared to have a significant indirect effect and to vary based on them. Typically, 3v3 and 4v4 recorded a greater heart load compared to 1v1 and 2v2. On the other hand, HR only varies for the large proportions of 3v3 and 4v4 players and only for the large and small 75 m^2 pitch compared to 150 m^2. It was already suggested by previous studies that the increase of pitch dimensions with a constant number of players can lead to an increase in HR [2, 11, 24]. Furthermore, in a study carried out on amateur players researchers observed higher values of % HR_{max} during SSGs with larger dimensions [21]. This is probably due to the fact that players have to cover longer distances with greater intensity [24]. In particular, players covered more distance from defense to attack [25]. The above findings are confirmed by a study by Casamichana and Castellano [2], which shows that reducing the dimensions of the pitch decreases the intensity of SSGs. In addition, it has been suggested that the increase in the number of players during SSGs provides more recovery time due to decreased active participation in the game. It was evident that during smaller sized matches, soccer players reached higher HR values [9]. On the other hand, the training load was shown to be both directly influenced by the dimensions of the pitch and indirectly by the proportion of players. In conclusion, SSGs with a larger number of players have a greater training load, as do those in a larger pitch dimension.

External load

The factors that constitute the external load – total distance and distance/minute – seemed to be influenced both by the larger dimensions of the pitch and by the larger proportions of players. An increase in the distance that needed to be covered was shown with an increase in the relative pitch size during all the game formats. This probably occurred due to the fact that the soccer players had the opportunity to cover longer distance/min as a result of greater available space [2].
After analyzing the data of five speed zones during the 4v4, 3v3, 2v2 and 1v1 formats with three different relative pitch sizes, it appeared that the ratio of players seemed to affect the distance travelled per minute, with the 4v4 format covering longer distances. In the first zone the dimensions of the pitch had no effect on the dependent variable. In speed zones 2 and 3 there was an increase in the covered distance with an increase of relative pitch size and an increase in the players' format. Greater covered distances were observed in 150 m^2 with more players. In speed zone 4 the covered distance was greater with a relative pitch size of 150 m^2/player compared with that of 75 m^2/player during the 3v3 and 4v4 format. In addition, there is a greater borrowed

distance on the pitches with more players. The 4v4 format consistently recorded the longest distances travelled compared to the other player ratios, with the 1v1 format recording the shortest. Similar results on the effect of pitch dimensions on speed zones had already been discussed by other researchers [28].

As far as the number of Sprints is concerned, what seemed to have an indirect effect is the proportion of players while there was no significant difference in the size of the pitches. The finding so far contradicts the existing literature, which claims that the number of Sprints is influenced by the size of the pitch [9].

Regarding the total number of decelerations, a greater number of decelerations (–5.00-–3.99 m/s^2) was recorded in the 3v3 format on a 150 m^2 pitch compared to the smaller 75 m^2 pitch, as well as in the 4v4 format on a 150 m^2 pitch compared to a 100 m^2 pitch. Therefore, it is evident that the number of decelerations is connected with an increase in available space [11]. Concerning the total number of decelerations (–3.00-–2.99 m/s^2), it appeared that the highest proportion of players recorded more decelerations with a greater difference in the larger 150 m^2 pitch. In the next zone (–2.00-–1.99 m/s^2) it was clearly demonstrated that the ratio of players affects the number of decelerations, with the least decelerations recorded in the 1v1 format and the most in the 4v4. In the last zone (–1.00-–0.99 m/s^2) it was also shown that the smaller proportion of players recorded a lower number of decelerations. Additionally, significant differences occurred in the 3v3 and 4v4 format at 75 m^2 compared to 150 m^2, where smaller pitches recorded a higher number of decelerations.

Regarding the results recorded on acceleration in the first three zones (0.50-0.99, 1.00-1.99, 2.00-2.99 m/s^2), there was a significant variation within the same pitch dimensions regarding the ratio of players. The 4v4 format consistently recorded the highest accelerations and the 1v1 recorded the lowest. The size of the pitches did not seem to make any difference in the number of accelerations. In the last zone (3.00-5.00 m/s^2) differences were recorded only in the 100 m^2 pitch. In particular, the 4v4 format recorded a higher number of accelerations compared to 1v1 and 3v3. The 2v2 format recorded more accelerations compared to 1v1. In the small pitch only the 4v4 format recorded more accelerations compared to the 3v3 format, while no significant differentiation was recorded in the large 150 m^2 pitch. Regarding the literature review, while there are some studies that typically evaluate the accelerations of athletes in team sports, they do not present results regarding the effect of the numerical ratio on them [18].

Conclusions

In summary, the purpose of this study was to investigate the importance of the players' ratio and the pitch dimensions for the internal and external burden experienced by semi-professional players during football training via small-sided games. What has emerged from the statistical analyses is that the higher proportions of players have a significant impact on the cardio load, the training load and the total distance. In contrast, the smaller pitches have a significant impact on the number of decelerations and accelerations, while they also affect the total number of sprints. The results of the study could by applied in the football training plan of semi-professional teams, with the aim of maximizing training results.

Limitations of the study and further research

A limitation of the study may be connected with the sample size; thus, in a future study data could be obtained from more football teams from the same division. In addition, it is important to evaluate other load factors such as lactic acid and subjective fatigue. The study could be carried out in another time phase, for example at the beginning of the league in order to set light on possible changes compared to the end of the season results. Moreover, it would be important in a subsequent design of the study to predict and evaluate the mental and psychological impact of football players in SSG. Furthermore, it would be important in further research to adopt the theoretical framework of reserve HR as a formula for an inter-individual comparison between football players.

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Conflict of Interest

The authors declare no conflict of interest.

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Effect of dual-task training on motor-cognitive interference among older women: implication for postural control during sit to stand in different visual conditions

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Abstract
Introduction. **Postural control difficulty during sit to stand (STS) is a common and costly problem in older adults. A potentially important strategy to enhance postural control through exercise intervention is to add cognitive components.** Aim of Study. **To examine the effect of STS dual-task training on postural control during STS, in eyes open (EO) and closed (EC) conditions in older women.** Material and Methods. **A total of 20 participants were randomly allocated into dual task (STS training and simultaneous dual-task) (n = 10) and single task (only STS training) (n = 10).** Results. **Significant differences were observed pre to post in dual-task training for velocity in mediolateral (ML) (p < 0.05) in EO condition, anteroposterior (AP) (p = 0.009), ML amplitude (p = 0.005), and AP velocity (p = 0.007) in the EC condition.** Conclusions. **These findings suggest that dual-task training is an effective at improving postural control of older people with history of falling during STS.**

KEYWORDS: aging, vision, sit to stand, dual task, postural control, motor-cognitive interference.

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Introduction

It has been reported that adults aged over 65 years will experience falling at least once a year [2] especially when they are required to perform a concurrent cognitive or secondary motor task while performing daily activities. Falling can be associated with age-related muscle weakness, impaired balance and gait and poor postural control [7, 13]. Postural control requires integrating proprioceptive afferent inputs and complex sensorimotor actions as well as cognitive regulations [10, 11]. Effective daily functioning requires people to share their attention resources between the cognitive and the postural requirements necessary to complete the tasks. Due to the aging process and prevalence of chronic diseases, older adults show some levels of decline when performing postural tasks while dual-tasking [3]. Studies have shown that the effect of cognition on postural control increases with aging and is a key characteristic of mobility problems in this population [4, 17, 26]. In situations such as making telephone calls while walking the interference between cognitive and motor tasks is greater in older adults compared with young healthy adults.

Although the ability to prioritize and allocate attention between two or more tasks becomes progressively compromised throughout aging, dual-task training has the potential to improve the ability of older adults to share attention between motor and cognitive tasks [1, 5, 15, 19, 20, 23, 25]. Motor and cognitive tasks can be simultaneously performed – when a concurrent attentional focus is required for both activities.

Transition from siting to standing is one of main components of daily living tasks, which requires appropriate postural adjustments to maintain stability in response to the internal and external perturbation. Thus, the impaired ability of older adults to allocate attention during the dual-task in sit to stand (STS) movement may increase the risk of falling. In dual-task training protocols, participants are usually asked to sometimes focus attention on motor and at times on cognitive task performance. However, it is important to highlight that a large part of the daily motor and cognitive tasks is simultaneously performed, especially during activities that require maintaining body balance in domestic activities, and in activities such as sit to standing and talking or solving mathematical calculations [1, 5, 15, 19, 20, 23, 25, 27]. Although the literature offers some support for benefits of dual-task training, an optimal training method for dual-task abilities in some functional situations such as STS is yet to be determined.

Yet little is known which practice strategies are most effective in improving concurrent performance of postural and cognitive tasks during the STS maneuver. To our knowledge, there is no study reporting the effects of dual-task training as a treatment modality on the clinical findings of older people with history of falling. Thus, it seems reasonable to propose a training protocol, in which participants sometimes perform a simultaneous dual task, such as STS and performing mathematical operations.

Additionally, postural control involves the interaction of the visual, vestibular and somatosensory systems, but it is more influenced by the visual system than by other systems [14]. Although research results imply the role of vision in setting motor responses during postural control, some studies have shown that eliminating visual information in eye-closed (EC) situations improves visuospatial mechanisms of postural control and provides better cognitive performance compared to eyes-open (EO) situations in darkness during postural control [3, 6, 8, 9, 14]. However, little is known about the role of visual sensory information in the influence of dual-task training on the interference between a cognitive task and postural control during STS movement.

Aim of Study

The aim of the present study was to investigate whether a dual-task STS training intervention is more effective than single-task STS training for improving postural control during STS under dual-task conditions under EO and EC conditions in community-dwelling older women.

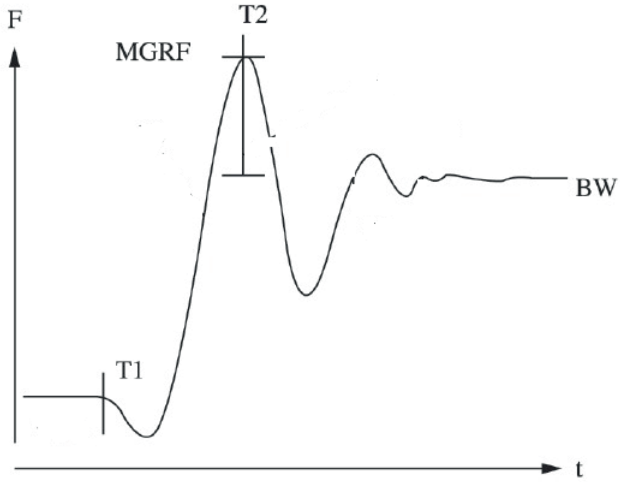
Material and Methods

A total of 20 elderly females were recruited in the local community according to the following inclusion criteria and they were randomly assigned to the dual-task (n = 10) and single-task (n = 10) STS training: being 65 years or older, having a history of at least one fall in the last three months, having efficient cognitive function to be able to communicate (a Mini-Mental State Examination score of 23 points and above) [9], walking independently 10 m, and being able to stand on both feet for at least 90 s without assistance. The exclusion criteria were having a severe hearing loss and/or visual impairment, uncontrolled hypertension, and vertigo. Exclusion criteria included the presence of a previous cognitive impairment or aphasia (determined by a clinical evaluation with a physiatrist), psychological disorder, an uncontrolled medical disease, or significant orthopedic pain or pain that limited participation in postural control testing. The protocol was reviewed and approved by the Institutional Review Board and Research Ethics Committee of the Ferdowsi University of Mashhad. All the participants provided their written informed consent before the selection procedure.

This study was a single-blind, randomized controlled trial, in which the participants were not aware of group type. Both groups received general physical programs (range of motion, strengthening, mat, and mobility exercises) and additionally STS exercises for 30 minutes per day, 5 days per week, for 4 weeks. All the participants were administered both general and STS training of the same amount and duration. The duration and intensity of this training were chosen based on previous studies. The participants in the single-task STS training group received activities under single-task conditions (only STS tasks were given). The participants receiving dual-task training practiced sit to standing tasks while simultaneously performing cognitive tasks, and they were instructed to maintain attention on both postural and cognitive tasks at all times. Examples of cognitive tasks included naming objects, counting n-back and remembering numbers.

Demographic characteristics (e.g., age, weight, dynamic balance ability, years of education, and employment status) were collected at the baseline. To evaluate the dynamic balance ability a test tool for the balance, the Berg Balance Test, was used at pre-test. It comprises a 5-point scale (0-4) composed of 14 items with a total score of 56 [3]. Postural control during a STS task was assessed before and after the 4 weeks of the training program while participants stood under EO and EC conditions. After explaining the sitting posture and movement pattern for the STS movement, the participants sat in an adjustable chair with legs shoulder-width apart, the trunk stretched vertically in a straight line and their hips, knees, and ankles held at 90° while the feet were on the force platform (Kistler Instrument Inc.) with an acquisition frequency of 100 Hz [8]. For all the tests the participants were instructed to stand upright from a seated position at a self-selected speed while their gaze was fixed in the normal plane of vision during the STS maneuver, they rested for approximately 2 seconds, and then sat down again. They performed three trials in succession with an interval of 2 seconds. The force platform provided a curve of vertical ground reaction force during the STS movement at 100 Hz for further analysis.

All the data were checked, amplified, and filtered before analysis using a digital Butterworth fourth-order low-pass filter with a 5 Hz cut-off frequency [16] using the MATLAB software (Mathworks Inc., Natick, MA, USA). Data normalization was performed using the body weight values. Analysis of data was performed for the preparation phase (F1), the beginning was determined by a decrease in vertical force greater than 2.5% relative to the weight of the feet on the platform, while the end was determined by the vertical peak force. Details of the division of STS movement into phases are presented in Figure 1. For this phase, the variables related to the center of pressure (COP) were calculated according to Duarte and Freitas's research [8]: i.e., the anteroposterior COP displacement amplitude (Amp AP), the mediolateral COP displacement amplitude (Amp ML), and the mean COP oscillation velocity (Vel AP, Vel ML).



Note: Preparation phase (T1-T2), BW – body weight, MGRF – maximum ground reaction force, T1 – start of movement, T2 – seat-off

Figure 1. The schematic representation of the Ground Reaction Force during STS movement

Outcome measures were first investigated applying descriptive and comparative statistical analyses. For comparison within and between the groups the data were analyzed by repeated measures ANOVA, the Mann–Whitney U-test and Student's-t test at $\alpha \leq 0.05$ according to the type and distribution of the recorded variables. All statistical tests were performed using the SPSS software version 25 for PC (IBM Corporation, Armonk, NY). A P value of < 0.05 was considered to be significant.

Results

Physical and demographic characteristics of the participants are given in Table 1. There were no statistical differences between the educational levels and the physical characteristics of the elderly in both groups. The mean and standard deviations for the all postural control variables of COP under the two vision conditions are shown in Tables 2 to 5. No significant changes in the amplitude and velocity of COP during the STS maneuvers were found between the EO and EC conditions before and after training. However, dual-task training showed significant changes in the Amp AP (U = 19.0; p = 0.005), Amp ML (U = 11.0; p = 0.001), Vel AP (U = 3.27; p = 0.02) and Vel ML (U = 2.61; p = 0.04) in the EO conditions (11.60 ± 0.62 vs 5.69 ± 0.81; p < 0.05) as well as (10.22 ± 0.74 vs 8.39 ± 0.93; p < 0.05). In the case of the dual task lower values were recorded for Vel ML compared with the control group (U = 2.99; p = 0.015). Additionally, significant differences were observed for pre- to post-test in dual-task training for the Amp AP (U = 4.1; p = 0.009), Amp ML (U = 4.7; p = 0.005), Vel AP (U = 4.4; p = 0.007) in EC condition. Significant differences in the amplitude and velocity of COP during the STS maneuver were found between the groups in the EC condition after training.

Table 1. Means ± SD for baseline demographic and clinical characteristics by groups

Characteristics	Single-task STS training (n = 10)	Dual-task STS training (n = 10)	P
Age	73.50 ± 0.93	74.20 ± 0.52	0.79
Women (n)	10	10	
Number of falls (previous year)	1.53 ± 1.51	1.15 ± 1.60	0.70
BBS (0-56)	50.00 ± 4.50	51.44 ± 3.61	0.53
Mini-Mental State Examination (0-30)	18.40 ± 1.68	23.5 ± 3.20	0.10

Table 2. Comparison between groups after intervention for amplitude of COP changes in ML and AP direction with EO

Variable	Group	Period	Paired t-test			Independent t-test		
			Means and standard deviation	Statistics	Sig.	Mean rating difference	Statistics	Sig.
Amplitude of COP changes in the AP direction with EO (mm/s)	Single-task STS training	pre-test	2.99 ± 0.9	–0/85	0/443	–0/8 ± 2/31 1/68 ± 4/07	2/64	0/27
		post-test	3.87 ± 0.6					
	Dual-task STS training	pre-test	3.78 ± 0.4	–5/91	0/2			
		post-test	7.85 ± 0.8					
Amplitude of COP changes in ML direction with EO (mm/s)	Single-task STS training	pre-test	5.75 ± 0.65	–3.26	0.31	–5.08 ± 0.48 –10.66 ± 0.49	2.25	0.49
		post-test	10.83 ± 0.8					
	Dual-task STS training	pre-test	7.15 ± 0.31	–5.80	0.20			
		post-test	17.81 ± 0.51					

*Significance level less than 0.05
Note: COP – center of pressure, ML mediolateral, AP – anteroposterior, EC – eyes-closed, EO – eyes-open, STS – sit to stand

Table 3. Comparison between groups after intervention for velocity of COP changes in ML and AP directions with EO

Variable	Group	period	Paired t-test			Independent t-test		
			Means and standard deviation	Statistics	Sig.	Mean rating difference	Statistics	Sig.
Velocity of COP changes in the AP direction with the EO (mm/s)	Single-task STS training	pre-test	10.00 ± 0.62	1.62	0.18	4.30 ± 0.94 9.18 ± 0.87	–1.24	0.24
		post-test	5.69 ± 0.81					
	Dual-task STS training	pre-test	17.58 ± 0.22	3.27	0.02*			
		post-test	8.39 ± 0.93					
Velocity of COP changes in the ML direction with the EO (mm/s)	Single-task STS training	pre-test	11.23 ± 0.46	–0.77	0.15	–7.88 ± 0.62 11.89 ± 0.89	–2.99	0.01*
		post-test	25.20 ± 0.47					
	Dual-task STS training	pre-test	25.85 ± 0.85	2.61	0.04*			
		post-test	13.16 ± 0.55					

*Significance level less than 0.05
Note: COP – center of pressure, ML mediolateral, AP – anteroposterior, EC – eyes-closed, EO – eyes-open, STS – sit to stand

Table 4. Comparison between groups after intervention for amplitude of COP changes in ML and AP directions with EC

Variable	Group	period	Paired t-test			Independent t-test		
			Means and standard deviation	Statistics	Sig.	Mean rating difference	Statistics	Sig.
Amplitude of COP changes in AP direction with EC (mm/s)	Single-task STS training	pre-test	6.80 ± 0.10	0.89	0.42	2.60 ± 0.47 2.44 ± 0.59	0.05	0.95
		post-test	4.20 ± 0.72					
	Dual-task STS training	pre-test	6.16 ± 0.92	4.10	0.009*			
		post-test	3.72 ± 0.80					

Amplitude of COP changes in ML direction with EC (mm/s)	Single-task STS training	pre-test	11.88 ± 0.77	–0.21	0.83	–1.65 ± 0.05 5.93 ± 0.04	0.60	0.55
		post-test	13.53 ± 0.71					
	Dual-task STS training	pre-test	9.57 ± 0.08	–4.77	0.005*			
		post-test	15.49 ± 0.78					

*Significance level less than 0.05
Note: COP – center of pressure, ML mediolateral, AP – anteroposterior, EC – eyes-closed, EO – eyes-open, STS – sit to stand

Table 5. Comparison between groups after intervention for velocity of COP changes in ML and AP directions with EC

Variable	Group	period	Paired t-test			Independent t-test		
			Means and standard deviation	Statistics	Sig.	Mean rating difference	Statistics	Sig.
Velocity of COP changes in the AP direction with EC (mm/s)	Single-task STS training	pre-test	11.34 ± 0.63	0.69	0.52	1.21 ± 0.92 4.99 ± 0.76	–1.87	0.09
		post-test	9.92 ± 0.23					
	Dual-task STS training	pre-test	13.06 ± 0.65	4.42	0.007*			
		post-test	8.06 ± 0.44					
Velocity of COP changes in ML direction with EC (mm/s)	Single-task STS training	pre-test	21.76 ± 0.20	–0.98	0.38	–4.58 ± 0.45 –9.25 ± 0.65	–0.59	0.56
		post-test	26.35 ± 0.85					
	Dual-task STS training	pre-test	21.38 ± 0.73	–1.54	0.18			
		post-test	30.99 ± 0.68					

*Significance level less than 0.05
Note: COP – center of pressure, ML mediolateral, AP – anteroposterior, EC – eyes-closed, EO – eyes-open, STS – sit to stand

Discussion

The aim of the present study was to investigate whether a dual-task STS training intervention would improve postural control during the STS maneuver to a higher extent when compared to single-task STS training in the EO and EC conditions. With respect to the results, during the STS maneuver the Amp ML, Vel AP and Vel ML in the EO condition were decreased (11.60 ± 0.62 vs 5.6 9 ± 0.81) as well as (10.22 ± 0.74 vs 8.39 ± 0.93). In the dual task lower values were found for Vel ML compared with the single task group under the EO conditions, which was consistent with those reported in other studies. They reported a significant improvement in postural stability of healthy older people [22]. Araújo et al. [1], in a systematic review of the dual-task effect, including seven studies of 194 participants, suggested evidence for the positive impact of combining balance training for enhancing postural control of the elderly population at the risk of falling. Hiyamizu et al., incorporated the Stroop task in a dual-task training duration of two sessions per week for 3 months [12]; however, Li et al. used an n-back counting task with a training duration spread [18]. This finding suggests that older adults are able to enhance their STS postural control under a concurrent motor-cognitive task only after specific STS training. A dual task acutely directs the attention toward an external source of attention (e.g., n-back, random letter generation tasks), while performing a primary task. According to the constrained action hypothesis, this attentional change might allow the motor systems to function in an automatic manner, resulting in more effective performance. It is, therefore, not surprising that repetitions of STS maneuvers concurrently with a cognitive task had a positive effect on postural sway outcome measures over time. It appears that for older individuals with a history of falling, the practice would lead to a lesser attention demand of a task. Variables such as COP velocity and amplitude are the most sensitive parameters for the diagnosis of the postural control deficit [24]. With respect to results there is no significant baseline difference between the dual- and single-task training group. While subjects in the dual task group decreased postural sway after intervention, for the control group the same values were found. Considering the present findings, it appears that the activities (balance cognitive tasks performed by the participants in the dual-task training groups) were much more difficult than the single tasks given to the participants in the ST group. Therefore, the postural control of the participants in the dual-task training groups were continually challenged and this may have resulted in reduced postural sways during STS maneuvers. In addition, some studies have also suggested that dual-task training may act as a cognitive therapy for patients with attentional deficits, because certain centers of the brain associated with dual-task processing showed less activation post-training and reduced processing demands [12, 25].

Although evidence suggests that cognitive-motor training compared to single-task interventions offers greater benefits to older adults with respect to the risk of falling [23], little is known about the effect of specific dual-task STS training. Neither studies considered a training program that prioritized STS activities combined with cognitive tasks. Taking into account that in daily living activities sit to stand maneuvers take place concomitantly with talking on the cell phone, thinking about a shopping list, etc., it is important to develop protocols that combine dual-task training to improve postural control. During the STS maneuver the Amp AP, Amp ML, and Vel AP decreased from the pre- to post-test period under the EC condition after dual-task training. Based on previous studies, vision is more important for postural control than the vestibular and proprioceptive senses in healthy individuals [6, 21]; therefore, body sway increases when vision is interrupted [15]. It seems that dual-task training enhances the proprioceptive function that compensates for interrupted vision, improving the balance ability of older adults with a history of falling. However, there was no significant change in postural sways between the EO and EC conditions, both pre and post training.

Conclusions

These findings demonstrated that dual-task training improves the proprioceptive function that compensates for interrupted vision, improving the balance ability of older adults with a risk of falling or a history of falling. Therefore, our findings suggest that dual-task training focused on balance control and the cognitive function improved postural control during sit to standing maneuvers. The results of the current study revealed that dual-task training may increase the effectiveness of STS exercise by enabling more sensory inputs during the exercise in older adults with a history of falling. Cognitive dual-task training can be applied easily and simply without the burden of time and cost, so it can be effectively used as a rehabilitation aid for older adults in clinical practice.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Table 1. Descriptive statistics and comparative analysis of maximal oxygen uptake (VO_2max in ml/kg·min^{-1}) between genotypes of the I/D *UCP2* gene polymorphism

UCP2		DD					ID					II			
Sex	*N*	$\bar{x}$	*SD*	*Min*	*Max*	*N*	$\bar{x}$	*SD*	*Min*	*Max*	*N*	$\bar{x}$	*SD*	*Min*	*Max*
F	42	45.65	*6.14*	32.30	59.00	36	45.66	*7.18*	30.60	59.80	7	45.07	*7.60*	35.00	54.80
M	72	54.01[a]	*6.20*	40.30	79.00	70	55.60	*7.32*	42.30	76.80	12	59.07[a]	*9.04*	49.70	74.90

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