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Original Article

The increase of VO2 max variation and the specific biochemical parameters in soccer players after a pre-season training program

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Abstract:
The purpose of this study was to examine the effect of a 56 days preparation training program, regarding parameters associated with cardiovascular risk indicators, aerobic ability and anthropometric characteristics of professional soccer players. The sample consisted of 25 professional players who competed in teams of the Greek Super League Soccer Championship. All athletes were measured on two levels, one before the start of preparation and one after the end of preparation. The parameters examined were the maximum oxygen uptake (VO_2 max), anaerobic power and anthropometric characteristics (weight and percentage fat composition). Furthermore, the biochemical parameters of the lipid profile, such as total cholesterol (T-C), high density lipoprotein (HDL-C) the HDL2, the HDL3, triglycerides (TG), the low density lipoprotein (LDL-C) and VLDL were evaluated. Statistical analysis of data was performed using the statistical program SPSS (version 15.0). A comparison of the variables measured before and after training preparation was made with the t-test for lateral distribution repeated measurements. The level of significance was set at $p<0.05$. The study results showed that coaching preparation drastically reduced footballers' body fat (t = -4,97, df = 58, p <0,002). Moreover, there was a remarkable improvement in the anaerobic capacity of athletes (t = -2,97, df = 58, p <0,02), as assessed with this maximum power in the Wingate test and the players' aerobic capacity (t = 9 , 98, df = 58, p <0,005), as assessed by the maximal oxygen uptake on the treadmill . In terms of the indicators of the lipid profile, the values of T-C compared with those before the start of preparation (t = 9,73, df = 58, p <0,001) dramatically decreased. The values of t HDL-C significantly increased too, compared with those before the start of preparation (t = 4,39, df = 58, p <0,001). The same happened with the values of HDL2 (t = 5,01, df = 58, p <0,001) and HDL3 (t = 6,90, df = 58, p <0,001). On the other hand, the values of the TG, compared with those before the start of training (t = 9,26, df = 58, p <0,001), significantly decreased. Regarding the LDL-C levels it was observed that they appeared considerably reduced compared to those before the start of training (t = 9,02, df = 58, p <0,001), while the same happened to the values of VLDL (t = 9, 54, df = 58, p <0,001). The study results showed a significant effect of training preparation in all subjects' above indicators, this should be evaluated by all those involved in the training of players.
Key words : Soccer, preparation period, VO_2 max, lipid profile

Introduction

Soccer is a dynamic sport that is especially demanding and requires stamina, strength, speed, coordination, flexibility and jumping. It also requires a good amount of energy for the best possible performance by an athlete and it is characterized by changes in rhythm between high and low intensity. In a soccer game the aerobic spaces are interspersed with the anaerobic spaces (lactate and non-lactate) which lead the athlete to extreme metabolic conditions. For instance, an athlete during a soccer game consumes about 1360 btus (Bangsbo, 1994). Furthermore, it is known that the energy requirements are varied, according to the athletes position (Di Salvo & Vand Gozzi, 1998). Performance in soccer depends on many attributes, with the main ones being the elements of physical condition. One of those is aerobic stamina, which plays an important role, not only in resisting weariness, but also in fast recovery after aggravation. By progressively developing the methods of training and technology, the physical condition of athletes has been improved. Today, soccer players cover distances between 9-12 kilometers per game (Stolen et a.l, 2005, Souglis et al., 2013), with their pulse being between 150-190 per minute (Bangsbo, 1994).

The soccer player is a multi-layer athlete with various skills, in aerobic and anaerobic ability. Aerobic ability, which is the most representative parameter of cardiovascular function, and is calculated by VO_{2max}, differs in terms of category (amateurs, professionals) and in terms of spaces. Greek professional players have been found to have values of 54,8 ± 4.9 ml/kg/min (Tokmakidis et al., 1986), while the highest values ever

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recorded were in German athletes with 62.0 ± 4.5 ml/kg/min (Hollmann et al., 1981). Also, we need to note that in terms of stamina, the maximum possible intake of oxygen (% VO_2 max) at the anaerobic threshold (Weineck, 1997). The percentage of body fat the soccer player has also plays a decisive factor in performance and high values reduce the maximizing of athletic ability. It has been reported that as the level of performance rises, the percentage of body fat reduces (Ekblom, 1994). In Greek soccer players, there have been reported values of 9,2% (Tokmakidis et al., 1986).

Many epidemiological studies suggest that systematic aerobic exercise brings about a range of positive changes in the levels of lipids and lipoproteins in the blood of the male and female population. Aerobic exercise changes the concentrations of TG, T-C, HDL-C, and LDL-C in the blood (Pronk, 1993; Apostolidis et al., 2014). Because of the positive changes through aerobic exercise, many studies took place in clinical populations. Kelley et al. (2005), in their meta-analysis examined the effect of systematic aerobic exercise in lipids and lipoproteins in overweight and fat adults and realized that the effects were positive, as important changes were documented in both groups, with individuals with higher body-fat percentage presenting the greatest effects. In another study by Kelley et al. (2004), they examined the effect of aerobic training in the lipodemic profile of adults with cardiovascular disease and they recorded a substantial reduction of TG and an increase of HDL-C after an interventional protocol of aerobic exercise. Leon & Sanchez (2001) reach the same conclusions, as they found out a reduction in TG and LDL-C and increase in HDL-C. Also, Kraus et al. (2002) concluded that participation in active exercise reduces the lipodemic profile of people, including the reduction of HDL-C, as well as sub-categories of HDL, without substantial weight loss. Halverstand et al. (2007) realized that elder people who trained for 24 weeks significantly reduced their T-C, TG and LDL-C values, while increasing HDL-2 and HDL-3.

Concerning aerobic exercise and its effect in athletes, data from various studies report lower TG levels in physically active individuals and primarily in endurance athletes, compared to people whose sport does not include aerobic exercise (Aellen et al., 1993; Pronk, 1993; Durstine & Haskell, 1994). Furthermore, epidemiological studies from the last decades, concerning aerobic exercise, report that this kind of training, as well as sustained training reduce T-C (Countinho and da Cunha1989; Oyelola & Rufai, 1993; Apostolidis et al., 2014) and LDL-C (Krustrup et al., 2009), while pointing out that individuals who exercise aerobically or work in a physically demanding job, increase their HDL cholesterol compared to individuals who don't (Kokkinos et al., 1995; Apostolidis et al., 2014). On the other hand, it is clear that exercise benefits the lipodemic profile in adults and children (Berlin et al., 1990; Kyle et al., 1991; Saakshahti et al., 1999). Changes in the lipodemic profile of athletes that lead to reduced danger for coronary disease have also been reported, after a program of sustained exercise (Farrell & Barboriak, 1980; Hartung et al., 1981) and after sustained periods of exercise (Enger et al., 1980; Berg et al, 1981). Reduction in the serum of whole cholesterol (T-C), of triglycerides (TG), low density lipoprotein (LDL-C), as well as high density lipoprotein (HDL-C) have been reported after a program of sustained exercise with an intensity between 70-85% VO2 max (Durstine et al., 1983). The physiological mechanisms, responsible for those findings are not yet determined, but data from recent publications suggest that beneficial effects from exercise are found in an athlete's serum for several days afterwards. Thus, physical exercise is considered today an important factor of preventing cardiovascular disease in adults (Bouchard & Despres, 1995; Vaccaro & Mahon, 1989).

Soccer is an especially dynamic and demanding sport, which calls for a high aerobic ability and great energy inventories, for increased performance during playing (Bangsbo, 1994; Reilly & Borrie, 1992). While in a game, athletes are subject to significant strain, which influence body health and the functioning of their organism, as well as their performance (Sotiropoulos et al., 2008). The purpose of this essay is to examine the effects of a two-month training program in parameters that have to do with cardiovascular danger, aerobic ability and anthropometric characteristics of professional soccer players.

Material and methods

Sample

Twenty-five soccer players participated in the study, from teams of the first Greek national league. The players were healthy and not under any medication. During the training season they did not receive any dietary complement besides the carbohydrates they received before, during and after practice. Also, smoking athletes were excluded.

Experimental Planning

The athletes had to visit the lab twice. The first time there was a blood sampling after an all night fast and, after that, measurements took place to determine their maximum intake of oxygen and aerobic ability. The second time the exact same measurements were repeated, with the difference that two months of training in soccer had intervened.

Assessment of (anthropometric) characteristics

Each individual visited the lab in the morning after an all night fast, with no alcohol or caffeine for 24 hours. During both visits, body mass (Beam Balance 710, Seca, UK), body height (Stadiometer 208, Seca, UK) and body fat were measured with a treadmill (Harpenden Skinfold Caliper HSK-BI).

Determination of maximum oxygen intake

The testing of VO_2max was made on a treadmill (Technogym, Runrace 2001, Italy), by using a portable gas analyzer ($K4b^2$, Cosmed, Italy), which was calibrated before each measurement by using a known mix of oxygen and carbon dioxide. The protocol of ergometric testing included running for a minute with a beginning speed of 7km/h. After that, the subject continued to run at 8km/h for 30 seconds. From that point on, the speed of the treadmill was increased by 0,5km/h for every 30 seconds until exhaustion. The criteria of exhaustion were the respiratory quotient (R>1.15), the maximizing of Heart Rate by age (M.H.R.) and the stabilization or small reduction (< 2 ml/kg Σ.M./min) of oxygen intake (VO2) by further increasing exercise (Astrand & Rodahl, 1986). During that test M.H.R., maximum speed at exhaustion (Tmax), anaerobic threshold (AT), measured as the VO_2 responding to AT ($VO_{2A.T.}$) were measured and also the speed at which $T_{A.T.}$ took place, heart rate (H.R..$_{A.T.}$), percentage of MHR (%M.H.R.$_{A.T.}$), as well as the percentage of VO_2max in A.T. (%$VO_2max_{A.TT.}$). $VO_{2A.T.}$ was determined through respiratory parameters, by calculating R, the volume of CO2 (V_{CO2}), the volume of pneumonic ventilation (V_E) and the respiratory equal to oxygen (V_E/VO_2) (Wasserman et al 1973; Yoshida et al 1981). Cardiac frequency was measured through telemetric method (Polar a_1, Kempele, Finland).

Anaerobic Testing

To measure anaerobic ability, the Wingate test was used (Bar-Or, 1987), modified to last 10 seconds, as in soccer a player usually does not make an anaerobic effort for greater than 10 seconds (Stolen et al 2005). The cycloergometer that was used (Monark 834 Ergometric) was connected to a computer with the right software for automatically recording mechanical force. The subjects made an effort of max. 10 seconds with resistance of 75 grams per kilogram, after being appropriately familiarized with the procedure. The beginning of the test occurred once the subjects had reached maximum pedaling speed without endurance ($Tmax_{Wing}$). During testing, besides of $Tmax_{Wing,}$ values of absolute (M.F.) and relative maximum force (M.F.) were measured, these were expressed in Watts and in Watts/Kg B.W. Likewise, absolute minimum (A.T.) and relative minimum force (R.M.F.) were also measured.

Training Program

The training program that the athletes followed had a duration of 56 days and was carried out during pre-season. In Chart 2, the structure of the programmer is detailed, as well as the training sections that were included in each training unit. Based on the training structure, the intent was to include all factors that participate in a soccer player's physical condition, in order to synthesize an harmonic and multi-layered physical condition, accustomed to the needs, specifics and kinetic standards of the sport (Hoff, 2005). Specific focus was given to improving aerobic ability, strength, explosiveness and neuromuscular coordination. Technique training was combined with stimulation for improving neuromuscular coordination, proprioception and aerobic stamina. During the third phase of the program, which lasted two weeks, the forming of the players took place so that there would be time for replenishment from the two previous phases that were characterized by a relatively large training volume. This period had the purpose of preparing players for the official matches, as well as their participation in ergometric reassessment testing (Svensson & Drust, 2005).

Parameters	
Days	56
Training Units	88
Double Practice	32
Single Practice	56
Morning Practice	37
Evening Practice	51
Duration of Practice	70´-90´(According to training units per day)
Low Intension Aerobic *(Training Units)*	22 Through technique training
Aerobic Training at the Threshold *(Training Units)*	18
Speed Training *(Training Units)*	12 Through matches and tactics
General Strength Training *(Training Units)*	14
Explosiveness Training Explosive power Plyometric Training *(Training Units)*	12
Anaerobic Stamina - Sustained Speed Training *(Training Units)*	8
Neuromuscular Coordination Training *(Training Units)*	10
Proprioceptive training *(Training Units)*	8
Technique Practice *(Training Units)*	22 (Combined to proprioception, neuromuscular coordination and tactics, and with intermediate breaks of aerobic training
Technique Practice *(Training Units)*	24
Friendly Matches	8
Official Cup Matches	-

Biochemical Analyses

The blood samples of the athletes were taken from the vasilical and vasilical vein of the arm at 9-9.30 a.m. after a 12 hour fast. The volume of blood received each time was 5ml. The blood samples were left to condense and after that they were centrifuged at 1500xg for 10 mins to produce the serum, which was subsequently refrigerated to be used for analysis. Determination of each parameter was made through photometric methods in a spectrophotometer U-1100 by Hitachi (Japan). To calculate TG and TC a number of kits by the company BEST (Athens, Greece) were used. To calculate HDL-C, an additional lipoprotein submersion kit was used by Bohringer (Mannheim, Germany). For hyper-centrifuging, the A-100 head was used, with an angle of 30 degrees to the rotation axis. A hyper-centrifuge was used by the company BECKMAN AIRGUGE. To determine homocysteine the ELISA method was used of the company AXIS and to determine isoprostane the method ELISA of the company ASSAY DESIGNS/STRESSGEN was used. All the biochemical parameters were determined in double with the use of the control serum by Böhringer.

The calculation of a given parameter in all samples occurred in the same day to reduce day to day fluctuations. The fluctuation agents for TG, TC and HDLC were 1.8% , 1.2 % and 1.2% accordingly. Middle range was calculated from the values of each volunteer's sample, to be used for further processing.

Statistical Analysis

The statistical analysis of the data occurred by using the SPSS (Version 15.0) software. Comparison of the values recorded before and after the 52 day training was done using the bilateral distribution t-test for repeated measurements. The level of importance was set at p>0.05.

Results

Anthropometric and physiological characteristics

The preparation of the players did not significantly change their body weight (t = -1,13, df = 58, p < 0,293), but resulted in a significant reduction of their body fat (t = -4,97, df = 58, p < 0,002). Also, there was a significant increase in their anaerobic ability (t = -2,97, df = 58, p < 0,02), as it was measured through maximum power in the Wingate test and in their aerobic ability (t = 9,98, df = 58, p < 0,005), as it was measured through maximum oxygen intake on the treadmill.

Table 1. Anthropometric and physiological attributes after two-months soccer training.

Variable	Before Training	After Training	t value	p value
Age	24,6 ± 3,6	24,6 ± 3,6		
Height (cm)	182,3 ± 5,4	182,3 ± 5,4		
Weight (kg)	79,0 ± 5,8	78,2 ± 4,7	-1,138	0,293
% Fat	9,9 ± 2,0	7,7 ± 1,1	-4,97	0,002*
Maximum Power (W)	963,6 ± 102,0	1049 ± 97,1	2,97	0,02*
VO_{2max} (ml/Kg/min)	53,6 ± 7,7	64,4 ± 5,7	9,98	0,005*

* Statistically important difference compared to pre-training:; VO_{2max}: Maximum Oxygen Intake,

Blood Lipids

Cholesterol levels (Graph 1) after two months practice season were significantly reduced compared to pre-training (t = 9,73, df = 58, p < 0,001).

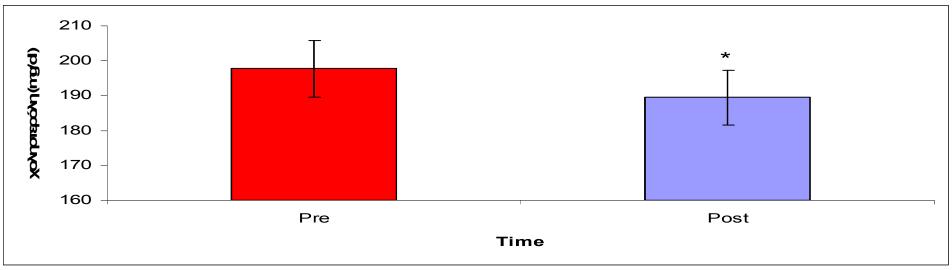

Fig. 1. Differences in cholesterol levels after three months of soccer training.

HDL values (Graph 2) after two months of training were significantly reduced compared to pre-training. (t = 4,39, df = 58, p < 0,001).

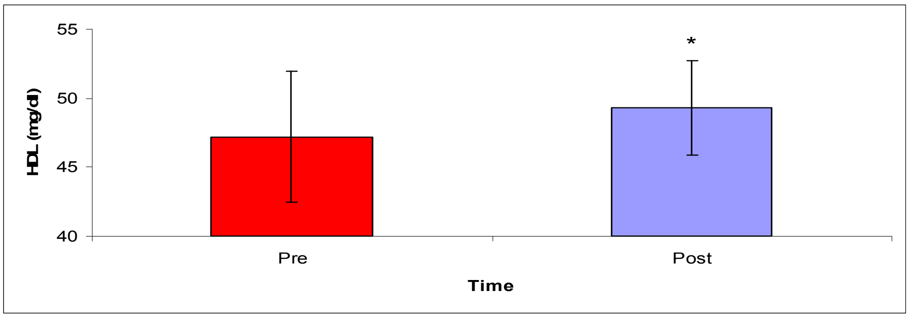

Fig.2. Differences in HDL values after two months of soccer training.

HDL2 values (Graph 3) after two months of training were significantly reduced compared to pre-training. (t = 5,01, df = 58, p < 0,001).

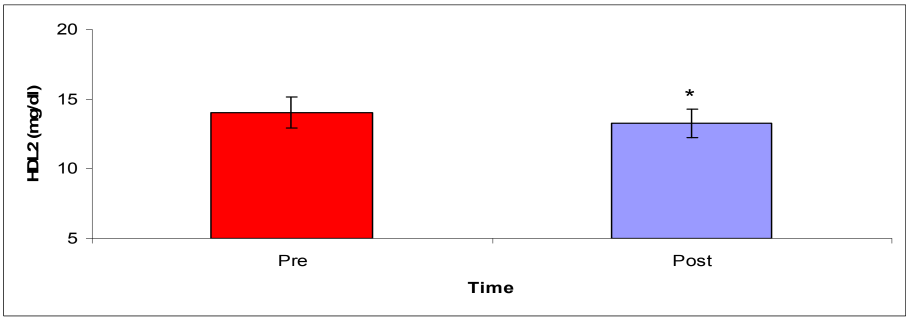

Fig. 3. Changes in HDL2 values after two months of soccer training.

HDL3 values (Graph 4) after two months of training were significantly reduced compared to pre-training. (t = 6,90, df = 58, p < 0,001).

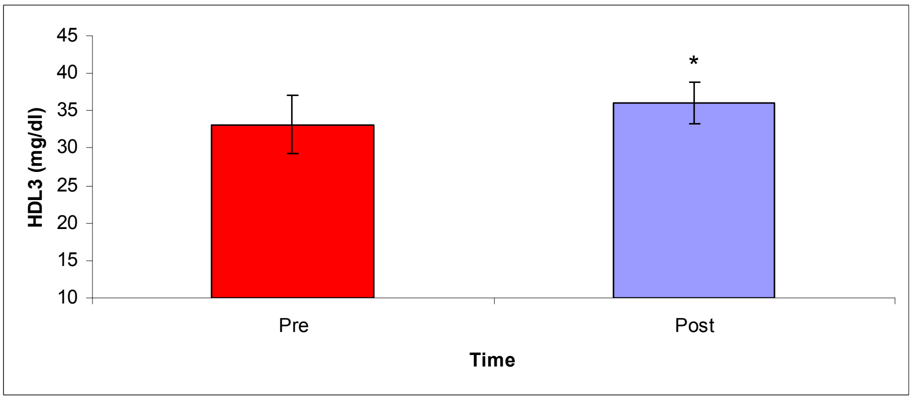

Fig. 4. Changes in HDL3 values after two months of soccer training.

TG levels (Graph 5) after two months of training were significantly reduced compared to pre-training (t = 9,26, df = 58, p < 0,001).

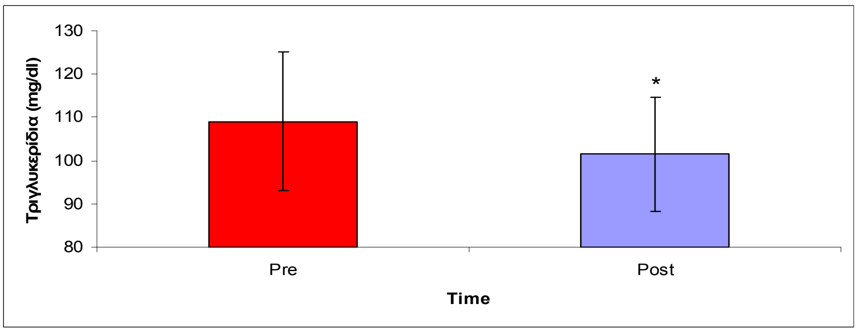

Fig. 5. Changes in triglyceride levels after two months of training were significantly reduced compared to pre-training

LDL values (Graph 6) after two months of training were significantly reduced compared to pre-training (t = 9,02, df = 58, p < 0,001).

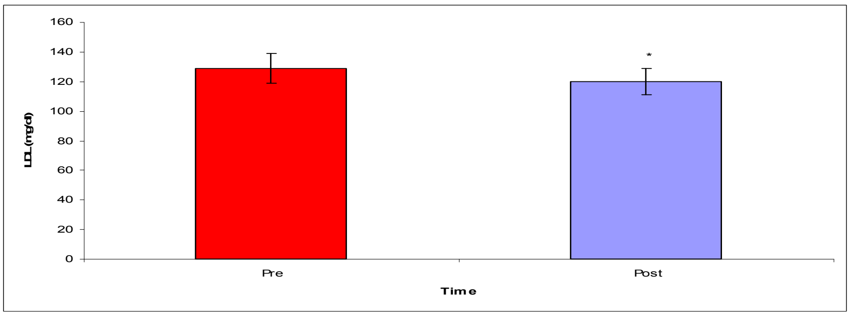

Fig.6. Changes in LDL levels after two months of training were significantly reduced compared to pre-training

VLDL values (Graph 7) after two months of training were significantly reduced compared to pre-training (t = 9,54, df = 58, p < 0,001).

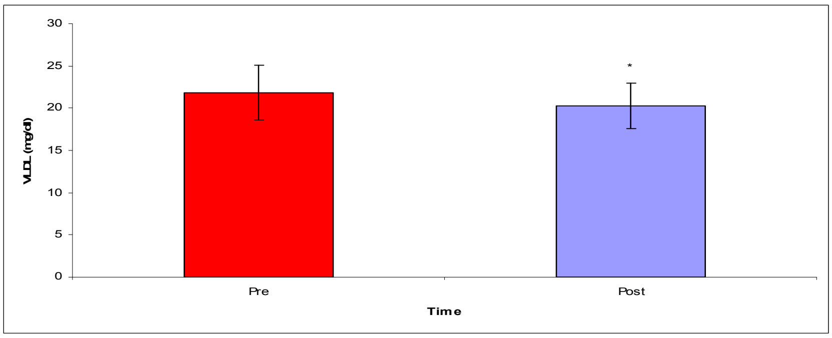

Fig. 7. Changes in VLDL levels after two months of training were significantly reduced compared to pre-training

Discussion

Soccer is a sport characterized by the rapid activation of both aerobic and anaerobic metabolism. Energy generation through aerobic metabolism is found to be at around 90% of total energy that is consumed during the match (Bangsbo, 1994). Levels of lactic acid before a soccer match are 3-9 mmol/L and at many instances they may reach over 10 mmol/L (Bangsbo, 1994), which means that the players' effort is intense. Furthermore, in order for the players to withstand that kind of intensity, they have to train accordingly. This extreme form of training and soccer players' effort in a match probably led to the improvement of the parameters examined within this study.

The results showed that the two-month training resulted in an altered body structure for the athletes. Although there was not a significant reduction in body weight (1%), there was an important reduction in body fat (22%). This means that the maximum reduction was in body fat, while non-fat body mass was increased. More specifically, body fat was reduced by 1,8 kg, while non-fat body mass was increased by about 1 kg. Those results agree with other studies which show that exercise has a positive impact in changing body structure (Krustrup et al., 2009).

Maximum intake during pre-season was below the values recorded for elite athletes (Hoff , 2005). However, the improvement of aerobic ability was about 20%, so the middle range of those values (~65 ml/kg/min) was inside the margins for athletes of that level (55-68 ml/kg/min) (Helgerund et al., 2001; Hoff, 2005; Hoff & Helgerund, 2004; Stolen et al., 2005). It has been reported that the anaerobic threshold of soccer players stands at about 77-90% of maximum cardiac activity, so the intension of a match can improve aerobic ability.

Furthermore, the training program consisted of about 16 practices, with the goal of improving aerobic training, so it can be said that this primary goal of improving aerobic ability was achieved. The bibliography suggests that the number of capillaries can be increased even up to 22% after a targeted football training program, which is conducted in small training squares (Krustrup et al., 2009) and this can be a reason for the increase in aerobic ability. The training program can be characterized as successful, since it was able to increase anaerobic ability as well. The improvement in maximum power was about 9% and statistically important. The levels of maximum power were high enough for football players, according to the limited data available in that particular field of expertise (Davis et al., 1992).

As far as the lipidemic profile of the athletes, the pre-season training resulted in improvement (reduction of T-C, TG, LDL-C, VLDL and increase in HDL-C, HDL2 and HDL3). The reduction of TC is attributed to the reverse transfer of cholesterol from the serum to the liver, as shown by the HDL-C molecules and it appears to be increased in individuals with a specific physical condition (Brites et al., 2004). About the triglycerides, their reduction after the program is attributed to the fact that football enhances metabolic rhythms in an athlete's organism. It is well known that intense training which takes place under aerobic circumstances calibrates the organism to seek energy through burning fatty acids (Berg et al., 1994). The increased fueling of fatty acids requires an increased hydrolysis of triglycerides through activating lipoprotein lipase, in order for the provision of substrates for oxidation to be possible and finally lead to the energy.

Although at first this is countered by the increased triglyceride production (Ahlborg & Felig, 1982), continuous exercise results in dropping triglyceride values, as a result of sustained activity of lipoprotein lipase (Sady et al., 1986). As for LDL-C, it is connected more to body weight, rather than the athletes' maximum aerobic ability (Berg & Keul, 1985). The exact mechanism for its reduction is not clearly documented. It is believed that this effect happens through the increased catabolism of triglyceride-rich molecule precursors of LDL-C, VLDL-C and IDL-C, as well as LDL-C itself, because of the increased need of the organism in fatty acid to produce energy Koozehchian et al. (2014).

Previous studies have reported cholesterol values similar to the ones mentioned in this study (Lehtonen & Viikari, 1980; Farrel et al., 1982; Apostolidis et al., 2014). However, training resulted in improving the lipidemic profile of the athletes. It has been reported that systematic aerobic training can improve the lipidemic profile (Halverstadt et al., 2007). Frequent exercise can bring positive changes for people with a normal lipidemic profile, as well as for those with high lipoproteins and with dyslipidemia. In general, the most frequent changes observed, have to do with the reduction of triglycerides, reduction of cholesterol, increase in HDL-C cholesterol and increase in enzymic activity (lipoprotein lipase, LPL, , acyltransferase lecithin, cholesterol, LCAT, protein of transport esterified cholesterol, CETP) in the metabolism of lipoproteins. Systematic training enhances the reverse transfer of cholesterol and can be further enhanced through a diet of low fat and the reduction of body weight. That way, either directly (e.g by increasing LPL activity) or indirectly (e.g reducing body weight), training can improve the trainee's lipidemic profile. However, there are very few studies which recorded the effect of a systematic training program in football and its effect on that lipidemic profile. In a fairly recent study, where young athletes trained for six days a week for a year, with some of those targeted at soccer, it was found that the lipoprotein and lipidemic profile of the participants was better and the cardiovascular danger significantly reduced (Brites et al., 2004). The reduced atherogenetic ability was considered to be caused by higher cholesterol outtake from the vessels because of exercise.

Training resulted in increasing HDL-C, HDL2 and HDL3. Increased concentrations of HDL after systematic training is connected to the reduced catabolism of lipoprotein, as well as its increased synthesis by A-I HDL (Thompson et al., 1997; Apostolidis et al., 2014). This can also be attributed to diet, weight loss and genetic disposition of the participants Koozehchian et al. (2014). Another important factor is that HDL-C molecules recruit circulating molecules of atherosclerotic cholesterol and triglycerides from the serum and take them out of circulation after they deposit them in the liver. Those activities seem to increase during exercise (Krustrup et al., 2009; Apostolidis et al., 2014) as well as soccer, which is one parameter that appears partly in this study, in the statistically important reduction of cholesterol in the athletes serum. Also, Tambalis et al. (2009) reached the same results and they mention that systematic aerobic training can increase HDL-C levels when it is intense enough.

Those positive changes in the lipidemic profile of soccer players probably protect them against arteriosclerosis and coronary heart disease (CHD) disease. This is a general conclusion connected to the positive results of exercise for coronary heart disease, which has a significant importance and must be positively used.

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