ORIGINAL ARTICLE
UDC: 61:79
https://doi.org/10.2298/VSP180725159A

Effect of long-term strenuous training on the plasma phospholipid fatty acid composition in handball players

Efekat dugotrajnog napornog vežbanja na masnokiselinski profil fosfolipida plazme kod rukometaša

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Abstract

Background/Aim. Consensus on the exercise effect on the fatty acid metabolism has not been reached, and probably depends on the type of sports (aerobic, anaerobic or mixed). The aim of this study was to investigate effect of long-term handball training on the body composition, lipid profile and the plasma phospholipid fatty acid composition in female and male younger players. **Methods.** Seventeen female and 15 male active handball players, aged 16–20 years, who competed at the national/international level, were enrolled in the study. A control group was established from healthy, sedentary individuals (13 females and 19 males, aged 17–21 years), comparable to the athletes in terms of age, sex and body mass index. **Results**. In both groups of handball players a higher percentage of palmitoleic acid and alpha linolenic acid (18:3, n-3), were found and lower percentage of oleic acid and docosahexaenoic acid (22:6, n-3), when compared with corresponding control group. On the other hand, the lower level of stearic acid and estimated activity of plasma elongase was detected in female players than in sedentary women. Furthermore, higher proportion of linoleic acid (18:2, n-6), n-6 polyunsaturated fatty acids (PUFA) and total PUFA was found only in female players in comparison to the control group. **Conclusion.** The observed differences between handball players and sedentary individuals showed that handball training influenced lipid and fatty acid metabolism. Follow-up of these changes could indicate potential need for supplementation or nutritional intervention in young handball players.

Key words:
body composition; lipid metabolism; fatty acids; sports; sex factors.

Apstrakt

Uvod/Cilj. Konsenzus o uticaju treniranja na metabolizam masnih kiselina nije postignut, a taj uticaj verovatno zavisi od tipa sporta – aerobno, anaerobno ili mešovito vežbanje. Cilj ove studije bio je da se ispita efekat dugotrajnog, aktivnog treniranja rukometa na telesnu kompoziciju, profil lipida i masnih kiselina fosfolipida plazme kod mlađih kategorija rukometaša oba pola. **Metode.** U studiju je bilo uključeno 17 devojaka i 15 mladića, starosne dobi od 16 do 20 godina koji treniraju rukomet i takmiče se na nacionalnom i internacionalnom nivou. Kontrolnu grupu činilo je 13 devojaka i 19 mladića starosti od 17 do 21 godine, koji su bili uporedivi sa sportistima po godinama, polu i indeksu telesne mase. **Rezultati.** Procenat palmitoleinske i alfa-linolenske kiseline (18:3, n-3) bio značajno viši, dok je procenat oleinske i dokozaheksaenske kiseline (22:6, n-3) bio značajno niži u fosfolipidima plazme kod obe grupe sportista u odnosu na kontrolnu grupu. Sa druge strane, niži nivo stearinske kiseline i procenjene aktivnosti elongaze, ali i visok nivo linolne kiseline (18:2, n-6), ukupnih n-6 masnih kiselina, kao i ukupnih polinezasićenih masnih kiselina, utvrđen je kod rukometašica u odnosu na ispitanice iz kontrolne grupe, dok u grupi muškaraca nisu utvrđene takve razlike. **Zaključak.** Utvrđene razlike između rukometaša i rukometašica, sa jedne strane, i kontrolne grupe, sa druge strane, ukazale su na to da treniranje rukometa utiče na metabolizam lipida i masnih kiselina. Praćenje tih promena moglo bi ukazati na moguću potrebu za suplementacijom kod mladih rukometaša i rukometašica.

Ključne reči:
telo, sastojci; lipidi, metabolizam; masne kiseline; sport; pol, faktori.

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Introduction

Beneficial effects of regular physical activity on health are well established [1]. However, long-term strenuous training could have the opposite effect by production of proinflammatory cytokines and promotion of low grade inflammation. Previous studies have shown that sports with high degree of stressful physical exertion (e.g. soccer and volleyball), are accompanied by unfavorable plasma lipid and lipoprotein profiles, while sports with low levels of stressful exercise, such as swimming, appear to have a beneficial effect on plasma lipids [2].

Beside alternations in the levels of triacylglycerol (TG), total cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol in the circulation, chronic exercise leads to significant changes in the fatty acid (FA) composition of blood and tissue phospholipids [3, 4]. As elements of all natural membranes, FA are required for several basic functions, playing pivotal roles in regulation of intracellular signaling pathways, gene expression and production of important lipid mediators [5]. Although FA composition in biological membranes depends on the dietary intake, many other factors, including physical activity, may influence their metabolism [6–8]. Alterations in the FA profiles of plasma and erythrocytes phospholipids were found in elite water polo, football, basketball players and boxers when compared with sedentary subjects [9–11]. In addition, changes were not similar in different groups of athletes, suggesting that FA composition may depend on type of sport [9].

Handball is a globally popular team sport with almost 20 million players in the world. Due to its fast-paced game involving a lot of running, jumping, turning and slamming, it is a great workout for the whole body. Thus it is related to boosting the body's agility and flexibility, building up muscle tone and strength and improving cardiovascular function and oxygen supply [12, 13]. However, prolonged intense exercise promotes reliance on lipids as a primary fuel source, that is also connected with increased rate of harmful lipid peroxidation when compared to moderate or no physical activity [14]. Although the effect of exercise training on the FA composition of total lipids and different lipid classes have been studied [15–17], consensus on the effect of exercise on FA metabolism has not been reached, and probably depends on the type of sports (aerobic, anaerobic or mixed). Considering all these facts, the aim of this study was to investigate whether handball training modifies body composition, lipids profile and plasma phospholipid FAs composition in young female and male players.

Methods

Subjects

Seventeen female and 15 male active handball players aged 16–20 years, who competed at the national/international level, were recruited from elite sport clubs in Belgrade and Kragujevac, Serbia. The study was conducted during the period of preparatory training prior to the next competition season. A control group was established from healthy, sedentary individuals (13 females and 19 males, aged 17–21 years), comparable to the athletes in terms of age, sex and body mass index (BMI). All subjects were apparently healthy at the recruitment and during the study, and none of them was taking any drugs, or dietary supplements that might have influenced the lipid profile results. General data, such as age, duration of regular daily training, period of time of weekly training, dietary habits and use of supplements were obtained from the subjects through standardized questionnaires under supervision of a trained nutritionist. Female study participants reported regular menstrual cycles (26–32 days) and those who were taking oral contraceptives were excluded. All of them were included in the study in the early follicular phase of the menstrual cycle. The study protocols were approved by the Ethics Committee of the Faculty of Medical Sciences, University of Kragujevac, Serbia in accordance with the Declaration of Helsinki and principles of Good Clinical Practice. All subjects gave written informed consent to participate in the study.

Anthropometric measurements

Standing height was measured in participants without shoes and socks, to the nearest 0.1 cm by a wall mounted stadiometer (Perspective Enterprises, Kalamazoo, MI). For measuring body weight (to the nearest 0.1 kg), BMI, percentage of body fat, fat mass, fat free mass and total body water, Tanita body composition analyzer (TBF-300, Tanita Corp., Tokyo, Japan) was used.

Analytical methods

Blood samples were taken in the morning after a 12 hrs fast, and 18 hrs after the end of the last training bout. Glucose, cholesterol and triglyceride concentrations were measured in the serum using automated enzymatic methods (Roche Diagnostics, Mannheim, Germany), on Cobas c111 analyzer (Roche, Basel, Switzerland).

Total lipid extract was prepared as described previously [10]. One-dimensional thin-layer chromatography in a neutral solvent system (petrol ether: diethyl ether: acetic acid 87:12:1 v/v) on Silica Gel GF plates (C. Merck, Darmstadt, Germany) was performed to isolate phospholipid fractions. Phospholipids were subjected to trans-esterification and obtained FA methyl esters were analyzed by the gas chromatograph Shimadzu 2014 (SHIMADZU, Kyoto, Japan) fitted with a capillary column (Rtx 2330, RESTEK, USA) as described previously [18]. The individual FA methyl esters were identified from the retention times of authentic standard mixtures (Sigma Chemical Co., St. Louis, MO, USA) and/or polyunsaturated FA (PUFA-2) standard mixture (Supelco, Inc., Bellefonte, Pennsylvania, USA). The results were expressed as the relative percentage of total identified FAs. Product-to-precursor ratios were used to estimate activities of certain enzymes involved in FA biosynthesis: 18:0/16:0 for elongase activity, 18:1/18:0 ratio for delta-9-desaturase (Δ9-desaturase) activity, 20:3/18:2 ratio for delta-6-

desaturase (Δ6-desaturase) and elongase activity, 20:4/20:3 ratio for delta-5-desaturase (Δ5-desaturase) activity.

Statistical analysis

Statistical analysis was performed using the statistical package SPSS 20.0 for Windows. The results are presented as means ± standard deviation. Normality was tested using the Shapiro-Wilk test before statistical analysis. For all variables which showed normal distribution, statistical comparisons of means were performed using the unpaired Student's *t*-test. For those which showed non-normal distribution [Δ6-desaturase, alpha linolenic acid (ALA) and eicosapentaenoic acid (EPA)], the Mann-Whitney *U*-test was performed. Differences were considered significant at *p*-values of < 0.05.

Results

The anthropometric characteristics and basic biochemical parameters of the study subjects are presented in Table 1. All anthropometric parameters, including height, weight, BMI and body fat were similar in both female groups. Although the level of all biochemical parameters was within reference ranges, concentrations of glucose and triglycerides in the serum were higher and lower, respectively in female players than in control women, as shown by the Student's *t*-test.

On the other hand, sportsmen had higher height, weight, fat free mass, and total body water, as well as lower body fat mass than control men. In addition, we found no difference in studied biochemical parameters between male athletes and control subjects.

FA composition of plasma phospholipids of the study participants are presented in Table 2. Among saturated FA (SFA), only percentage of stearic acid (18:0) was significantly lower in female handball players than in the control group. The percentage of oleic acid (18:1, n-9) was lower, and that of palmitoleic acid (16:1, n-7) was higher in both groups of athletes when compared to controls. In addition, female players had higher proportion of linoleic acid (LA, 18:2, n-6), n-6 PUFA, total PUFA than sedentary women, while higher ALA (18:3, n-3) and lower percentage of docosahexaenoic acid (DHA, 22:6, n-3) were observed in both groups of players in comparison to the control groups. The Student's *t*-test was used for all comparisons except ALA, which was analyzed by the Mann-Whitney *U*-test.

As shown in Table 3, the estimated activity of plasma elongase was lower in female handball players than in sedentary subjects, whereas estimated activities of desaturases were similar among the examined groups.

Discussion

It has been well established that long-term intense physical training modulates lipid profile of many tissues, not only concentration and distribution of lipid classes but also their FA composition [4]. We have previously shown that FA profiles in plasma and erythrocyte phospholipids differ between sportsmen and sedentary subjects [10, 11], as well as that type of regular training may affect metabolism of FA in elite athletes [9]. Here we examined the effects of handball training on plasma phospholipid FA profile in young players.

Different anthropometric parameters (Table 1) including body fat (both % and kg), fat free mass (kg) and total body water (kg) between male players and controls were expected due to intense trainings and in line with our previous results [9, 10]. Because of different body constitution, these changes in female athletes were not significant. Namely, women generally have higher % of body fat than men, due to sexual hormones, and this % markedly varies among women, including handballers. Thus, the standard deviation is higher and there was no statistically significant difference in body composition between athletes and the control group. Moreover, Bayios et al. [19] have published that Greek female handball players were shorter and had higher levels of body fat than basketball and volleyball players, and that their body composition was even close to general female population in Greece. They concluded that hours of training and sport-specific physiological demands during the game could explain the observed differences.

Table 1

The anthropometric characteristics of male and female handball players

Parameter	Male handball player	Control	Female handball player	Control
Age (years)	18.47 ± 1.06	19.05 ± 0.85	16.89 ± 1.00	17.91 ± 1.38
Height (cm)	192.73 ± 6.32***	182.44 ± 6.56	172.11 ± 7.64	171.18 ± 4.40
Weight (kg)	90.66 ± 14.96***	78.13 ± 10.04	64.36 ± 8.71	63.93 ± 8.72
BMI (kg/m^2)	24.37 ± 3.80	23.41 ± 2.12	21.78 ± 2.30	21.78 ± 2.07
Body fat (%)	9.66 ± 2.20***	14.70 ± 2.69	20.69 ± 4.94	24.09 ± 5.11
Fat mass (kg)	8.06 ± 2.99***	13.42 ± 4.55	14.34 ± 4.05	15.65 ± 4.99
Fat free mass (kg)	81.25 ± 9.38***	65.84 ± 6.62	51.00 ± 6.98	48.30 ± 5.53
Total body water (kg)	59.58 ± 6.87***	48.21 ± 4.85	37.43 ± 5.13	35.36 ± 4.05
Glucose (mmol/L)	4.67 ± 0.34	4.33 ± 0.35	4.40 ± 0.28*	4.17 ± 0.33
Triglycerides (mmol/L)	0.96 ± 0.30	0.99 ± 0.34	0.46 ± 0.13**	0.81 ± 0.30
Cholesterol (mmol/L)	4.07 ± 0.42	4.16 ± 0.94	3.95 ± 0.49	4.40 ± 0.66

Data are presented as a mean ± standard deviation.
BMI – body mass index.
***p* < 0.05, ****p* < 0.01, *****p* < 0.001 compared to the control group.**

Table 2

Plasma phospholipid fatty acid composition in male and female handball players

Fatty acid (%)	Male handball player	Control	Female handball player	Control
SFA				
16:0	26.39 ± 2.24	25.84 ± 1.59	27.95 ± 1.53	27.50 ± 1.44
18:0	15.26 ± 1.25	15.78 ± 1.44	13.91 ± 1.13*	15.43 ± 1.40
Total SFA	41.65 ± 1.42	41.63 ± 2.33	41.87 ± 1.29	42.93 ± 1.49
MUFA				
16:1, n-7	0.53 ± 0.17**	0.34 ± 0.12	0.46 ± 0.09**	0.39 ± 0.09
18:1, n-9	8.87 ± 1.13*	9.82 ± 1.10	8.51 ± 0.33*	8.85 ±1.07
18:1, n-7	1.56 ± 0.22	1.42 ± 0.24	1.45 ± 0.17	1.41± 0.16
Total MUFA	10.93 ± 1.22	11.58 ± 1.23	10.49 ± 0.84	10.65 ± 1.22
n-6 PUFA				
18:2, n-6	26.46 ± 2.68	26.10 ± 2.03	29.88 ± 2.24*	27.87 ± 2.58
20:3, n-6	3.31 ± 0.58	2.90 ± 0.59	2.74 ± 0.74	2.71 ± 0.68
20:4, n-6	13.16 ± 2.46	12.78 ± 1.94	10.90 ± 1.52	11.08 ± 1.59
22:4, n-6	0.70 ± 0.18	0.62 ± 0.14	0.50 ± 0.12	0.55 ± 0.15
Total n-6 PUFA	43.64 ± 2.14	42.38 ± 2.78	44.02 ± 1.34*	42.21 ±1.75
n-3 PUFA				
18:3, n-3	0.37 ± 0.16***	0.13 ± 0.04	0.26 ± 0.10**	0.12 ± 0.04
20:5, n-3	0.38 ± 0.08	0.34 ± 0.13	0.25 ± 0.09	0.20 ± 0.07
22:5, n-3	0.65 ± 0.15	0.73 ± 0.14	0.51 ± 0.12	0.54 ± 0.15
22:6, n-3	2.36 ± 0.52**	3.23 ± 0.97	2.60 ± 0.59*	3.19 ± 0.56
Total n-3 PUFA	3.69 ± 0.69	4.19 ± 1.31	3.56 ± 0.80	4.02 ± 0.68
Total PUFA	47.32 ± 1.42	46.56 ± 3.02	47.57 ± 1.40**	45.40 ± 2.60
n-6/n-3 ratio	12.17 ± 1.98	10.52 ±2.72	12.72 ± 5.34	10.85 ± 2.00

Data are presented as a mean ± standard deviation.
SFA – saturated fatty acids (16:0 – palmitic acid; 18:0 – stearic acid); MUFA – monounsaturated fatty acids (16:1, n-7 – palmitoleic acid; 18:1, n-9 – oleic acid; 18:1, n-7 – vaccenic acid); PUFA – polyunsaturated fatty acids (18:2, n-6 – linoleic acid; 20:3, n-6, – dihomo gamma-linolenic acid; 20:4, n-6:4 – arachidonic acid; 22:4, n-6 – adrenic acid; 18:3, n-3 – alpha-linolenic acid; 20:5, n-3 – eicosapentaenoic acid; 22:5, n-3 –docosapentaenoic acid; 22:6, n-3 – docosahexaenoic acid).
***p* < 0.05, ****p* < 0.01, *****p* < 0.001 compared to the control group.**

Table 3

The estimated plasma desaturase and elongase activities in male and female handball players

Enzyme	Male handball	Control	Female handball	Control
Elongase (18:0/16:0)	0.59 ± 0.10	0.61 ± 0.06	0.50 ± 0.06*	0.56 ± 0.07
Δ9- desaturase (18:1/18:0)	0.59 ± 0.11	0.63 ± 0.08	0.62 ± 0.10	0.58 ± 0.13
Δ6- desaturase and elongase (20:3, n-6/18:2, n-6)	0.13 ± 0.03	0.11 ± 0.03	0.09 ± 0.03	0.10 ± 0.03
Δ5- desaturase (20:4, n-6/20:3, n-6)	4.10 ± 1.01	4.55 ± 1.03	4.29 ± 1.26	4.30 ± 1.18

Data are presented as a mean ± standard deviation.
***p* < 0.05, compared to the corresponding control group.**

Furthermore, reduced plasma TG levels, which are used as energy sources during exercise, were found, but only in female athletes, the finding in accordance with the literature data [20]. Reduced plasma TG levels, which are used as energy source during exercise, were found in only female athletes.

Even though glucose levels in both examined groups were within referent values, we detected higher level of glucose in female athletes when compared to the control group. Plasma glucose concentration can increase in response to intermittent sport activity due to an increase in circulating catecholamines [21, 22]. Catecholamine-stimulated glycogenolysis results in an elevated plasma glucose level even exceeding resting values [21], which returns to the basal level after a few hrs recovery period [23]. Since glucose was determined 18 hrs after the last bout of exercise, we think that this difference can be a natural difference between two groups, unrelated to sport, especially as we did not find the same in males. Nevertheless, it should be checked comparing glucose levels in other handball and control groups.

Our results on FA composition of plasma phospholipids (Table 2) showed lower level of stearic acid and estimated elongase activity in female players than in the sedentary women. This is contrary to our previous study where female football players had higher level of stearic acid than controls, suggesting the effects of type of exercise on the elongase activity [9]. Increased SFA in plasma and/or erythrocytes is posi-

tively associated with the development of diabetes [24] and coronary heart disease [25], but this effect can be attributed to palmitic acid rather than stearic acid, which even exerts cardioprotective effects [26]. The lower level of stearic acid might be explained by the effect of handball training on elongase included in synthesis of stearic acid. Since we have not observed differences in the levels of stearic acid in male players nor in the estimated elongase activity, we can assume that the effect of exercise on the FA profile in plasma phospholipids is gender dependent. Still, further research is required to elucidate the relationship between exercise and modulation of activities of enzymes included in FA synthesis.

Unlike SFA, the impact of handball on monounsaturated fatty acids (MUFA) plasma phospholipids is similar in both groups of athletes. Namely, we found a significantly higher level of palmitoleic acid and lower level of oleic acid in both handball groups than in the control groups. These results are in line with our previous results on female athletes [9], but in male football and basketball players no differences were found [6, 10, 27]. Regarding beneficial cardioprotective effect of oleic acid [28], our results indicate the importance of increased dietary intake of olive oil as the best source of oleic acid. Furthermore, level of linoleic acid, and thus n-6 PUFA and total PUFA in plasma phospholipids was significantly higher in female players than in sedentary women. However, proportions of LA considerably vary between groups of athletes. For instance, LA and n-6 PUFA were decreased in female football players [9], increased in male basketball players [10], and similar to controls in male players in our study and in the study by Andersson et al. [6]. This is important since LA is precursor of the other n-6 PUFAs, including arachidonic acid which is a strong proinflammatory mediator [29].

Furthermore, handball players had higher levels of ALA than control groups. As precursor of n-3 PUFA family, ALA can reduce systemic inflammation by decreasing synthesis of inflammatory cytokines and stimulating synthesis of antiinflammatory eicosanoids [29]. Higher level of ALA, which we observed, could be of special importance in handball players, since strenuous exercise promotes synthesis of proinflammatory cytokines, and elite athletes often have altered immune response [30]. However, lower level of DHA, found in both athletes groups, suggest possibly decreased conversion of ALA to long chain n-3 PUFA – EPA and DHA, that could be a reason for elevated ALA proportion. Considering strong antiinflammatory properties of EPA and DHA and their importance not only for sport performances, but also for health, in general, our results indicate the need for nutritional intervention and/or n-3 PUFA supplementation in handball players.

Conclusion

The observed differences between handball players and sedentary individuals as well as between female and male players can be attributed to handball training and gender differences, although the mechanism underlying these changes requires further investigations. Since millions of people train handball, investigation and follow-up of lipid and FA profiles in handball players would indicate potential need for supplementation early in their career to avoid far-reaching consequences for their health.

Acknowledgement

This work was supported by the Project 41030 financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

Conflict of interests

The authors declare that they have no competing interests.

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Received on July 25, 2018.
Revised on September 17, 2018.
Revised on September 24, 2018.
Accepted on September 28, 2018.
Online First October, 2018.