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

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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 (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).

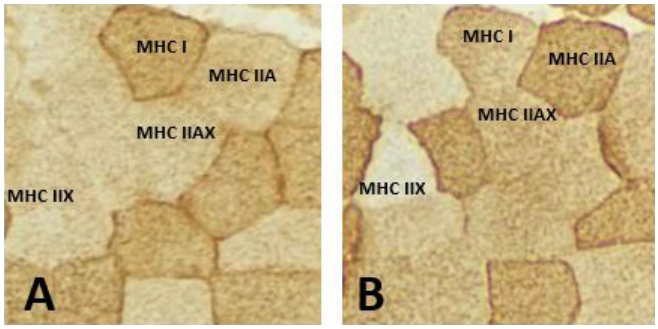


Figure 1. Immunohistochemical staining of serial cross-sections with: A) antibody A4.840 and B) antibody N2.261, for the identification of muscle fibre type distribution

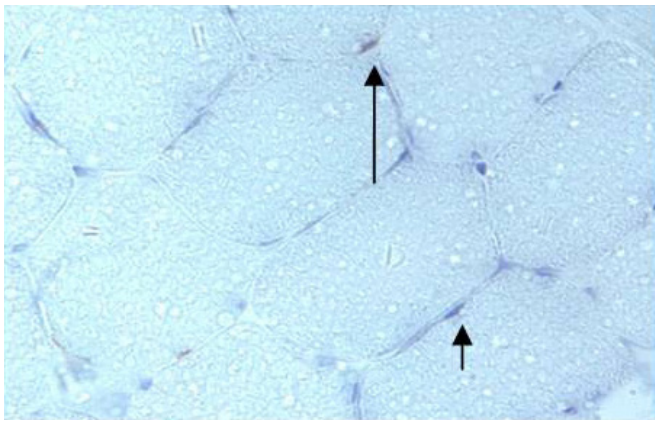


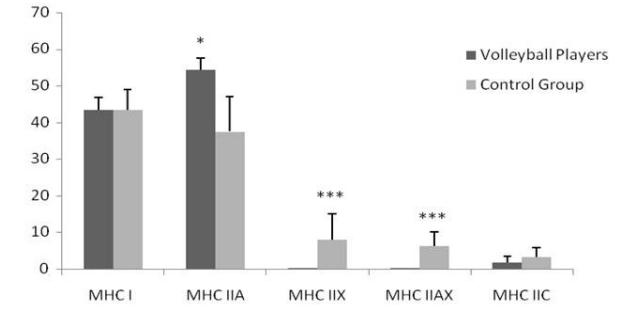
Figure 2. Immunohistochemical staining with antibody CD 56 and counterstained with Mayer's hematoxylin for the visualisation of myonuclei (short arrow) and satellite cells (long arrow)

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.

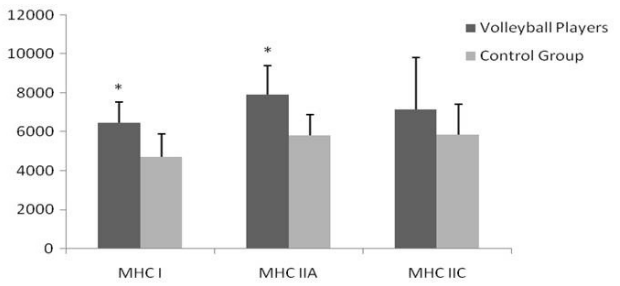
Results

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



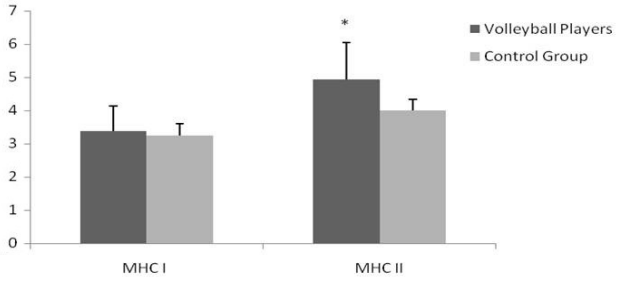
* $p < 0.05$, *** $p < 0.001$

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



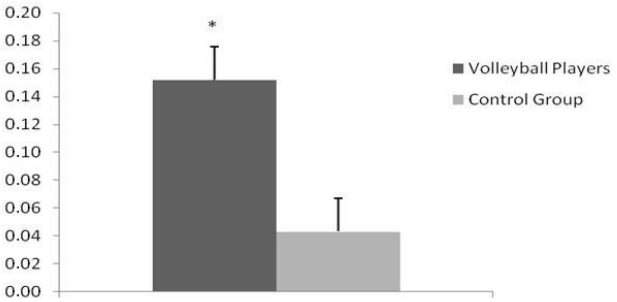
* p < 0.05

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.

References

1. Adams GR, Hather BM, Baldwin KM, Dudley GA. Skeletal muscle myosin heavy chain composition and resistance training. J Appl Physiol. 1993;74(2):911-915.
2. Andersen JL, Aagaard P. Effects of strength training on muscle fiber types and size; consequences for athletes training for high-intensity sport. Scan J Med Sci Sports. 2010;20(Suppl 2):32-38.
3. Andersen JL, Aagaard P. Myosin heavy chain IIX overshoot in human skeletal muscle. Muscle Nerve. 2000;23(7):1095-1104.
4. Balasas DG, Christoulas K, Stefanidis P, Vamvakoudis E, Bampouras TM. The effect of beach volleyball training on muscle performance of indoor volleyball players. J Sports Med Phys Fitness. 2018;58(9):1240-1246.
5. Balasas DG, Kellis S, Christoulas K, Bampouras TM. An off-season plyometric and resistance training programme to improve vertical jump height in adolescent female volleyball players. J Sci Sport Exer. 2021. https://doi.org/10.1007/s42978-021-00136-y.
6. Blomstrand E, Celsing F, Fridén J, Ekblom B. How to calculate human muscle fibre areas in biopsy samples – methodological considerations. Acta Physiol Scand. 1984; 122(4):545-551.
7. Bottinelli R, Betto R, Schiaffino S, Reggiani C. Unloaded shortening velocity and myosin heavy chain and alkali light chain isoform composition in rat skeletal muscle fibres. J Physiol. 1994;478:341-349.
8. Bottinelli R, Schiaffino S, Reggiani C. Force-velocity relations and myosin heavy chain isoform compositions of skinned fibres from rat skeletal muscle. J Physiol. 1991;437:655-672.
9. Cheek DB. The control of cell mass and replication. The DNA unit – a personal 20 year study. Early Hum Dev. 1985;12:211-239.
10. Folland JP, Williams AG. Morphological and neurological contributions to increased strength. Sports Med. 2007; 37:145-168.

11. Forthomme B, Croisier JL, Ciccarone G, Crielaard JM, Cloes M. Factors correlated with volleyball spike velocity. Am J Sports Med. 2005;33(10):1513-1519.
12. Gilliver SF, Degens H, Rittweger J, Sargeant AJ, Jones DA. Variation in the determinants of power of chemically skinned human muscle fibres. Exp Physiol. 2009;94:1070-1078.
13. Holm S. A simple sequentially rejective multiple test procedure. Scan J Stat. 1979;6(2):65-70.
14. Kadi F. Adaptation of human skeletal muscle to training and anabolic steroids. Acta Physiol Scand (Suppl). 2000;646:1-52.
15. Kadi F, Eriksson A, Holmner S, Butler-Browne GS, Thornell LE. Cellular adaptation of the trapezius muscle in strength-trained athletes. Histochem Cell Biol. 1999; 111(3):189-195.
16. Kadi F, Eriksson A, Holmner S, Thornell LE. Effects of anabolic steroids on the muscle cells of strength-trained athletes. Med Sci Sports Exer. 1999;31(11):1528-1534.
17. Kadi F, Schjerling P, Andersen LL, Charifi N, Madsen JL, Christensen LR, et al. The effects of heavy resistance training and detraining on satellite cells in human skeletal muscles. J Physiol. 2004;558:1005-1012.
18. Kadi F, Thornell LE. Concomitant increases in myonuclear and satellite cell content in female trapezius muscle following strength training. Histochem Cell Biol. 2000;113(2):99-103.
19. Kountouris P, Drikos S, Aggelonidis I, Laios A, Kyprianou M. Evidence for differences in men's and women's volleyball games based on skills effectiveness in four consecutive olympic tournaments. Compr Psychology. 2015;4(9):1-7.
20. Kraemer WJ, Patton JF, Gordon SE, Harman EA, Deschenes MR, Reynolds K, et al. Compatibility of high-intensity strength and endurance training on hormonal and skeletal muscle adaptations. J Appl Physiol. 1995; 78:976-989.
21. Lexell J, Taylor CC. Variability in muscle fibre areas in whole human quadriceps muscle: how to reduce sampling errors in biopsy techniques. Clin Physiol. 1989;9(4): 333-343.
22. Lidor R, Ziv G. Physical characteristics and physiological attributes of adolescent volleyball players-a review. Ped Exer Sci. 2010;22:114-134.
23. McCall GE, Byrnes WC, Dickinson A, Pattany PM, Fleck SJ. Muscle fiber hypertrophy, hyperplasia, and capillary density in college men after resistance training. J Appl Physiol (1985). 1996;81(5):2004-2012.
24. Ruxton GD. The unequal variance t-test is an underused alternative to Student's t-test and the Mann–Whitney U test. Behav Ecol. 2006;17(4):688-690.
25. Sheppard JM, Gabbett TJ, Taylor KL, Dorman J, Lebedew AJ, Borgeaud R. Development of a repeated-effort test for elite men's volleyball. Int J Sports Physiol Perform. 2007;2(3):292-304.
26. Sinha-Hikim I, Artaza J, Woodhouse L, Gonzalez-Cadavid N, Singh AB, Lee MI, et al. Testosterone-induced increase in muscle size in healthy young men is associated with muscle fiber hypertrophy. Am J Physiol Endocrinol Metab. 2002;283(1):E154-E164.
27. Sleivert GG, Backur RD, Wenger HA. Neuromuscular differences between volleyball players, middle distance runners and untrained controls. Int J Sports Med. 1995;16(5):390-398.
28. Smerdu V, Karsch-Mizrachi I, Campione M, Leinwand L, Schiaffino S. Type IIx myosin heavy chain transcripts are expressed in type IIb fibers of human skeletal muscle. Am J Physiol. 1994;267(6 Pt 1):C1723-C1728.
29. Sotiropoulos K, Drikos S, Papadopoulou SD, Barzouka K. Characterizing adaptations of serve indicators in top-level male volleyball among seasons. Int J Sport Sci Coach. 2021;16(3):784-792.
30. Ziv G, Lidor R. Vertical jump in female and male volleyball players: a review of observational and experimental studies. Scan J Med Sci Sports. 2010;20(4):556-567.