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CONTENTS

ORIGINAL ARTICLE

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Muscle fibre type, size and satellite cell pool in male volleyball players

DIMITRIOS G. BALASAS[1], ATHANASIOS MANDROUKAS[1], YIANNIS MICHAILIDIS[1], SOTIRIOS DRIKOS[2], KONSTANTINOS SOTIROPOULOS[2], KOSMAS CHRISTOULAS[1], THOMAS I. METAXAS[1], THEODOROS M. BAMPOURAS[3]

Abstract
Introduction. **Longitudinal volleyball training stimuli can cause an increase in muscle strength that is brought about by neurological and morphological adaptations, such as changes in muscle fibre composition percentage and cross-sectional area (CSA).** Aim of Study. **The aim of this study was to examine the biological adaptations of volleyball-players in terms of muscle fiber type composition, cross-sectional area, myonuclei and satellite cell pool in comparison to physically active controls.** Material and Methods. **Ten professional volleyball-players (VG) and five physically active-persons (CG) participated in this study. Muscle biopsies were obtained from the vastus-lateralis of the dominant leg.** Results. **Immunohistochemical analysis revealed that although MHC I and MHC IIC muscle fibre distribution was not different between the groups, MHC IIX and MHC IIAX were totally absent in VG and appeared only in the CG. The cross-sectional area revealed a slightly different pattern as both MHC I and IIA were larger for the volleyball players. In accordance, MHC II myonuclei number was moderately larger in the volleyball players, while the satellite cells and their ratio to number of fibres had a large and very large difference, respectively.** Conclusions. **In conclusion, our study reveals that volleyball training-induced hypertrophy for both type I and II muscle fibres in the vastus lateralis of volleyball players and resulted in a specific shift in muscle fibres containing MHC II isoforms. This hypertrophy of the muscle fibres is associated with an increase in the myonuclear number and satellite cells.**

KEYWORDS: volleyball, muscle fibre, cross-sectional area, satellite cells.

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Introduction

Team sports, such as volleyball, require not only high-level technique in all aspects of the game, but also efforts characterised by maximum speed, strength and power, which determine most of the winning-point phases of the match [22]. It has been shown previously that increased jumping height is a performance indicator for high-level volleyball players [30] and a distinguishing variable among players of different levels [11] due to its importance in performing specific game tasks, such as block, spike [25] and jump serve [19, 29].

In order to enhance performance of these tasks, strength and/or plyometric training have always complemented volleyball training. Such modes of longitudinal training stimuli can cause an increase in muscle strength that is brought about by neurological and morphological adaptations, such as changes in muscle fibre composition percentage and cross-sectional area (CSA) [10, 20]. Indeed, the differences in muscle fibre composition percentage and CSA characteristics can determine muscle performance. A high content of mitochondria and an increased number of capillaries characterise slow-twitch fibres (type I) which exhibit long excitation time and low maximum shortening velocity. On the other hand, fast-twitch fibres (type II) are characterised by a high ATPase activity, high content of creatine phosphate and glycogen, and consequently short excitation time and high maximum shortening velocity [7, 8]. Therefore, the force-velocity curve of type II fibres shows a less steep decrease with increasing velocity compared to type I fibres and higher maximum mechanical power [8, 12].

In line with the above, it has been reported that 19 weeks of heavy resistance training caused a decrease in the percentage of type IIB (IIX, as presented by Smerdu et al. [28]), and a concomitant increase in the percentage of type IIA fibres, suggesting that heavy resistance training affects MHC composition in skeletal muscle, mainly showing adaptations in genetic expression [1]. In contrast, type I fibres seemed not to be affected by strength training, as no changes in the distribution of these type of fibres were observed after strength training [3].

It has been well established that increases in CSA occur after strength training [10]. The duration of training seems to create a specific response, as 12 weeks of strength training increased fibre CSA for both type I (slow) and II (fast) muscle fibres [23], which seems to be a common finding in "longitudinal" training studies [10]. In contrast, training for 6-10 weeks caused a preferential hypertrophy of type II fibres specifically [10].

In terms of myonuclear adaptation, Kadi and Thornell [18] reported that 10 weeks of resistance training resulted in an increase in myonuclear and satellite cell numbers in the trapezius muscle, concluding that the additional myonuclei seem to assist the enlargement of skeletal muscle fibres. The same lead author, in a comparison between high-level powerlifters and untrained subjects, revealed that the number of satellite cells as well as myonuclear numbers were significantly higher in athletes compared to the controls [15, 16].

Despite the popularity of volleyball worldwide only a few studies have been published concerning muscle characteristics of volleyball players. For example, Sleivert et al. [27] reported a type II muscle fibre size difference between volleyball players (and middle-distance runners) in comparison to untrained subjects. As volleyball requires explosive strength and power [4] it seems that a higher proportion of type II fibres, with an increased CSA of type II fibres and an increased myonuclear type II number and satellite cell pool, would possibly match the morphological and immunohistochemical profile of long-term volleyball training. Thus, this study aimed to track the biological adaptations of volleyball players in terms of muscle fibre type composition, CSA, myonuclei and satellite cell pool in comparison to physically active controls.

Material and Methods

Participants

Ten volleyball players (age 26.5 ± 4.6 years, height 1.88 ± ± 0.05 m, body mass 86.7 ± 9.0 kg, 12.6 ± 4.8 years of volleyball training), free from injuries or any medication that could affect the study, volunteered to participate in this study. All were professionals competing at the second highest Greek League (A2) and trained every day with volleyball-specific training and twice a week with resistance training. Measurements were taken at the in-season period, two weeks before the league's Christmas break. Additionally, five physically active participants (age 21.3 ± 1.0 years, height 1.80 ± 0.03 m, body mass 80.1 ± 9.4 kg), defined as taking part in at least 150 minutes of moderate-intensity exercise per week were recruited from the student population to serve as controls. The local ethical committee of the Department of Physical Education and Sports Science approved the study in accordance with the ethical standards in sport and exercise research and the Declaration of Helsinki. All the subjects were informed of the procedures, possible risks and benefits and provided their written consent before participation in the study.

Muscle biopsies

A physician using Weil-Blakesley's choncotom technique obtained muscle biopsies from the middle portion of the vastus lateralis muscle of the dominant leg. Prior to the procedure local anesthesia was applied in the skin penetrating the underlying fascia. The muscle tissue was embedded in an embedding medium (Jung Tissue Freezing) and immediately frozen in isopentane, pre-cooled in liquid nitrogen and stored at –80°C until analysed. On average, 440 ± 186 muscle fibres were classified in each sample of each participant.

Immunohistochemistry

Serial transverse sections of 5-7 μm in thickness were cut using a microtome at –22°C and mounted on glass slides. Muscle biopsies were air dried, rinsed for 20 min in phosphate buffered saline (PBS) and incubated for 20 min with diluted normal horse serum. Sections were incubated overnight at +4°C with the primary monoclonal antibodies (mAbs) diluted in bovine serum albumin (BSA). The day after, the slides were washed in PBS for 20 min and incubated for 1 hour with the diluted biotinylated horse antimouse secondary antibody (Vector BA-9200, Burlingame, California). The slides were then washed for 20 min in PBS and incubated for 1 hour with a Vectastain ABC reagent. In order to visualise the primary antibody binding, the diaminobenzidine (DAB) substrate kit for peroxidase (Vector, SK-4100, Burlingame, California) was used. MHC expression was assessed using well characterised mAbs tested against human MHC I (mAb A4.840) and MHC I and IIA (mAb N2.261) [18]. The mAb A4.840 strongly stained type I fibres, whereas type IIA, IIAX and IIX remained unstained (Figure 1A). The mAb N2.261 strongly stained type IIA fibres, whereas type I and IIAX fibres were equally weakly stained and type IIX fibres were unstained (Figure 1B). Type IIC fibres were strongly stained with mAb N2.261 and moderately stained with mAb A4.840. CSA of muscle fibres was measured using TEMA image analysis system

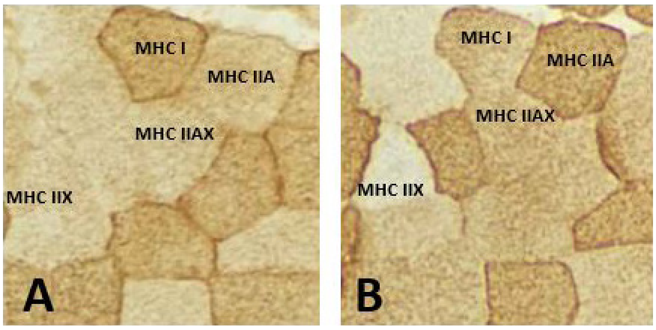


Figure 1. Immunohistochemical staining of serial cross-sections with: A) antibody A4.840 and B) antibody N2.261, for the identification of muscle fibre type distribution

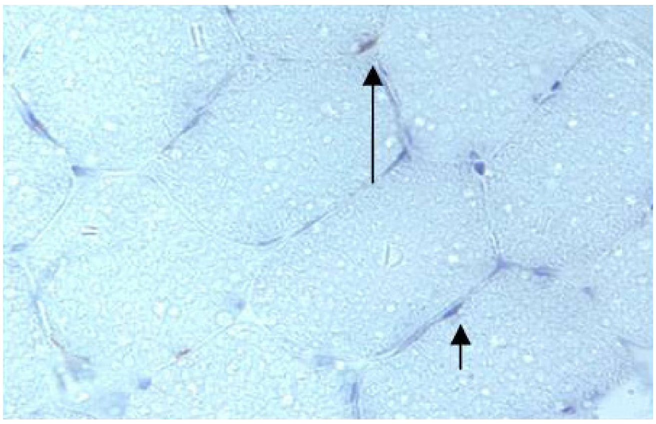


Figure 2. Immunohistochemical staining with antibody CD 56 and counterstained with Mayer's hematoxylin for the visualisation of myonuclei (short arrow) and satellite cells (long arrow)

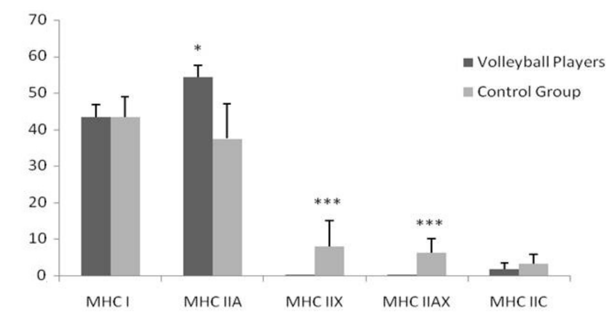
(Scanbeam, a/s, Handsund, Denmark). Satellite cells were analysed using a monoclonal antibody directed against the neural cell adhesion molecule (NCAM/CD56) (Becton Dickinson, San Jose, California) [18]. To visualise satellite cells the sections were counterstained with Mayer's hematoxylin. Satellite cells were stained brown and images were acquired with a digital camera (SPOT Insight, Diagnostics Inc., Sterling Heights, Michigan) connected to a light microscope (Nikon Eclipse E400, Badhoevedorp, The Netherlands). Satellite cells were visualised at high magnification (objective, X40 or X60) (Figure 2).

Statistical analysis

Following confirmation of normality of data distribution, Welch's t-test was used to compare the two groups [24], with Holm–Bonferroni correction for multiple pairwise comparisons [13], and the adjusted p values are reported. All data are presented as mean ± SD. Significance was set at $p < 0.05$ and all the statistical analyses were conducted using SPSS v25.0 software.

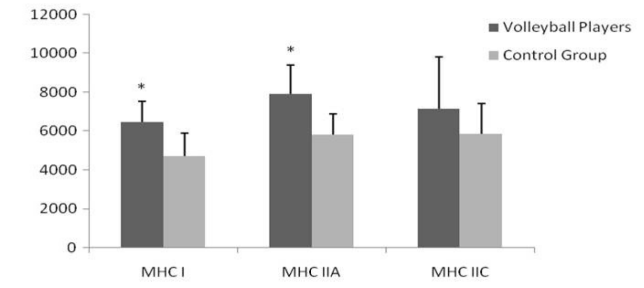
Results

The results suggest that volleyball players' muscle morphology shows some considerable differences when compared to physically active controls. Immunohistochemical analysis revealed that although MHC I and MHC IIC muscle fibre distribution did not differ between the groups, MHC IIX and MHC IIAX were totally absent in volleyball players and appeared only in the control group (MHC IIX 8.2 ± 7.0% and MHC IIAX 6.5 ± 3.8%) (Figure 3). The cross-sectional area revealed a slightly different pattern, as both MHC I and IIA were larger for the volleyball players (Figure 4). In accordance, MHC II myonuclear number was moderately higher in the volleyball players



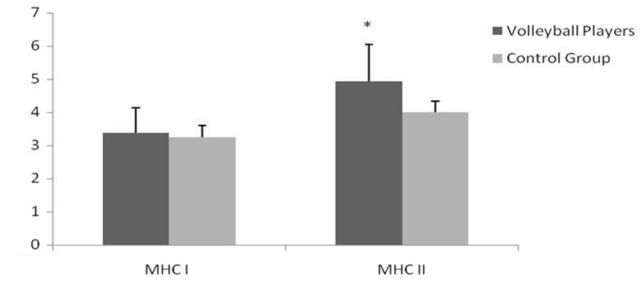
* $p < 0.05$, *** $p < 0.001$

Figure 3. Mean muscle fibre type distribution (%) of volleyball players (darker bars) and control group (lighter bars). Vertical bars denote SD



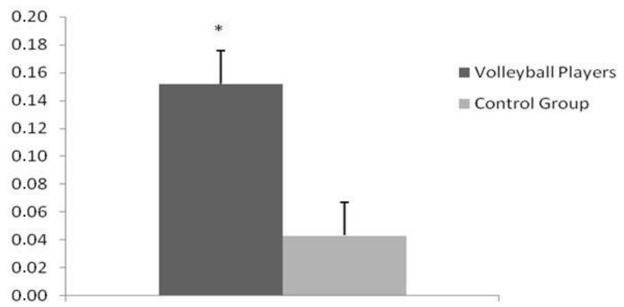
* $p < 0.05$

Figure 4. Cross-sectional area of volleyball players and control group



* $p < 0.05$

Figure 5. Number of myonuclei per MHC I and MHC II of volleyball players and control group



* $p < 0.05$

Figure 6. Satellite cell number per muscle fibre of volleyball players and control group

(Figure 5), while the satellite cells and their ratio to the number of fibres showed a large and very large difference, respectively (Figure 6).

Discussion

Volleyball requires explosive movements including a high number of jumps, which are considered critical to successful performance [30]. Indeed, good jumping performance is required for blocking, spiking or serving [25, 29] and it distinguishes the better players [11]. As a result, training modes such as strength and plyometrics are routinely used for volleyball training [5]. Longitudinally, this is a process that triggers special adaptations within the musculoskeletal system resulting in an enhanced physical performance. Our study revealed that the vastus lateralis of volleyball players is characterised by a higher proportion of MHC IIA and a total absence of MHC IIX and MHC IIAX in comparison to the control group, whereas MHC I and MHC IIC showed no difference. This finding provides evidence that the conversion capacity from MHC IIX and IIAX into muscle fibres containing MHC IIA is fully utilised in well-trained volleyball players.

It appears that volleyball players have converted all MHC IIX and IIAX fibres to MHC IIA, which resulted in this very large difference between the groups. Similar findings have been reported in other studies, which showed an increased proportion of fibres IIA with a concomitant decrease in the percentage of type IIX fibres after heavy resistance training. It suggests that this type of training affects MHC composition in skeletal muscle, mainly showing adaptations in genetic expression [1, 19]. Moreover, it has been described that type IIB fibres (IIX as presented by Smerdu et al. [28] constitute a pool of fibres that shift into IIA when recruited systematically. Regarding fibres expressing MHC I and the absence of differences between the volleyball players and the controls, our results are consistent with previous research, which suggested that there were minimal if any at all, changes in the number of fibres expressing MHC I after strength training [2].

It has been well established that resistance training can increase fibre CSA, with a) increased hypertrophy for both type I and II muscle fibres after longitudinal studies, and b) preferential hypertrophy of type II fibres only in shorter training protocols lasting 6 to 10 weeks [1]. For example, McCall et al. [23] reported that 12 weeks of strength training increased fibre CSA for both type I (slow) and II (fast) muscle fibres. In the present study the volleyball players' group appeared to have significantly higher CSA both in MHC I and MHC IIA fibres in comparison to the control group. Given the above studies, it may be reasonably assumed that the long-term volleyball training adaptations elicited from explosive training (e.g. strength, plyometrics) led to an increase in both muscle fibre type I and II CSA. Notwithstanding the lack of studies examining the muscle characteristics of volleyball players using muscle biopsies, our findings contradict findings by Sleivert et al. [27] who reported no difference in the CSA of volleyball players when compared to the controls and a higher type II / type I fibre area ratio per sample biopsy of volleyball players in comparison to the controls. However, this difference may have been caused by a methodological difference, as in the study by Sleivert et al. [27] the mean number of muscle fibres per biopsy used was 56, while in the present study the respective number was 440. Such a low muscle fibre number [6] is likely to have resulted in a larger standard error [21], potentially masking any differences in CSA, something that the authors themselves identified. The standard error is considerably reduced with numbers of muscle fibres >100; it is worth noting that the same applies to both type I and type II fibres [21].

Our study revealed that the myonuclear number of MHC II in volleyball players also increased. It seems that an acquisition of additional myonuclei occurred in order to support MHC II fibre hypertrophy [18]. Kadi [14] observed a high number of myonuclei in hypertrophied muscles of elite powerlifters and athletes using anabolic steroids. Furthermore, Sinha-Hikim et al. [26] showed that the myonuclear number per fibre was significantly correlated with muscle fibre cross-sectional area after 20 weeks of strength training and concurrent steroid intake. Finally, in a comparison between high-level powerlifters and untrained subjects Kadi et al. [15, 16] revealed that the number of satellite cells as well as myonuclear numbers were significantly higher in athletes than the controls. The mechanism responsible for these changes in skeletal muscle fibres is connected with the regulation of protein expression of defined cytoplasmic volume and within all multinucleated cells by each nucleus [9]. In the hypertrophic muscle fibres, the nuclear to cytoplasmic ratio was maintained by increased nuclear content. In mature muscle fibres, myonuclei are unable to undergo mitosis. Hence, satellite cells activate and subsequently incorporate into muscle fibres during hypertrophy [15, 16].

Our study showed no significant differences in the myonuclear number of fibres expressing MHC I, which possibly means that the nuclear to cytoplasmic ratio of these fibres was maintained by the existing myonuclear content. Thus, the hypertrophy that MHC I may have underwent was not sufficient to trigger proliferation of satellite cells and form new myonuclei. Additionally, our study revealed that there is an increased number of satellite cells in the vastus lateralis of volleyball players compared to the controls. It seems that the activation and proliferation of satellite cells led to an increase in satellite cell number. Our findings are consistent with other studies showing that activated satellite cells provide more stem cells in skeletal muscles [14, 17].

Conclusions

In conclusion, our study reveals that longitudinal volleyball training-induced hypertrophy for both type I and II muscle fibres in the vastus lateralis of volleyball players resulted in a specific shift in muscle fibres containing MHC II isoforms. The hypertrophy of muscle fibres is associated with an increase in the myonuclear number and the number of satellite cells.

Conflict of Interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

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High-intensity physical performance parameters in soccer

DANIEL ROJANO ORTEGA[1], MIGUEL ÁNGEL MARTÍN SIMÓN[2]

Abstract

Introduction. **High-speeds distances covered during a soccer match are considered a good indicator of team success, especially for some playing positions. These distances vary markedly among different playing positions, and match congestion typically results in a reduction of high-intensity activities. However, the different playing levels might influence those results.** Aim of Study. **1) To analyze the differences in the total distance covered during the match, and the high-intensity physical performance parameters between the various playing positions in the four highest-ranking teams in the 2018 FIFA World Cup. 2) To follow the evolution of those parameters throughout the competition.** Material and Methods. **Match data, reported by FIFA, of the four teams classified for the semi-finals in the 2018 World Cup were used. Differences between the different playing positions and the different phases were analyzed.** Results. **The distances covered at high speeds and the number of sprints performed during a soccer match may differentiate team performance. The distance covered at 20-25 km/h, at speeds exceeding 25 km/h, and the total number of sprints were significantly lower ($p < 0.05$) for central defenders; they were higher for the wide-midfielders, though not significantly likely due to the low sample size. No significant differences were observed between the different phases in most of the study variables.** Conclusions. **Understanding the differences between the different playing positions in the highest-ranking teams of the competitions may help coaches to carefully design players' rotations and include different soccer-specific drills for each playing position to contribute to team success. Together with a good technical and tactical strategy, promoting post-match recovery may be the key for team success.**

KEYWORDS: recovery, sprints, high running speed, congested matches.

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Introduction

Aside from technical demands, understanding the physical requirements of elite soccer players has received growing interest in the last decades [7, 8, 11]. A professional soccer player covers on average 9-12 km throughout a match [12, 17], but that amount varies depending on the playing position and the running speed [6, 18, 21, 24].

Sprinting, along with other physical attributes, is a desirable attribute associated with success in a soccer match [5, 14, 22], and the distance covered at high speeds seems to be a better indicator of success than the total distance covered, especially for some playing positions [2, 19]. According to Ugalde-Ramirez [24], high-speed running performed by players of lateral positions may differentiate team performance.

Different game tactics during a soccer match can affect the physical demands of the players [9], including the number of sprints performed during the match, the amount of distance covered at high speeds, and the maximum speed achieved. In any case, excluding goalkeepers, professional soccer players perform on average more than 20 sprints per match [21, 24], cover more than 200 m at sprinting speeds [3], and reach top speeds of approximately 30 km/h [17, 24].

Previous studies reported that total distance covered during a match and distances covered at high or very high running intensities vary widely among the different playing positions [7, 11, 17, 21, 24]. Data analyzed in those studies included several or all teams in one of the strongest leagues in the world [7, 17, 21], data from 58 teams playing in the European Champions League and the UEFA Cup [11], and data from all matches of the 2018 FIFA World Cup [24]. However, the highest-ranking teams of those leagues and competitions were not analyzed separately to determine if there were differences between those teams and the other participating teams.

As the match progresses, the amount of high-intensity running usually decreases as a result of fatigue, regardless of playing position or level [7, 19]. Similar results were reported for the total distance covered and the number of sprints [21, 24]. Additionally, Palucci et al. [20] concluded that during a congested match (when the team played two times a week), the players presented a reduced number of high-intensity activities than during a non-congested match. Kovács et al. [16] also observed that in the play-off stage of the 2015-2016 Future Talents Cup International tournament match congestion resulted in a reduction in the total distance covered.

To our knowledge, no study has investigated the possible effect of fatigue on physical performance parameters during the FIFA World Cup, during which all matches are played within one month and players may not achieve a full recovery between matches. Therefore, the purpose of this study was two-fold: 1) To analyze differences in the total distance covered during the match, and high-intensity physical performance parameters between the various playing positions in the four highest-ranking teams in the 2018 FIFA World Cup; 2) To follow the evolution of those parameters throughout the competition.

Material and Methods

Design and participants

In this study, match data reported by FIFA for the 2018 FIFA World Cup was used [15]. The four teams that qualified for the semi-final matches were analyzed.

Due to level differences between the first and last classified of each group at the beginning of the competition, players from the strongest teams do not always play at full capacity. Therefore, only data from round of 16, quarter-finals and semi-finals were used for subsequent analysis. Players who performed at least 90 minutes during the game were selected for analysis, excluding goalkeepers. The players were classified into five different playing positions: central defenders, wide defenders, central midfielders, wide midfielders, and center-forwards. The study followed the ethical guidelines of the Declaration of Helsinki.

Measures

FIFA [15] reported 14 different physical activities for each player during the entire match, the first half, and the second half. For the purposes of this study only five of them over the duration of the entire match were used: total distance covered, distance covered at zone 4 (20-25 km/h), distance covered at zone 5 (>25 km/h), top speed, and total number of sprints. The data were obtained with a reliable real-time optical tracking system at 25 frames per second [23, 24].

Statistical analysis

Statistical analysis was performed using the SPSS software package for Windows, v. 22.0 (SPSS Inc., USA). To determine differences between the different playing positions, data from the four teams and the three phases were pooled and the means and standard deviations of all variables were calculated. Data were tested for normality using the Shapiro–Wilk test. When this condition was fulfilled, one-way ANOVA with Bonferroni post-hoc tests was performed to determine significant differences between the five playing positions. When data were not normally distributed, the Kruskal–Wallis H tests with Dunn's post-hoc tests and Bonferroni correction were performed. To explore the differences of one group across the different phases of the competition data from the four teams were pooled and one-way ANOVA with Bonferroni post-hoc tests was performed. Results were considered statistically significant at $p \leq 0.05$.

Results

Differences between playing positions

Table 1 shows the means and standard deviations of all study variables, calculated with pool data from the four teams and the three phases and taking into consideration the specific playing positions. Significant differences between positions are also shown in Table 1. No significant differences were found for the top speed, whereas a significant effect of playing position was observed for the other variables. Post-hoc tests revealed

Table 1. Descriptive statistics and differences between the different playing positions

Variables	Central defenders	Wide defenders	Central midfielders	Wide midfielders	Centre-forwards
	n = 31	n = 17	n = 25	n = 9	n = 16
Total distance (m)	9141.97 ± 475.41[b,c]	10099.94 ± 828.44[a]	10745.84 ± 893.54[a,d,e]	9554.33 ± 1019.55[c]	9641.19 ± 839.68[c]
20-25 km/h distance covered (m)	344.19 ± 97.50[b,c,d,e]	583.65 ± 170.00[a]	572.76 ± 150.31[a]	609.67 ± 163.18[a]	528.81 ± 126.89[a]
>25 km/h distance covered (m)	124.87 ± 75.47[b,c,d,e]	257.76 ± 124.98[a]	177.64 ± 98.23[a]	326.89 ± 85.28[a]	297.69 ± 107.63[a]
Top speed (km/s)	28.40 ± 2.55	29.00 ± 1.94	28.60 ± 2.19	30.83 ± 1.61	28.76 ± 2.43
Number of sprints	21.45 ± 7.63[b,c,d,e]	37.59 ± 11.43[a]	31.88 ± 9.98[a]	40.11 ± 9.09[a]	32.75 ± 8.99[a]

[a] significantly different from central defenders ($p < 0.05$); [b] significantly different from wide defenders ($p < 0.05$); [c] significantly different from central midfielders ($p < 0.05$); [d] significantly different from wide midfielders ($p < 0.05$); [e] significantly different from centre-forwards ($p < 0.05$)

that the distance covered at 20-25 km/h, at speeds exceeding 25 km/h and the total number of sprints were significantly lower ($p < 0.05$) for central defenders than for the other playing positions. Additionally, the total distance covered was also significantly lower for central defenders than for wide defenders and central midfielders, while it was significantly higher for central midfielders than for wide midfielders and center-forwards.

The distance covered at 20-25 km/h, at speeds exceeding 25 km/h and the total number of sprints were greater for wide midfielders, but the differences were not significant likely due to low sample size.

Differences between phases of the competition

Table 2 shows the means and standard deviations of all the study variables in each phase of the competition, calculated with pool data from the four teams, both depending on the specific playing position and regardless of this position. Significant differences between the phases are also shown in Table 2. No significant differences were observed between the different phases in any of the study variables, whether the players were grouped or classified by position, except for the top speed of the center-forwards, which significantly increased ($p < 0.05$) from round of 16 to the quarter-finals.

Discussion

The first purpose of this study was to analyze differences in the total distance covered during the match and in the high-intensity physical performance parameters between the different playing positions in the four highest-ranking teams of the 2018 FIFA World Cup.

Table 2. Descriptive statistics and differences between different phases of the competition

Variables	Phase of the competition	All players	Central defenders	Wide defenders	Central midfielders	Wide midfielders	Centre-forwards
		n = 32,31,35	n = 11,10,10	n = 5,6,6	n = 8,8,9	n = 3,1,5	n = 5,6,5
Total distance (m)	round of 16	9652.25 ± 1003.97	9149.18 ± 512.90	10076.80 ± 1137.06	10448.13 ± 969.79	8959.133 ± 1271.30	9476.80 ± 907.10
	quarter-final	9899.29 ± 909.24	9206.60 ± 406.95	10302.83 ± 845.14	10729.13 ± 912.79	8918.00	9707.33 ± 536.53
	semi-final	9952.40 ± 1031.44	9069.40 ± 533.79	9916.33 ± 597.67	11025.33 ± 817.13	10056.60 ± 767.46	9726.20 ± 1188.25
20-25 km/h distance covered (m)	round of 16	478.59 ± 163.73	339.09 ± 72.19	606.40 ± 239.53	534.25 ± 131.98	544.67 ± 109.37	529.00 ± 119.67
	quarter-final	500.77 ± 173.12	341.50 ± 116.91	607.33 ± 152.90	590.88 ± 175.29	537.00	533.50 ± 99.65
	semi-final	514.86 ± 181.26	352.50 ± 110.04	541.00 ± 140.82	590.89 ± 153.21	663.20 ± 197.99	523.00 ± 183.21

Variables	Phase of the competition	All players	Central defenders	Wide defenders	Central midfielders	Wide midfielders	Centre-forwards
		n = 32,31,35	n = 11,10,10	n = 5,6,6	n = 8,8,9	n = 3,1,5	n = 5,6,5
>25 km/h distance covered (m)	round of 16	198.34 ± 130.36	126.36 ± 100.33	309.20 ± 138.29	190.50 ± 119.29	354.22 ± 80.70	164.80 ± 99.29
	quarter-final	184.32 ± 105.51	116.60 ± 48.58	260.50 ± 152.79	171.38 ± 96.87	292.00	220.33 ± 76.06
	semi-final	197.09 ± 109.22	131.50 ± 72.90	212.17 ± 78.66	171.78 ± 89.66	317.40 ± 101.53	235.40 ± 151.16
Top speed (km/h)	round of 16	29.14 ± 2.87	28.88 ± 2.64	29.90 ± 2.19	29.33 ± 3.36	31.95 ± 2.28	26.98 ± 2.54*
	quarter-final	29.08 ± 1.80	28.56 ± 2.02	29.35 ± 2.06	28.34 ± 1.38	30.17	30.48 ± 1.06*
	semi-final	28.34 ± 2.15	27.72 ± 3.01	27.89 ± 1.23	28.17 ± 1.42	30.29 ± 1.09	28.48 ± 2.42
Number of sprints	round of 16	29.22 ± 11.34	20.45 ± 5.75	38.20 ± 15.64	30.25 ± 8.80	38.00 ± 10.44	32.60 ± 9.71
	quarter-final	30.81 ± 11.48	21.70 ± 9.02	40.17 ± 11.99	32.50 ± 9.96	35.00	33.67 ± 8.52
	semi-final	31.31 ± 11.41	22.30 ± 8.60	34.50 ± 7.66	32.78 ± 11.84	42.40 ± 9.71	31.80 ± 10.76

* significant differences between round of 16 and quarter-final phases ($p < 0.05$)

Our main finding is that, excluding top speed, the high-intensity physical performance parameters were significantly lower for the central defenders than for the rest of the playing positions, while it was higher, but not significantly, for the wide midfielders.

Our results are not consistent with those reported by Di Salvo et al. [11] or Ugalde-Ramirez [24]. Di Salvo et al. [11] measured the total number of sprints in 67 European matches (the European Champions League and the UEFA Cup) from 2002 to 2006. The data of 20 different countries were analyzed and the total number of sprints differed between all the positions except wide defenders and center-forwards. In addition, the number of sprints was substantially smaller in all playing positions when compared to the present study. Ugalde-Ramirez [24] analyzed the data reported by FIFA for the 2018 World Cup and found no significant differences in the high-intensity physical performance parameters between the different playing positions.

Findings in this study, however, are very similar to those observed by Bradley et al. [7], Mallo et al. [17] and Rivilla-García et al. [21]. Bradley et al. [7] collected match performance data from players in the English FA Premier League. While they did not report significant differences between the different playing positions, their data clearly showed that the distances covered at 20--25 km/h and at speeds exceeding 25 km/h were smaller for central defenders and greater for wide midfielders, with no differences observed between the other positions in the study [7]. Mallo et al. [17] analyzed 111 matches of the Spanish First Division League and they observed that the distance covered at very high-intensity running was significantly smaller for central defenders and central midfielders, while it was greater, but not significantly, for wide midfielders. Rivilla-García et al. [21] calculated the number of sprints and the sprint distance per match with the data from 380 matches of the Spanish First Division in the 2013-2014 season. They concluded that the sprint distance per match and the number of sprints performed were significantly lower for central defenders and central midfielders, and higher, although non-significantly, for wide midfielders and wide defenders.

The discrepancies and similarities of the present study compared to previous studies can be attributed to the teams and the players analyzed. In this study only the four highest-ranking teams of the 2018 FIFA World Cup were analyzed, while Di Salvo et al. [11] used the data of 20 different countries and 67 European matches and Ugalde-Ramirez [24] used all data from the 2018 World Cup. This indicates that the high-intensity physical performance parameters do not adhere to the same pattern for the highest-ranking teams compared to the other teams participating in European and World soccer competitions. However, the physical characteristics as well as the physical and physiological demands of many players in the English FA Premier League and the Spanish First Division, being two of the strongest leagues in the world, must be similar to those for the best teams of our study, which explains the similar results found.

It was also observed that central defenders covered less distance per match than the other playing positions, but differences were only significant with central midfielders and wide defenders. For this parameter, our results are not in line with those reported by Bradley et al. [7], Mallo et al. [17] or Rivilla-García et al. [21]. Furthermore, the total distance covered in the different playing positions differs from many studies [4, 7, 10, 17, 21], and also varies between them. These different results suggest that the success of a soccer match is not affected by the total distance covered. However, soccer match success may rely on high-intensity performance parameters and, of course, on a superior technical and tactical strategy, as it is suggested by Di Salvo et al. [13] and Abbott et al. [1].

The second purpose of this study was to analyze the evolution of the total distance covered during the match and the high-intensity physical performance parameters of the four highest-ranking teams throughout the 2018 FIFA World Cup, from round of 16 to semi-finals. According to some authors [16, 20], match congestion may reduce the total distance covered and the number of high-intensity activities during the match. However, no significant differences were observed between the different phases, except for the top speed of the center-forwards. These results show that the highest-ranking teams of the 2018 FIFA World Cup suffered no detrimental effects on high-intensity physical performance parameters, even if the three matches analyzed were played within 10 days. Along with a superior technical and tactical strategy, this may be evidence why those four teams classified for the semi-finals.

Conclusions

The findings of this study confirm that the distances covered at high speeds and the number of sprints performed during a soccer match may differentiate team performance, especially in some playing positions. Our results show that the wide midfielders of the highest-ranking teams in the 2018 FIFA World Cup covered more distance at speeds exceeding 20 km/h and performed a higher number of sprints than the rest of the players. Understanding these differences may help coaches to carefully design players' rotations and include different soccer-specific drills for each playing position to contribute to team success. The findings of this study also revealed that the highest-ranking teams in the 2018 FIFA World Cup did not experience detrimental effects on high-intensity physical performance parameters due to congested matches. Together with a good technical and tactical strategy, promoting post-match recovery may be the key for team success.

Conflict of Interest

The authors declare no conflict of interest.

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The effects of a repeated sprint ability program on youth soccer players' physical performance

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Abstract

Introduction. **Soccer trainers in their effort to be effective by saving time for technical tactical training try to use methods that have positive effects on multiple physical abilities. High-intensity interval training (HIIT) is widely used in soccer.** Aim of Study. **The purpose of the study was to investigate the effect of a repeated sprint training (RST) program on the performance of soccer players under the age of 17.** Material and Methods. **Twenty-nine youth players participated in this study. Players were randomly separated into two groups: control group (CG, n = 14) and intervention group which performed RST program (IG, n = 15). The duration of the training program was four weeks. Sprint 10 m, 30 m, countermovement jump (CMJ), squat jump (SJ), Illinois agility test, Yo-Yo intermitted recovery test 2 (YYIRT2) and repeated sprint test (RSA: RSAbest, RSAmean, RSAdecrement) were measured pre and post of the training program.** Results. **The performance in 30 m, RSAbest, RSAmean and RSAdecrement improved in IG group (P = 0.049, η^2 = 0.171, P = 0.017, η^2 = 0.307, P = 0.002, η^2 = 0.622, and P < 0.001, η^2 = 0.774, respectively). The performances of the two groups differed in post measurement of 30 m, RSAbest and RSAmean (P = 0.044, η^2 = 0.160, P = 0.048, η^2 = 0.014, and P = 0.038, η^2 = 0.226, respectively).** Conclusions. **This study supports that a short-term program of HIIT can improve sprint and repeated sprint ability performance. The results reflect the training principle of the specialization of the stimulus. The improvement in performance presented in tests that had similar characteristics to training stimuli.**

KEYWORDS: youth, soccer, fitness, high intensity interval training.

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Introduction

Soccer is an intermittent sport characterized by periods of low, moderate and high intensity efforts [4]. The development of technology has made it possible to record actions carried out both during matches and training sessions of teams. Thus, in professional soccer players during a match cover 9-14 km, of which about 10% is covered with a speed of more than 19 km/h [26]. It results both from literature sources and from practice that the distances covered at high intensity are the most important for the performance of the player and for the result of the match [11]. Knowledge of the requirements of the "match" helped trainers to focus on important factors they need to develop in order to improve performance of their players. Such key factors are the ability to repeated sprints, endurance, the ability to sprint and power.

Trainers in their effort to be more effective by saving time for technical tactical training try to use training methods that have positive effects on multiple physical abilities. One such method is the high intensity interval training (HIIT) proposed by Buchheit and Rabbani [6]. This form of training includes five subcategories: 1) training using racing games, 2) interval training with sprints >20-30 s with long breaks (3 min), 3) training of repeated sprints <10 s, 4) HIIT long time (no maximum exercise for 2-4 min), and 5) HIIT short time (no maximum exercise for <45 s) [4].

Several studies have shown positive effects of these training methods on power, speed, high intensity running and the ability to repeat speeds in youth soccer players [3, 5, 8, 10, 16, 23]. However, results of the studies are difficult to compare as they use different training protocols (different characteristics of the charge: volume, intensity, break, density) and different ages of participants. For example, it is known that age and biological maturity can affect effectiveness of a fitness program [13]. Therefore, any research on the effect of HIIT on performance of teenage soccer players contributes to an overall picture. Thus, the purpose of this study was to study the effect of a short 4-week repeated sprint training (RST) program on physical abilities of youth soccer players under 17 (U17) of a certain biological age. The hypothesis of the study was that this short program would improve performance in repeated sprinting ability, speed, power and endurance.

Material and Methods

Design

This study used a two-group (intervention group, IG, and control group, CG), randomized controlled trial design to compare effects of a short RSA training program on U17 youth soccer players. The study was conducted during the in-season period for four weeks. Each group participated in four training sessions and one soccer game per week. The CG performed only the conventional soccer practice. The IG performed a RSA program twice a week, 72 hours apart. The two experimental groups had the same duration of training sessions (90 min). The RSA program was performed immediately after warm-up to ensure full neuromuscular activation, and the CG during the intervention carried out technical and tactical exercises. Two weeks before the study the soccer players familiarized with the tests in order to minimize the learning effect error. During the first visit after the two weeks the soccer players had their body mass, height and percentage of body fat measured. During the next two visits they performed fitness tests: countermovement jump (CMJ), squat jump (SJ), speed (10 m and 30 m), change of direction (Illinois agility test), Yo-Yo intermittent recovery test level 2 (YYIR2). The tests were repeated after four weeks of training and were conducted 48 hours after the last training session and with the same sequence each testing day. At the beginning of each testing session the soccer players performed a 15-minute warm-up and at the end a 10-minute cool-down period. Soccer players consumed water ad libitum to ensure proper hydration during training and testing.

Subjects

Power analysis was performed before the study by setting an effect size of 0.6, a probability error of 0.05 and a power of 0.9 for two groups and two measurements points (pre- and post-test). Power analysis (G*Power, version 3.1.9.2, Universität Kiel, Düsseldorf, Germany) estimations were based on studies that examined effects of training protocols on performance of soccer players [8]. The analysis indicated that 24 subjects were the smallest acceptable number of participants. The inclusion criteria to participate in the study were as follows: 1) no musculoskeletal injuries for ≥6 months prior to the study, 2) having participated in ≥95% of training sessions and seven or more of the intervention trainings for IG, and 3) not to be taking any medication. A total of 29 male youth soccer players under the age of 17 (U17) participated in the study. The players came from two local soccer academies. They did four training sessions and one game a week and all of them had at least 10 years of systematic soccer training. The players were randomly assigned into two groups, the intervention group (IG, n = 15) and the control group (CG, n = 14). All the participants were informed of the potential risks and benefits of the study and signed a consent form for their participation. For the research the requirements of the Research Code of Ethics of the Aristotle University of Thessaloniki were fulfilled in compliance with the Helsinki Declaration. The researcher conducting the assessment tests was blinded to the participants' allocated group. Participant characteristics are presented in Table 1.

Table 1. Participants' physical characteristics

	CG (n = 14)		IG (n = 15)	
	pre-training	post-training	pre-training	post-training
Age (years)	15.8 ± 0.2	15.9 ± 0.3	16 ± 0.2	16.1 ± 0.2
Height (cm)	177 ± 7	178 ± 6	176 ± 6	177 ± 6
Weight (kg)	65.7 ± 8.8	66.2 ± 8.6	66.4 ± 11.3	65.9 ± 11.9
Body fat (%)	15.4 ± 5.3	15.8 ± 5.8	16.5 ± 5.5	15.9 ± 6.2

Note: CG – control group, IG – intervention group

Intervention program

The intervention program lasted four weeks with a frequency of two times a week with two days being 48 hours apart. The total duration of the sessions was 90 minutes. In addition to the soccer teams' program, IG also performed the intervention program, while CG participated exclusively in the teams' athletic program. The intervention lasted 12-20 minutes (9 min in the first week to 22 min in the fourth week) and took place immediately after the warm-up period. The characteristics of the RSA program are presented in Table 2.

Table 2. Description of the four weeks of repeated sprint ability training program and features of each session

Week	Sessions	RST				Rest between	
		Set	Reps	Meters	Speed	Sprint (s)	Sets (min)
1st	1st	2	6	40	max	20	4
	2nd	2	6	40	max	20	4
2nd	3rd	3	6	40	max	20	4
	4th	3	6	40	max	20	4
3rd	5th	4	6	40	max	20	4
	6th	4	6	40	max	20	4
4th	7th	4	6	40	max	20	4
	8th	4	6	40	max	20	4

Note: RST – repeated sprint training

Anthropometric measurements
Body mass was measured to the nearest 0.1 kg using an electronic digital scale with the participants in their underclothes and barefoot. Standing height was measured to the nearest 0.1 cm (Seca 220e, Hamburg, Germany). Body fat percentage was estimated based on the sum of four (biceps, triceps, suprailiac, subscapular) skinfold thicknesses measured with a specific caliper (Lafayette, Ins. Co., Indiana) on the right side of the body as described [22]. Estimation of body density was calculated according to the Durnin and Rahaman [9] equation for males under the age of 16 and estimated by the equation of Siri [21].

Speed testing
A 30-m sprint test with 10-m splits (0-10 m were measured as well) was used to measure speed performance. Sprint testing was performed with the participants wearing soccer shoes on the synthetic grass of a soccer field. After a 5-second countdown the participants ran in front of three infrared photoelectric gates (Microgate, Bolzano, Italy) that recorded times at each gate. The participants sprinted from a standing starting position with the toe of the front foot approximately 0.3 m behind the first gate. Photocells were placed 0.6 m above the ground (approximately at hip level) to capture the movement of the trunk rather than a false signal because of a limb motion [18]. The coefficient of variation for test–retest trials was 3.2%.

Vertical jump testing
Participants performed two vertical jump tests. The first test was the squat jump (SJ) where the players from a static half-sitting position (90° angle knee bend) performed a maximum vertical jump. The second test was the countermovement jump (CMJ). Participants started the jump from an upright position, making a quick preliminary movement by bending their knees and hips at 90°, followed by an explosive jump upwards extending their knees and hips. The jumps were performed with the hands on hips. Participants wore athletic shoes and made two attempts in each jump. The jump height was measured using with the Chronojump electronic leap mat (Chronojump, Boscosystem, Barcelona, Spain). The coefficients of variation for the test–retest trials were three and 2.8% SJ and CMJ, respectively.

Illinois agility test
Participants started from an upright position, 30 cm behind the gate. From position A (starting position) they sprinted to position B, where they turned out of the cone at position C and continued with zig-zags to position D and returned in the same way to position C. From this position they sprinted to position E, where they turned out of the cone sprinting to position F (finish position) (Figure 1). At the starting and closing points there were photocell-reflector gates (Microgate, Bolzano, Italy). The coefficient of variation for the test–retest trials was 3.9%.

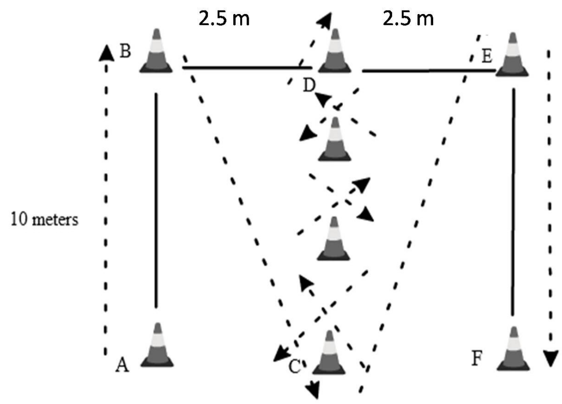


Figure 1. Description of the Illinois agility test

Repeated sprint ability test
The RSA test included 6 × 40 m (20 + 20 m sprints with 180° turns) shuttle sprints separated by 20 s of passive recovery (19). Participants started from an upright position, sprinted for 20 m, touched a line with a foot

and came back to the starting line. At the starting line there was one photocell-reflector gate [19] (Microgate, Bolzano, Italy). Immediately after the warm-up each soccer player performed a single sprint of 20 + 20 m with a turn of 180°. This time was used to test the players' maximum effort in the RSA test. If the time of the first sprint in the RSA test was 3% greater than the single sprint test, the test was terminated and the subjects were required to repeat the RSA test with maximum effort after 5 minutes. Three seconds before the start of each sprint the subjects assumed the ready position and waited for the acoustic start signal. The best time (RSAbest – the best time of the six sprints), mean time (RSAmean – the average time of the six sprints) and decrement (RSAdec = RSAmean/RSAbest – the rate of performance decrement) were determined [19].

Yo-Yo intermitted recovery test level 2

The YYIR2 test consisted of 2 × 20 m intervals of running interspersed by regular short rest periods (10 s). Furthermore, signals were given by a CD-ROM to control the speed. The player run 20 m forward and he adjusted his speed so as to reach the 20-m marker exactly at the time of the signal. Additionally, a turn was made at the 20-m marker and the player ran back to the starting marker, which was to be reached at the time of the next signal. Then the player had a 10 s break to run slowly around the third marker, which was placed 5 m behind him. He had to wait at the marker until the next signal. The course was repeated until the player failed to complete the shuttle run two times in a row. The first time, when the start marker was not reached a warning was given ("yellow card"), while at the second one the test was terminated ("red card"). The last running interval that a player had completed before being excluded from the test was recorded and the test result was expressed as the total running distance covered in the test [7].

Statistical analysis

The SPSS software (version 18.0, SPSS Inc., Chicago, IL) was used for all analyses. Data are presented as means ± standard deviation (SD). In addition, confidence intervals (CIs) were reported for fitness variables. Data normality was verified by the 1-sample Kolmogorov–Smirnov test. Therefore, no non-parametric test was necessary. The two-way analysis of variance (ANOVA) (trial × time) with repeated measurements was performed. Wherever a significant difference was found, post hoc LSD was applied. Furthermore, the effect size via η^2 was calculated. The significance level was set at $P < 0.05$. As mentioned earlier, power analysis was performed to estimate the smallest acceptable number of participants to analyze the interaction between group and time points of measurements.

Results

The two groups did not differ in chronological age, height, body fat and all other fitness tests at the beginning of the study. Also, soccer training and the intervention program did not affect the participants' anthropometric profile (Table 1).

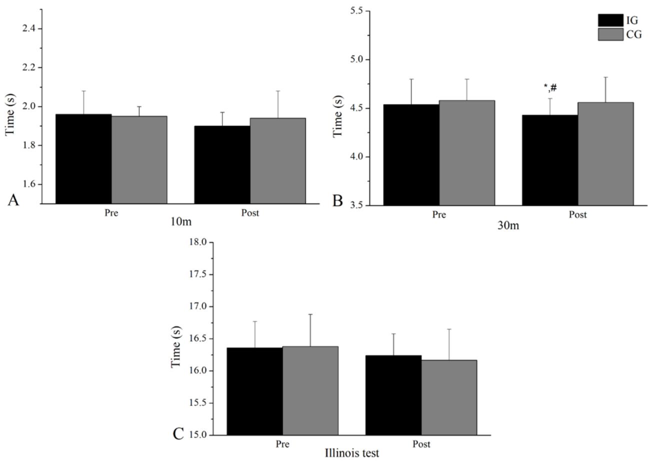
After the intervention the IG participants demonstrated a reduction of sprint time in the 30 m sprint (IG: 2.4%, $P = 0.049$, $\eta^2 = 0.171$). No differences were observed in the 10 m results (IG: 3.6%, $P = 0.454$, $\eta^2 = 0.047$). In the CG results no changes were observed between the pre- and post-test measurements for both tests, 30 m (CG: 0.4%, $P = 0.216$, $\eta^2 = 0.039$) and 10 m (CG: 0.5%, $P = 0.632$, $\eta^2 = 0.024$). Differences between the groups were found in post measurements for the 30 m sprint ($P = 0.044$, $\eta^2 = 0.160$). The differences are presented in Figure 2. The confidence intervals of all the measurements are presented in Table 3.

Table 3. Confidence intervals of fitness measurements

	Confidence interval			
Variable	IG	CG	Pre	Post
30-m (s)	4.21-4.76	4.46-4.68	4.42-4.71	4.33-4.65
10-m (s)	1.83-2.06	1.89-2	1.91-2	1.84-2
SJ (cm)	25.49-33.85	25.84-33.21	26.13-31.56	27.28-33.41
CMJ (cm)	28.34-37.09	25.59-35.11	28.64-34.33	30.21-35.95
Illinois test (s)	15.89-16.71	16.04-16.51	16.11-16.63	15.96-16.45
YYIR2 (m)	611-796	207-872	409-750	473-854
RSAbest (s)	7.26-7.59	7.33-7.56	7.39-7.63	7.23-7.47
RSAmean (s)	7.63-8.09	7.65-7.98	7.8-8.08	7.57-7.9
RSAdec	1.03-1.06	1.03-1.05	1.05-1.06	1.03-1.04

Note: CG – control group, IG – intervention group, SJ – squat jump, CMJ – countermovement jump, YYIR2 – Yo-Yo intermittent recovery test level 2, RSAbest – the best time of the six sprints, RSAmean – the average time of the six sprints, RSAdec – the rate of performance decrement

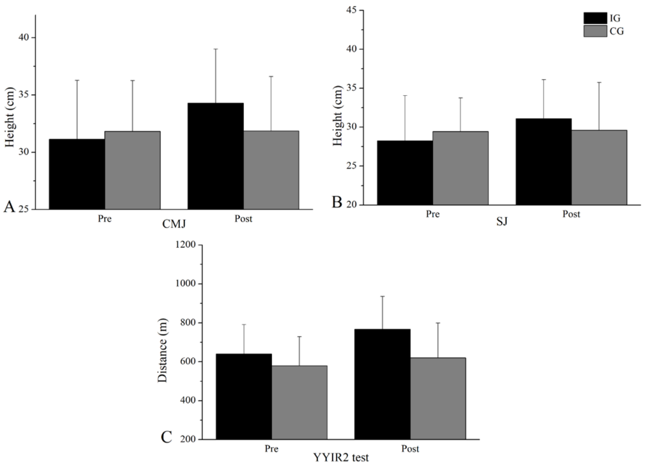
The performance of both groups did not change in the Illinois agility test (IG: $P = 0.075$, $\eta^2 = 0.104$, CG: $P = 0.124$, $\eta^2 = 0.088$). No differences were observed between the groups at the post measurement ($P = 0.902$, $\eta^2 = 0.001$). The differences are presented in Figure 2.



Note: CG – control group, IG – intervention group
* denotes significant differences with Pre (P < 0.05); # denotes significant differences with CG (P < 0.05)

Figure 2. Performance changes in: A) 10 m, B) 30 m, C) Illinois agility test

Jumping performance for both groups between pre- and post-test measurements was not changed. The IG results showed an increment of 10% both in SJ (P = 0.056, η^2 = 0.237) and in CMJ (P = 0.063, η^2 = 0.258). The performance of CG in SJ changed by 1.6% (P = 0.223, η^2 = 0.078) and in CMJ by 0.1% (P = 0.869, η^2 = 0.022). Additionally, no differences were observed between the groups in post-test measurements for SJ (P = 0.956, η^2 = 0.015) and CMJ (P = 0.736, η^2 = 0.006). The differences are presented in Figure 3.

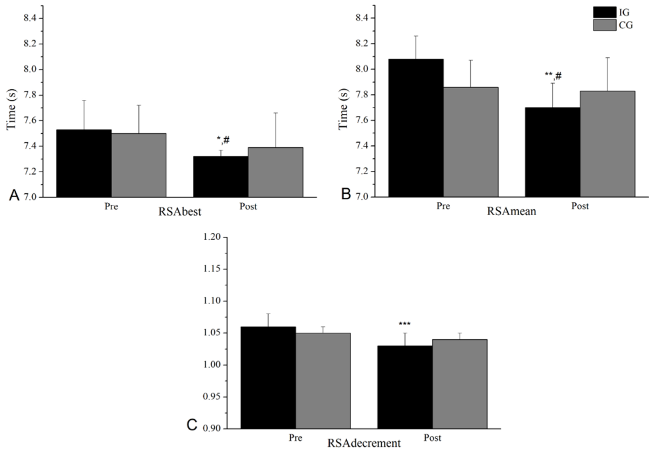


Note: CG – control group, IG – intervention group, CMJ – countermovement jump, SJ – squat jump, YYIR2 – Yo-Yo intermittent recovery test level 2

Figure 3. Performance changes in: A) countermovement jump, B) squat jump, C) Yo-Yo intermittent recovery test level 2

No changes were observed for both groups in the post-test measurement for the YYIR2 test (IG: 20%, P = 0.115, η^2 = 0.307; CG: 6.9%, P = 0.322, η^2 = 0.082). No differences were observed between groups. The differences are presented in Figure 3.

The IG group improved its performance in RSAmean (4.7%, P = 0.002, η^2 = 0.622). The change in RSAbest was 2.8% (P = 0.017, η^2 = 0.288), while in RSAdecrement it was 2.8% (P < 0.001, η^2 = 0.774). In CG no changes were observed in all the variables of the RSA test (RSAbest: 1.5%, P = 0.386, η^2 = 0.096; RSAmean: 0.4%, P = 0.625, η^2 = 0.116; RSAdecrement: 0.95%, P = 0.784, η^2 = 0.126). Differences between the groups were observed in post-test measurements of RSAmean (P = 0.048, η^2 = 0.226) and RSAbest (P = 0.038, η^2 = 0.014). The differences are presented in Figure 4.



Note: CG – control group, IG – intervention group, RSAbest – the best time of the six sprints, RSAmean – the average time of the six sprints, RSAdec – the rate of performance decrement
* denotes significant differences with Pre (P < 0.05); ** denotes significant differences with Pre (P < 0.01); *** denotes significant differences with Pre (P < 0.001); # denotes significant differences with CG (P < 0.05)

Figure 4. Performance changes in: A) RSAbest, B) RSAmean, C) RSAdecrement

Discussion

From the results of the study it appeared that this type of training did not affect the participants' performance. However, the IG had the tension to improve its performance in the YYIR2 test. A recent study comparing RSA programs in terms of recovery time showed that a shorter time (15 to 30 s) had a greater effect. More specifically, the program with the 15 s rest between the sprints further improved the performance in YYIRT2 [16]. Perhaps the shorter break activates to a greater extent both the anaerobic mechanism and the aerobic mechanism in the body's effort to respond to the continuous maximum stimuli without adequate recovery [4]. In a more recent study comparing repeated sprint training (RST) programs performed in a straight line or with a change of direction no changes in performance were recorded in the YYIR1 test [1]. Sanchez-Sanchez et al. [20] compared the effect of different aerobic levels in young footballers on the effectiveness of RSA programs. The results showed that the programs improved performance of YYRT1 in players with lower aerobic capacity, which however did not differ from those with high aerobic capacity. As mentioned above, the impact of the programs is difficult to compare as they differed in many characteristics of the load. However, this type of training places a greater strain on the body's anaerobic mechanism, with footballers receiving a greater neuromuscular load and the respiratory chain of the aerobic system being less burdened. It seems that this type of training is not the most suitable for improving aerobic capacity [4].

In the present study it was shown that IG improved its performance. In a similar study reported recently, Tønnessen et al. [25] observed an improvement in performance in the RSA test. Also improvements in indicators of RSA tests are reported by recent studies [1, 8, 16, 20]. However, there are also studies that do not report any improvement in performance in RSA after a repeated sprint training program [10, 14, 15]. The similarity of the RSA evaluation tests to the form of training may be a reason for an improvement in this ability. Also, the ability of RSAs depends on metabolic, nervous and mechanical factors [2]. Training programs that can improve one or more of these factors at the same time cause improvement in the RSA.

Based on the results it seems that IG improved its performance in the 30 m test. The literature shows that the impact of RST programs on the RSA is not clear. More specifically, there are studies that report significant improvement in a 30-40 m sprint [8, 25], but also studies that did not observe any improvement in performance [3, 16, 17]. Beato et al. [1] studied the incorporation of a change of direction in RST and while they noticed no improvement in the sprint (>20 m), they observed that the group that trained with a change of direction improved its acceleration in the 10 m test. The improvement may be due to various mechanisms. The RST training may have increased muscle metabolites, such as phosphocreatine, leading to better performance. Also neuromuscular changes such as increment in muscle fiber recruitment, firing frequency and motor unit synchronization can lead to improved performance [24].

In the present study no changes were observed in jumping performance. Based on literature sources it may be concluded that the jumping ability does not improve after the application of RST [12]. This method of training stimulates neuromuscular factors that could affect the jumping ability; however, it seems that the stimulus is not sufficient to act in this way. Since the goal is to improve jumping ability, more targeted contents in the reactive strength and stretch-shortening cycle (SSC) need to be applied.

No changes were observed for agility performance. In the available literature there are studies, which findings are in agreement with ours [1]. However, some studies mentioned improvement in the performance of soccer players in agility tests [8]. Agility tests include accelerations and decelerations, therefore the use of the stretch-shortening cycle (SSC) phenomenon is crucial in this performance. As in the jumps, a more specialized training stimulus is required in order to have an improvement in performance.

As mentioned above, each of the five different types of high intensity interval training causes different adjustments in athletes. Also, differences of programs in the characteristics of the load may explain the different findings. Finally, another factor that makes it difficult to compare the results is the different evaluation tests used in the studies.

Conclusions

In conclusion, in this study a significant improvement in performance was observed in the 30 m test, the RSAbest, the RSAmean and the RSAdecrement after the implementation of a HIIT-RST program that was applied two times a week for four weeks. The use of HIIT training is particularly widespread, as it improves performance in a short period of time, saving time for technical and tactical training. However, the lack of improvement in some tests indicates a relationship between the type of load and the adjustment achieved.

Conflict of Interest

The authors declare no conflict of interest.

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Differences between boys and girls in applied variables to assess motor skills

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Abstract
Introduction. Physical fitness is the capacity to perform different activities involved in everyday life. Aim of Study. The main goal of this study was to investigate gender differences among second graders in the physical activity level, physical fitness and fundamental movement skills. Material and Methods. In doing so, a sample of 78 children (35 girls, 43 boys, overall mean 7.34 ± ± 0.53 years) was measured using NPAQ (Netherland Physical Activity Questionnaire), FMS-Polygon, BOT-2 (Bruininks–Oseretsky Test of Motor Proficiency), F3 test and BMI. Results. The between-subject t-test was applied to determine whether a significant difference exists between the genders. Differences were found between boys and girls in applied physical fitness tests in two tests: F3 (t = 3.05; p = 0.003) and FMS-Polygon (t = –2.7; p = 0.007), while no significant differences were observed for BOT-2, physical activity (KA), sedentary activity (SA) and BMI. Conclusions. The applied between-subject t-test showed significant differences between the genders. Such differences were found between boys and girls in applied physical fitness tests in two tests F3 (t = 3.05; p = 0.003) and FMS-Polygon (t = –2.7; p = 0.007), while no significant differences were observed for BOT-2, physical activity (KA), sedentary activity (SA) and BMI.

KEYWORDS: children, physical activity, motor skills, sedentary activity, fundamental movement skills.

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Introduction

Physical fitness, defined as the capacity to perform physical activity, includes integrated measures of most body functions (skeletomuscular, cardiorespiratory, hematocirculatory, psychoneurological and endocrine-metabolic) involved in everyday physical life activity and physical exercise [24]. Therefore, physical fitness includes two categories: skill-related fitness described based on agility, speed, coordination, balance, power and reaction time, and health-related fitness such as cardiorespiratory fitness, muscular endurance, muscular strength and flexibility [21]. Studies report that a higher motor skill proficiency during childhood positively impacts physical activity among young people [6, 7]. Furthermore, increased physical activity is likely to have positive effects on preschool health and skill-related fitness qualities [14]. Moreover, a high level of physical fitness can also increase health outcomes and prevent chronic diseases, musculoskeletal problems and psychological health issues. It has been treated as a powerful marker of health [24]. Moreover, the acquisition of fundamental movement skills at preschool age is one of the most important predictors of physical fitness because of the natural form of movement in preschoolers, which is important in further specific sports skills [12]. Insufficiently stimulated and underdeveloped motor skills and knowledge at a young age may lead to decreased or delayed development of motor creativity [19].

Furthermore, previous studies had identified gender differences in physical fitness, which may to a greater extent affect adolescents [9]. Thus preschool-age boys have better results on some tests such as standing broad jump, shuttle run and arm hanging, while girls have better results in coordination and manual dexterity; nevertheless, the differences were small [18]. Researchers have reported that boys spend more time in various physical activities during the day than girls [14, 17]. Some studies have also reported a significant difference between genders in locomotor skills, where boys performed better in object control skills [5, 16]. In contrast, girls tend to have better locomotor skills [16]. Consequently, despite some gender differences some researchers state that there are no significant differences between genders in physical fitness [5]. Therefore, there is a lack of evidence in previous studies in identifying gender differences in this context. Therefore, this study aimed to investigate gender differences among second graders in their physical activity level, physical fitness and fundamental movement skills.

Material and Methods

Participants

The sample included 78 children (35 girls, 43 boys) with an average age of 7.34 ± 0.53 years. Participants for this study were recruited from two elementary schools attending the second grade according to the nine-year curriculum in Bosnia and Herzegovina. With the school principal's consent to conduct the research, a meeting was held with the parents of the respondents. Children with health problems and motor disorders were not included. All the participant's parents signed written consent forms and were fully informed of the purpose of the study. The research was conducted fully in accordance with the Declaration of Helsinki and approved by the institution's Ethics Committee.

Measures and procedures

Two experienced observers took all measurements at the beginning of June for five consecutive days before training at approximately the same time of the day between 9:00 a.m. and 10:30 a.m. during physical education classes. The FMS-Polygon test was taken three times and all the other tests were repeated once.

Gender was the independent variable analyzed in this study. Additional dependent tests included six quantitative variables. For the evaluation of physical activity and sedentary activity of children, parents were asked to complete the NPAQ [22] determining psychometric characteristics. The NPAQ consists of two sections: assessment of (1) physical activity, and assessment of (2) sedentary activities. The section that evaluates activity contains seven statements that parents must answer using a Likert scale (1-5) regarding how much they agree with the statement, with the total score being the mean value of all answers (KA). The part that evaluates the child's inactivity contains only two questions related to the average daily time spent doing sedentary activities (watching television and using a computer). The total is calculated by summing the two responses (SA). To assess fundamental movement skills an (3) obstacle polygon was used [29]. An obstacle polygon was designed with its reliability and validity proved [20]. More precisely, the polygon is based on the scientifically founded hierarchy of fundamental movement skills and includes the following tasks: passing and catching a volleyball against the wall as representing motor skills for object manipulation, jumping over sponge obstacles 50 cm high as representing motor skills to overcome obstacles, lifting and carrying 3 kg medicine balls as representing motor skills to overcome resistance and a 20-m run as representing motor skills to master space. The result required to overcome the polygon (FMS-Polygon) was recorded with a pair of photocells. In addition to the FMS-Polygon, a short form was used to assess the degree of motor skills acquisition, (4) Bruininks-Oseretsky Test of Motor Proficiency, second edition (BOT-2) [8]. The short version consists of 14 tests covering all 8 motor areas. The scoring system depends on the individual test (range of 2 to 13-point scales). The sum of all results gives the total motor quotient. Furthermore, children were tested in (5) F3 test (running distance in 3 minutes). Performance was determined from the running distance in meters covered in 3 full minutes in an track outdoor field. To gain a more detailed view of morphological features of the observed sample (6) the body mass index (BMI) was also calculated.

Statistical analysis

All data were presented as means ± standard deviation, minimum and maximum results. Gender differences were evaluated using the t-test for independent samples. Statistical analyses were performed using the Statistica 13.2 data analysis software system (Dell Inc., Tulsa, OK, USA). The type one error was set at $\alpha = 5\%$.

Results

Means ± SD values, minimum and maximum values in both observed groups were presented (Table 1).

Table 1. Descriptive statistics for parameters between genders

Variables	Total	Male	Female	Min	Max	p
	M ± SD	M ± SD	M ± SD			
F3 (meters)	479.56 ± 38.87	491.1 ± 36.41	465.40 ± 37.55	396	568	<0.001
BOT-2 (sum of point scale)	63.69 ± 5.94	63.47 ± 6.59	4.10 ± 0.50	46	75	0.71
Physical activity (hours)	4.01 ± 0.63	4.10 ± 0.50	3.89 ± 0.74	1.57	5	0.16
Sedentary active (minutes)	147.44 ± 67.47	150.23 ± 65.88	144.00 ± 70.18	30	390	0.68
FMS-Polygon (seconds)	25.51 ± 2.81	24.75 ± 2.73	26.46 ± 2.65	20.89	33.66	<0.01
Body mass index (kg/m^2)	17.71 ± 2.68	17.55 ± 2.73	16.79 ± 2.59	12.95	28.21	0.22

The t-test was applied to determine whether a significant difference exists between the genders. As can be seen in Table 1, there were significant difference between male and female first graders in F3 ($t = 3.05$; $p = 0.003$) and PBMZ ($t = –2.7$; $p = 0.007$), while no significant differences were observed for BOT-2, KA, SA and BMI.

Discussion

This study confirmed that there are general gender differences in physical fitness. Differences between boys and girls in applied physical fitness tests were identified in two tests, F3 and FMS-Polygon, while BOT-2, NPAQ and BMI showed no significant differences in performance related to locomotor and manipulative motor skills in children aged 6-7 years [20]. These findings are in agreement with the frequently observed gender differences in aerobic performance [11, 27]. Previous research studying aerobic capacity assumed that body composition has an impact on gender differences [9]. In this case, the F3 test confirms the distance between boys and girls. Accordingly, Armstrong and Welsman [3] claimed that these differences are due to changes in lean-body weight, body fat, hemoglobin and hormonal changes, but increase during puberty. To put it differently, girls have a higher level of leptin, which is strongly related to adipose tissue [26]. Also, some authors compared heart size with an aerobic difference, because girls have smaller heart sizes, indicating a smaller stroke volume [20].

Additionally, in terms of muscle mass boys also have an advantage over girls, which leads to gender differences [2]. Furthermore, girls have more imbalances in the development of a high burst of power [4]. For the most part, these claims are not so readily apparent in preschoolers and first graders, as all explanations have been speculative and rejected [13, 25].

Not only the F3 test, but also the FMS-Polygon, which can be characterized as a test assessing locomotor and manipulative motor skills, had shown gender differences, because in most cases boys are better in fundamental movement skills, where they are superior in object manipulation [28]. More precisely, they have an advantage over girls in strength and endurance [30]. Although this may be true, researchers concluded that this polygon test is also age-sensitive [10].

Furthermore, the BOT-2 test in the paper contains all motor skills, the sum of which gives the result of the total motor space, showed no gender differences. On the other hand, the differences were confirmed in the FMS-Polygon, which can be characterized as a test assessing locomotor and manipulative motor skills. Therefore, the obtained results certainly open the space for additional research that would determine the background responsible for the skills that differentiate boys and girls in fundamental movement skills. Based on previous knowledge, it can be argued that gender differences in fundamental motor skills are primarily defined by sports activities that children practice [23]. Nevertheless, similarly as the NPAQ test, BMI does not significantly affect performance in locomotor and manipulative motor skills in children aged 6-7 years [20].

The presented results suggest a careful observation of gender as a predictor of physical activity in early elementary school age. Correspondingly, from the observed six areas of physical fitness, differences were found in some tests, while in others there were no differences. It is in line with findings from previous research [15]. Therefore, it is an indicator of the need for individual analyses of different areas of physical fitness in the context of gender differences. In this way it would be possible to find the most effective teaching models for physical education as sports training in elementary school pupils in relation to gender differences [1].

Conclusions

The present study results indicate that differences in boys and girls were identified mainly in aerobic performance due to body composition. Also, differences are observed in fundamental movement skills characterized by strength and endurance, where boys have an advantage. Although we can say that the results of this study are generally accepted facts, they are often neglected in practice. This is especially true for the implementation of teaching physical education in the lower grades of elementary school, through which due to organizational shortcomings gender differences can be neglected. Moreover, differences can also be observed in sports clubs of younger age groups, where girls and boys train together. This practice certainly has many advantages, but it is still necessary to consider gender as a crucial factor in specified areas of physical fitness.

Consequently, the practical value of this paper is to point out once again the complexity of the approach to the implementation of physical education programs in view of gender differences and various aspects of physical fitness.

Therefore, it is obvious that gender cannot be unambiguously viewed as a predictor of physical fitness, but it is necessary to take into account the specifics of individual factors of fitness and observe them individually. This approach will ensure the most complete development of all areas of physical fitness in boys and girls.

Conflict of Interest

The authors declare no conflict of interest.

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Planned load reduction strategies to maintain optimal repetitions for hypertrophy training in leg press for women

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Abstract
Introduction. According to the ACSM's position stand on resistance training, there is strong evidence (category A) that the optimal number of repetitions to optimize hypertrophy should be between 8 and 12 repetitions per set for each exercise. Aim of Study. We investigated which intensity scheme would maintain 8-12 repetitions during repeated sets of resistance exercise performed until muscular failure. Material and Methods. Twenty-eight resistance-trained women (age = 26.1 ± 6.6 years, body mass = 66.2 ± 5.94 kg, height = 165 ± 6 cm) were tested over a five-week period. In week 1 the subjects were tested for 10RM leg press. In weeks 2-5 the subjects completed four sets of leg presses performed until muscular failure, with 60 s inter-set rest intervals in a randomized, counterbalanced order. Set 1 of each bout was performed at 10RM, with differing intensity for sets 2-4 as follows: (CON) 10RM load for all sets, (RED5) 5% load reduction after each set, (RED10) 10% load reduction after each set, and (RED15) 15% load reduction after each set. Results. The number of repetitions completed differed ($p < 0.001$) depending on the conditions. Repetitions were reduced below set 1 in sets 2-4 under CON ($p < 0.05$) and for sets 3-4 ($p < 0.05$) in RED5. RED10 and RED15 resulted in increased repetitions during sets 2-4 (p 60%) of sets in the range of 8-12 repetitions, where both CON and RED15 resulted in <50% of sets in the range of 8-12 repetitions. Time under tension (TUT) was kept within a 20-70 s per set window for most sets (95% CI) for RED10 and RED15. Conclusions. Load reductions of 5-10% in subsequent sets should allow for the maintenance of 8-12 repetitions for most sets of resistance exercise, with load reductions of 10% more likely to maintain optimal TUT.

KEYWORDS: **resistance training, volume, intensity, hypertrophy.**

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Introduction

Training to optimize muscle hypertrophy is a common goal for health and performance. Several training variables (intensity level, numbers of sets, number of repetitions per set, movement tempo, rest intervals between sets and sessions, exercise order, etc.) have been proposed as necessary to optimize gains in muscle hypertrophy [5, 7, 8, 14].
The number of repetitions per set, for example, has been typically proposed as a vital program component ensuring optimum hypertrophy. The ACSM points to support from evidence category A (evidence from well-designed randomized control trials) that provide a consistent pattern of findings in the population, for which the recommendation is made [1, 2, 7] that the optimal number of repetitions to optimize hypertrophy should be between 8 and 12 repetitions per set for each exercise. This proposal is shared by several other authors [4, 9, 17]. Related to the number of repetitions performed, the concept of total time under tension (TUT) placed on muscles during an exercise bout is

thought to be key to muscle hypertrophy as well, with TUT recommendations to optimize hypertrophy ranging from 20-70 s per set over multiple sets [12-14].

The ability to complete a given number of repetitions, of course, is dependent on the load lifted. It has anecdotally been proposed that loads lifted must be sufficient to accrue a certain degree of fatigue during each work set, independent of the number of repetitions performed [3, 9]. Thus some researchers have proposed that no fixed number of repetitions should be established; instead trainees should rather work to failure (voluntary exhaustion) during every work set [3]. The case for maintaining repetitions in the 8-12 range is enhanced (vs lifting heavier loads for fewer repetitions or lighter loads for more repetitions) by TUT recommendations for achieving hypertrophy [14], which tend to coincide with the 8-12 range. It is still controversial which method would be more efficient to optimize muscle hypertrophy. The major difficulty has been to develop a methodology that allows for an efficient comparison of the two proposals [15, 17].

When training to failure, volume and intensity have an inverse relationship. Thus, when choosing the number of repetitions to perform, the load must allow for completion of all planned repetitions in each set. This format is difficult to fulfill, as has been amply demonstrated in the literature that fatigue from previous sets performed reduces the number of repetitions completed per set and consequent TUT [9, 15, 16]. The length of the rest interval between sets crucially affects performance, with the obvious inverse relationship between the rest interval and the amount of work that may be performed [17]. A fairly short (1-2 min) inter-set rest intervals is recommended to optimize hypertrophy during resistance exercise [1].

Planned load reductions, in which the load lifted is reduced by a fixed amount over several sets, have been investigated as a strategy to allow for the maintenance of repetition numbers over multiple sets in the face of accumulated fatigue. Several studies have shown that load reductions in the range of 5-15% per set can preserve or even increase the number of repetitions completed over several successive sets [7, 17]. In theory, maintaining repetitions within this range should provide a superior stimulus for hypertrophy, though the only training study investigating load reduction to date [5] found equal results between constant loading and either 5 or 10% load reduction over a 16-week period.

Aim of Study

Thus, several studies have been carried out to verify which would be the best load reduction condition to allow for the maintenance of repetitions in the optimal hypertrophy range [5, 7, 8, 15]. However, only one study [17] employed women subjects, creating a gap in knowledge and making it difficult for athletes and coaches to adopt the method. The purpose of this study is to investigate how much load reduction (in relative terms) is necessary to maintain the majority of repetitions per set and TUT within a target zone (8 to 12 repetitions, 20-70 s) for the leg press exercise in women with a 1-minute interval between sets.

Material and Methods

Experimental approach to the problem

The current study was conducted over five weeks; during the first week anthropometric measures (e.g. height, body mass) were collected and 10RM leg press tests were repeated 72 hours apart to verify reliable loads. During each of the succeeding four weeks the subjects performed one resistance exercise session that involved performance of leg press. During each session one of the following randomly ordered loading condition was applied: a) a constant 10RM load for all the four sets (CON), b) a 5% reduction following each set (RED5) (i.e. 10RM, 95% of 10RM, 90% of 10RM, and 85% of 10RM), c) a 10% reduction following each set (RED10), and d) a 15% reduction following each set (RED15).

The 1-min rest interval between sets has been previously recommended as a training strategy to maximize hypertrophy [1]. The premise behind is that shorter rest intervals increase metabolic and hormonal response to exercise [4, 5], which may have a positive impact on stimulating greater muscle protein synthesis. Multiple sets to failure with short rest intervals are often practiced in hypertrophy training.

Subjects

Twenty-eight women (age = 26.1 ± 6.6 years; body mass = = 66.2 ± 5.94 kg; height = 165 ± 6 cm; BMI = 24.4 ± ± 1.5) with min. two years of recreational resistance training experience participated in the current study. All the subjects were characterized by the following training history: consistent participation in a resistance training program during the two previous years with a minimum training frequency of three sessions per week; one hour per session; three to five sets per exercise; six to fifteen repetitions per set; leg press experience; experience in repetitions maximum sets (to failure); and 1 to 2 minutes rest intervals between sets. Additional exclusion criteria were: a) subjects could not be using drugs or nutritional supplements that could affect repetitions performance within min. three previous months; b) subjects could not exhibit bone, joint or muscular problems that could limit the effective execution of leg press exercise; and c) subjects could not be performing any extraneous structured exercise for the duration of the study. All the participants read and signed an informed consent form, which thoroughly explained the testing procedures; the experimental procedures were approved by the Unig Campus V Ethics Committee and were in accordance with the Declaration of Helsinki by the World Medical Association (WMA) as a statement of ethical principles for medical research involving human subjects.

Procedures

Each subject completed one exercise session per week for five weeks. Each exercise session was conducted on a consistent day and time each week. A strength and conditioning specialist supervised each exercise session to ensure proper technique and provide spotting and verbal encouragement.

Week 1 was the preparatory period, during which 10RM loads were established for the leg press according to previously published procedures [4]. The 10RM for each exercise was assessed two times with 72 hours between tests. Before the 10RM tests each subject completed 5 minutes of low-intensity aerobic activity (i.e. jogging/walking). Two warm-up sets preceded testing of each exercise at 50% of the perceived 10RM load for 10 repetitions each. After the warm-ups sets were completed, the load was increased to the perceived 10RM and one set was performed to voluntary exhaustion (i.e. muscle failure). The same spotters closely supervised each 10RM attempt and the subjects were instructed to give a verbal signal when voluntary exhaustion was reached. If fewer than or more than 10 repetitions were accomplished during a given 10RM attempt, the load was adjusted during the next testing session. Load adjustments during these sessions followed this plan – in cases of fewer than 10 repetitions lifted, the load was reduced by 10%. In cases where the subject continued beyond a 10th repetition, testers stopped the subject on the 11th repetition and a subjective determination, from 5-10%, was made for increased load on the second session. Several (three) subjects required a third testing session to establish a 10RM. In these cases an additional 72 h rest was provided before the final 10RM determination.

The 10RM loads established during the preparatory period were used to design subsequent testing sessions. Weeks 2, 3, 4, and 5 were the data collection period, during which the subjects completed one lower-body testing session per week under one of the following load conditions: (a) constant load for all sets (CON), (b) 5% load reduction after each set (RED5), (c) 10% load reduction after each set (RED10), and (d) 15% load reduction after each set (RED15). The conditions were randomized and counter-balanced to control order effects.

Each testing session during the data collection period began with 5 minutes of low-intensity aerobic activity (i.e. jogging/walking). Two warm-up sets were performed at 50% of the predetermined 10RM for 15 repetitions each.

Three minutes after the warm-up sets four consecutive sets were performed to the point of voluntary exhaustion (i.e. full repetition maximums). The subjects were allowed exactly 1 minute of rest between sets. The rest intervals were precisely controlled through the use of a handheld stopwatch.

Proper execution of the leg press was defined as follows: subjects started in an initial position of 0° knee flexion (extended knees) and then participants were asked to lower the sled by bending their knees and flexing their hips in a controlled manner up to a position of 90° knee flexion and 60° hip flexion, which was set as the turnover point. The subjects' feet were positioned a shoulder-width apart at the middle point on the leg press footplate. Repetitions were performed at a cadence determined by a metronome (2 s between sounds) – with a goal tempo of 2/0/2/0, meaning 2 s each for eccentric and concentric movements and no pause in the transition phase [12].

Statistical analysis

All data are presented as means ± standard deviation. Reliability of the 10RM loads for leg press exercise was assessed with the intraclass correlation (ICC) and reliability was described as 'excellent' for ICC values in the range of 0.8-1.0 and 'good' for 0.6-0.8, whereas values below 0.6 were 'poor' [10]. Repetitions completed were assessed with a two-way mixed model analysis of variance (4 sets × 4 load conditions) with repeated measures. Multiple comparisons were made according to Bonferroni's method with a significance level $p < 0.05$. Volume load (total repetitions completed × load) was calculated and compared between the applied conditions. Statistical analyses were completed using JAMOVI 1.8 software (Sydney, Australia).

Results

We observed a significant difference in the number of repetitions performed depending on the conditions

(ANOVA repeated measures with the Greenhouse–Geisser sphericity correction; $p < 0.001$; $\eta^2 = 0.857$) (Table 1). Sphericity was not assumed (Mauchly's W $p < 0.001$).

Table 1. Number of repetitions per set (mean ± SD [95% CI]) at four conditions: CON (without load reduction), RED5 (with 5% load reduction for each set), RED10 (with 10% load reduction for each set), RED15 (with 15% load reduction for each set)

Condition	1st set	2nd set	3rd set	4th set
CON	11.2 ± 1.0 [10.8 to 11.7]	7.3 ± 0.8 [7.0 to 7.7] (a)	5.8 ± 1.2 [5.3 to 6.4] (a,b)	4.2 ± 1.0 [3.7 to 4.6] (a,b,c)
RED5	11.6 ± 0.9 [11.2 to 12.0] (c,d)	8.4 ± 0.8 [8.1 to 8.8] (a,c,d,e)	7.1 ± 1.3 [6.5 to 7.7] (a,d,e)	5.4 ± 1.4 [4.7 to 6.0] (a,b,e,f,g)
RED10	11.2 ± 1.3 [10.6 to 11.8] (b,c,d,f,g,h)	9.2 ± 1.3 [8.6 to 9.8] (a,b,c d,e,h,i)	8.2 ± 1.2 [7.7 to 8.8] (a,c,d,e,h,i,j)	7.3 ± 1.1 [6.8 to 7.8] (a,c,d,e,h,j,k)
RED15	11.2 ± 1.0 [10.8 to 11.7] (l)	11.7 ± 1.4 [11.0 to 12.3] (l)	13.1 ± 1.4 [12.4 to 13.7] (m)	14.0 ± 1.4 [13.3 to 14.7] (m)

Statistical results from post hoc test (Bonferroni's correction): (a) difference from control 1st set ($p < 0.05$); (b) difference from control 2nd set ($p < 0.05$); (c) difference from control 3rd set ($p < 0.05$); (d) difference from control 4rd set ($p < 0.05$); (e) difference from RED5 1st set ($p < 0.05$); (f) difference from RED5 2nd set ($p < 0.05$); (g) difference from RED5 3rd set ($p < 0.05$); (h) difference from RED5 4th set ($p < 0.05$); (i) difference from RED10 1st set ($p < 0.05$); (j) difference from RED10 2nd set ($p < 0.05$); (k) difference from RED10 3rd set ($p < 0.05$); (l) difference from all other values ($p < 0.05$) except for CON and RED5 1st set; (m) difference from all other values ($p < 0.05$)

Table 2. Average number of repetitions (mean ± SD [95%CI]) across sets and volume load (sets × repetitions × load) for each condition: CON (without load reduction), RED5 (with 5% load reduction for each set), RED10 (with 10% load reduction for each set), RED15 (with 15% load reduction for each set)

	CON	RED5	RED10	RED15
Average repetitions per set (range)	7.2 ± 0.7 [6.9 to 7.5]	8.2 ± 0.7[a,c] [7.8 to 8.5]	9.0 ± 0.9[a,b,c] [8.6 to 9.4]	12.5 ± 1.0[a,b] [12.0 to 13.0]
Volume load completed per bout (kg)	5575 ± 540 [5326 to 5825]	5965 ± 481 [5743 to 6187]	6078±627[a] [5788 to 6368]	7410 ± 569[a,b,c] [7148 to 7673]

[a] difference with CON ($p < 0.01$); [b] difference with RED5 ($p < 0.01$); [c] difference with RED10 ($p < 0.01$)

Table 3. Time under tension (seconds) per set (mean ± SD [95%CI]) at four conditions: CON (without load reduction), RED 5 (with 5% load reduction for each set), RED10 (with 10% load reduction for each set), RED15 (with 15% load reduction for each set)

Condition	1st set	2nd set	3rd set	4th set
CON	44.9 ± 4.0 [43.0 to 46.7]	29.3 ± 3.4 [27.8 to 30.9] (a)	23.3 ± 4.8 [21.1 to 25.6] (a,b)	16.7 ± 3.9 [14.8 to 18.5] (a,b,c)
RED5	46.2 ± 3.4 [44.6 to 47.8] (c,d)	33.8 ± 3.1 [32.3 to 35.2] (a,c,d,e)	28.4 ± 5.3 [26.0 to 30.9] (a,d,e)	21.6 ± 5.7 [18.9 to 24.6] (a,b,e,f,g)
RED10	44.7 ± 5.2 [42.3 to 47.1] (b,c,d,f,g,h)	36.9 ± 5.2 [34.6 to 39.3] a,b,c.d,e,h,i)	32.9 ± 4.9 [30.6 to 35.1] (a,c,d,e,h,i,j)	29.1 ± 4.3 [27.1 to 31.1] (a,c,d,e,h,j,k)
RED15	44.9 ± 4.0 [43.0 to 46.7] (l)	46.7 ± 5.7 [44.1 to 49.3] (l)	52.2 ± 5.7 [49.6 to 54.9] (m)	56.0 ± 5.7 [53.4 to 58.6] (m)

Statistical results from post hoc test (Bonferroni's correction): (a) difference from control 1st set ($p < 0.05$); (b) difference from control 2nd set ($p < 0.05$); (c) difference from control 3rd set ($p < 0.05$); (d) difference from control 4rd set ($p < 0.05$); (e) difference from RED5 1st set ($p < 0.05$); (f) difference from RED5 2nd set ($p < 0.05$); (g) difference from RED5 3rd set ($p < 0.05$); (h) difference from RED5 4th set ($p < 0.05$); (i) difference from RED10 1st set ($p < 0.05$); (j) difference from RED10 2nd set ($p < 0.05$); (k) difference from RED10 3rd set ($p < 0.05$); (l) difference from all other values ($p < 0.05$) except for CON and RED5 1st set; (m) difference from all other values ($p < 0.05$)

The analysis of variance (ANOVA) showed a statistically significant difference ($p < 0.001$) when comparing the average number of repetitions performed per condition (Table 2) and TUT (Table 3).

Specifically, for sets 2-4 all RED conditions allowed for more repetitions performed and longer TUT than CON ($p < 0.001$). Total volume load lifted across all the sets was increased ($p < 0.01$) above CON in RED10 (+9%) and RED15 (+33%) (Table 2).

We also found a large effect size ($\eta^2 = 0.859$). Homogeneity of variance (Levene's $p = 0.186$) and the normality test (Shapiro–Wilk's $p = 0.211$) were adequate for ANOVA. Similar results were found in total load (sets × repetitions × load) ($p < 0.001$; $\eta^2 = 0.620$).

Discussion

The main finding of the present study is that short rest intervals (60 s) greatly reduce the training volume performed over successive sets, but this can be compensated by an adequate load reduction. These results were consistent with prior studies that examined exercises with a constant load [6, 14] and with load reduction in men [5, 7, 11] and women [17]. On the other hand, RED15 induced significant increases in repetitions per set.

In an earlier study on load reduction Willardson and Burkett [15] compared men's performance in back squat, leg curl and leg extension exercises. They found that back squat and leg curl required 15% load reductions per set to maintain repetition performance and load reductions were not necessary for leg extension. In another study women's performance on the bench press, wide grip front lat pulldown and back squat with 5, 10 and 15% load reductions was compared with a constant load [17]. In this case the authors found that for the wide grip front lat pulldown and back squat a 10% load reduction was necessary following the first and second sets to accomplish 10 repetitions on all three sets. For the bench press a load reduction between 10% and 15% was necessary. For the back squat a 15% load reduction resulted in an increase in the number of repetitions performed (9.8 ± 0.3 vs 14.0 ± 1.7 vs 14.5 ± 1.4).

In our study of the leg press CON (7.2 ± 0.7), RED5 (8.2 ± 0.7) and RED10 (9.0 ± 0.9) completed an average of <10 repetitions per set. This is an issue if the trainer or athlete intends to work 10RM loads with short rest intervals between sets. We can speculate that even shorter intervals (such as 30 or 45 s) will possibly unproductively reduce the volume of repetitions.

Similar to the prior findings of Willardson et al. [17] for the back squat exercise, the present study found the RED15 resulted in an increase in the number of leg press repetitions performed over consecutive sets. In both cases the increase in repetitions performed over successive sets led to repetition numbers higher than the 8-12 window recommended for hypertrophy, which may indicate that the load was lightened below optimal levels for developing hypertrophy.

Findings in the present study are similar to those of Medeiros et al. [7], who investigated load reduction in the leg press exercise in men under very similar conditions. In both studies four sets of leg press with 60 s rest were compared under CON, RED5, RED10 and RED15 conditions in recreationally trained subjects. Similarly to the present data, Medeiros et al. [7] found both RED5 and RED10 allowed for the maintenance of most repetitions within an 8-12 range.

Time under tension and repetition tempo are not as often addressed as the number of repetitions training variables; however, they are recognized as important contributors to muscle hypertrophy [14]. Logically TUT is related to the number of repetitions performed, while in the present study, in which repetitions were performed at a set (2/0/2/0) cadence, the pattern of change between TUT over sets was identical to the pattern seen with the number of repetitions (Table 3). Namely, RED5 and RED10 maintained TUT above levels seen on CON, while RED15 resulted in an increased TUT above all conditions. The optimal window for TUT has been suggested to be between 20 and 70 s per set [14]. Under RED10 and RED15 conditions most subjects (95% CI) were able to maintain this level of TUT over four successive sets, but not for CON and RED5.

In theory, load reduction should allow for the completion of a greater volume load lifted over time and consequently provides a greater stimulus to develop skeletal muscle hypertrophy. Todate, evidence supporting this theory is lacking. Lima et al. [5] provided the only longitudinal study comparing the effects of training with load reduction (RED5 and RED10) to constant loading. In their study recreationally-trained men completed 16 wks of $3d \cdot wk^{-1}$ training on two forms of bicep curls. At the conclusion of 16 wks no measurable differences were observed in muscle hypertrophy, 10RM bicep curl strength, or average volume load lifted throughout the intervention. The subjects who trained with a RED10 protocol during this study did achieve similar results to the other conditions, although while experiencing a lower perception of effort during training [5].

Conclusions

Our work has the novel aspect that it evaluated performance on leg press and focused on women subjects. The volume load lifted could be important for resistance training adaptations [2, 5, 9]. Counterintuitively, load reduction had more effect on the number of repetitions than on volume load, as all load reductions differed from CON in repetitions completed (Table 2). Of note in this experiment, RED10 produced a maintenance of repetitions in the 8-12 range, TUT within the recommended optimal window and a significantly higher volume load lifted than CON, all of which may possibly be advantageous for increasing muscle hypertrophy over chronic training periods. This result is not supported by previous studies [5, 7, 11, 15, 17], but as the number of studies is still low, an analysis of how the total weight lifted is affected by the sex of the participants needs to be further evaluated.

Conflict of Interest

The authors declare no conflict of interest.

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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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