



The effects of polyphenol-rich chokeberry juice on fatty acid profiles and lipid peroxidation of active handball players: results from a randomized, double blind, placebo controlled study

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Complete List of Authors:	Petrovic, Snjezana; Institute for Medical Research, University of Belgrade, Serbia, Centre of Research Excellence in Nutrition and Metabolism Arsic, Aleksandra; Centre of Research Excellence in Nutrition and Metabolism, Institute for Medical Research, University of Belgrade, Serbia Glibetic, Marija; Institute for Medical Research, University of Belgrade, Serbia, Centre of Research Excellence in Nutrition and Metabolism Cikiriz, Nikola; Institute of Hygiene, Military Medical Academy, Belgrade, Serbia, Department of Exercise Physiology, Jakovljevic, Vladimir; Faculty of Medical Sciences, University of Kragujevac, Department of Physiology Vucic, Vesna; Institute for Medical Research, University of Belgrade, Serbia, Centre of Research Excellence in Nutrition and Metabolism
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4 Snjezana Petrovic^a, Aleksandra Arsic^a, Marija Glibetic^a, Nikola Cikiriz^b, Vladimir
5 Jakovljevic^c, Vesna Vucic^a

6 ^aCentre of Research Excellence in Nutrition and Metabolism, Institute for Medical Research,
7 University of Belgrade, Serbia

8 ^bDepartment of Exercise Physiology, Institute of Hygiene, Military Medical Academy,
9 Belgrade, Serbia

10 ^cDepartment of Physiology, Faculty of Medical Sciences, University of Kragujevac, Serbia

11

12 Corresponding author:

13 Dr Vesna Vucic

14 Centre of research excellence in nutrition and metabolism

15 Institute for Medical Research

16 University of Belgrade

17 Tadeusa Koscuska 1

18 PO Box 102

19 11129 Belgrade

20 Serbia

21 E-mail address: vesna.vucic.imr@gmail.com

22 Tel: +381 11 303-1997

23 Fax: +381 11 2030-169

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26 Abstract

27 The effect of polyphenol-rich chokeberry juice consumption on plasma phospholipids fatty
28 acid profiles of 32 active male and female handball players was examined. This randomised
29 double-blind, placebo controlled study was conducted during the preparatory training in a
30 closed campus, where 18 players (8M, 10F) consumed 100 ml of chokeberry juice, while 14
31 players (7M, 7F) consumed placebo. Lipid status, glucose, thiobarbituric acid reactive
32 substances (TBARS) and percentages of fatty acids were assessed at baseline and at the end of
33 the study. Consumption of chokeberry juice induced decreases of C18:1n-9 and C18:3n-3 in
34 men, but no changes in female players. However, placebo controlled groups had reduced
35 proportions of mono- (C16:1n-7, C18:1n-7), and polyunsaturated fatty acids (PUFAs:
36 C18:3n-3, C20:5n-3, and C22:4 n-6) in males, as well as n-6 PUFAs and total PUFAs in
37 females after consumption. These results indicate that chokeberry juice had a weak impact on
38 attenuating the effect of intensive training in active handball players.

39 Keywords: handball, chokeberry juice, *Aronia melanocarpa*, polyphenols, fatty acids,
40 placebo, randomized controlled trial

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49 **1. Introduction**

50 Many athletes use various dietary supplements to improve their performances and to protect
51 their health from adverse effects of prolonged strenuous training. However, the effectiveness
52 and benefits of such supplementation are often questionable. It has been shown that athletes
53 under a regular training program exhibit a substantial increase in oxidative stress than healthy
54 sedentary people (Lafay et al. 2009). Although small amounts of reactive oxygen species
55 (ROS) are constantly being generated in cells during normal physiological processes and even
56 participate in different cell functions and immunity, overproduction of ROS causes oxidative
57 damages of lipids, proteins and DNA, chronic inflammation and is involved in many acute
58 and chronic diseases. The membrane phospholipids, especially polyunsaturated fatty acids
59 (PUFA) are prone to oxidative damage (Solans et al. 2000), so increased oxidative stress and
60 altered fatty acid (FA) status in plasma and erythrocytes phospholipids are commonly found
61 in elite athletes (Arsic et al. 2012, 2015; Tepsic et al. 2009, 2011). Accordingly,
62 administration of different antioxidants, such as selenium, vitamin E, vitamin C or
63 polyphenols is rather common and revealed that it was possible to improve exercise-induced
64 antioxidant response even in already adopted antioxidant status (Margaritis et al. 2003;
65 Morillas-Ruiz et al. 2005, 2006).

66 Berries are important dietary sources of polyphenols, in particular anthocyanins, which
67 are well known for their strong antioxidant activity (Szajdek and Borowska 2008). Among
68 berries, chokeberry (*Aronia melanocarpa* L.) showed the highest contents of polyphenols and
69 thus antioxidant capacity (Bermudez-Soto and Tomas-Barberan 2004; Zheng and Wang
70 2003). The most abundant polyphenols in chokeberry are procyanidins, anthocyanins and
71 phenolic acids. These polyphenols are largely responsible for many of the chokeberry
72 medicinal properties and physiological effects, given that their high content and distribution

73 pattern remain almost the same in juice and pomace, as in fresh berries (Kulling and Rawel
74 2008). A plenty of evidences arising from *in vitro*, animal and human studies reported that
75 chokeberry juice attenuates hyperglycemia and hypertriglyceridemia and reduces systolic and
76 diastolic blood pressure, as well as total cholesterol and LDL in serum, while increases level
77 of HDL2 cholesterol in hyperlipidemic people (Broncel et al. 2010; Skoczynska et al. 2007).
78 Beneficial effects of chokeberry juice on cellular antioxidant enzymes and erythrocyte
79 membrane FA status exerted through increased n-3 PUFA, total PUFA and average degree of
80 membrane phospholipids FA unsaturation in healthy women were also reported (Kardum et
81 al. 2014a). However, the effects of polyphenol rich chokeberry juice on FA status in elite
82 athletes have not been investigated so far.

83 As a strenuous intermittent sport, handball places an important stress on a players
84 aerobic and anaerobic metabolism and produces a marked state of oxidative stress (Marin et
85 al. 2011). Plasma FA status of handball players is also altered (Mougios et al. 1995). Taking
86 into account all these facts, this study was aimed to estimate the influence of four weeks
87 regular consumption of chokeberry juice on body composition and FA profile of plasma
88 phospholipids in young elite male and female handball players.

89

90 **2. Material and methods**

91 *2.1. Subjects*

92 The subjects were recruited from elite handball clubs from Belgrade and Kragujevac, Serbia.
93 The study included 15 male handball players, aged 16-20 years (18.5 ± 1.06 years), playing for
94 the Junior National Selection Team, and 17 female handball players, 16-19 years of age
95 (17.2 ± 0.93 years). All study participants were apparently healthy, had no active sports
96 injuries, did not use any medications or oral contraceptives, and were non-smokers.
97 Standardized questionnaires conducted under the supervision of a trained nutritionist were

98 used to collect general data, nutritional habits and use of dietary supplements. The athletes
99 who used any dietary supplements at least a month before the study, were excluded. All
100 female study participants reported regular menstrual cycles (26–32 days). All participants, (or
101 their parents if they were under the age of 18) signed an informed consent document. The
102 study was approved by the Ethical committee of Faculty of Medical Sciences, University of
103 Kragujevac.

104

105 2.2. Study design

106 The study was conducted after regular competition season, during the period of preparatory
107 training prior to the next competition season, and lasted for four weeks. The participants were
108 settled in a closed campus in Serbia, all having the same training and nutritional regime,
109 which excluded intake of berries. Regular training regimen before the study included
110 combination of aerobic, conditioning and strength exercise, once a day for 1.5 h. Campus
111 regime included similar combination of exercises, but of higher intensity, twice per day,
112 lasting 3 hours in total.

113 The research protocol started at 8 AM, after an overnight rest and fast, and before the
114 breakfast. After filling out standard sports medicine questionnaire and passing the standard
115 sports medicine examination, the blood samples were taken from the players.

116 The players (male or female, respectively) were randomly divided into two treatment
117 groups. The supplemented group ($n=8$ for male, $n=10$ for female) received 100 mL of
118 chokeberry juice (Aronia anti-oxi donated by Nutrika d.o.o Belgrade, Serbia), in the morning
119 during breakfast, for 4 weeks. The analytical analysis showed that 100 mL of the chokeberry
120 juice contained 586.7 ± 3.3 mg of total phenolics expressed as galic acid equivalents (Kardum
121 et al. 2014a), while the content of vitamin C was about 29 mg. In the same period, placebo
122 group ($n=7$ for male, $n=7$ for female) was given 100 mL of placebo, having the same content

123 of vitamin C but no polyphenols. The placebo was identical to the chokeberry juice in terms
124 of taste and appearance. Therefore, the participants were not aware of the difference in the
125 juice consumed. Both investigators and the trainers were also blind to group assignment.
126 Compliance was monitored by the trainers.

127

128 *2.3. Anthropometric measurements*

129 Standing height was measured to the nearest 0.1 cm on a wall-mounted stadiometer with
130 participants removing their shoes and socks (Perspective Enterprises, Kalamazoo, Mich.,
131 USA). Body weight to the nearest 0.1 kg and body composition were measured using Tanita
132 body composition analyzer (TBF-300, Tanita Corp., Japan).

133

134 *2.4. Serum samples and biochemical determination*

135 Blood samples were taken at the beginning of the study and after four weeks chokeberry juice
136 or placebo consumption during the preparatory training in campus. The samples were taken
137 into sample tubes for serum and ethylenediaminetetraacetic acid (EDTA) tubes for plasma.
138 Lipid status and glucose level were determined in sera of the athletes, on the same day the
139 samples were collected, using the automated enzymatic methods (Roche Diagnostics kits,
140 Mannheim, Germany), on Cobas e411 analyzer (Roche Diagnostics, Basel, Switzerland)
141 according to the manufactures' instruction. Samples from the EDTA tubes were centrifuged at
142 1500 g for 5 minutes, and the obtained plasma stored at -20°C until analysis.

143 The degree of lipid peroxidation in plasma was estimated by measuring of thiobarbituric acid
144 reactive substances (TBARS) using 1% TBA (Thiobarbituric Acid) in 0.05 NaOH, incubated
145 with plasma at 100° C for 15 min and read at 530 nm. Distilled water was used as a blank
146 probe. TBA extract was obtained by mixing 0.8 ml plasma and 0.4 ml TCA (Trichloro Acetic

147 Acid), then samples were put on ice for 10 min, and centrifuged for 15 min at 6000 rpm. This
148 method was described previously (Okhava et al. 1979).

149

150 *2.5. Analysis of plasma phospholipids fatty acid composition*

151 Plasma lipids were extracted by the method of Folch et al. (1957) using a chloroform–
152 methanol mixture (2:1 v/v), as previously described (Petrovic et al. 2014). The 2,6-di-tert-
153 butyl-4-methylphenol (BHT; 10 mg/100 ml) was added as an antioxidant. The phospholipids
154 fraction was derived from the lipid extract by one-dimensional thin-layer chromatography
155 (TLC) on Silica Gel GF plates (Merck, Darmstadt, Germany), using neutral lipid solvent
156 system of hexane: diethyl ether: acetic acid (87:12:1). Methyl esters of phospholipids fatty
157 acids were obtained by transmethylation, as already reported by Tepsic et al. (2009). Gas
158 Chromatography (GC) using Shimadzu GC 2014 (Shimadzu Co, Tokyo, Japan) equipped
159 with a flame ionization detector and Rtx 2330 fused silica gel capillary column, (60 m x
160 0.25 mm x 0.2 μ m) (Restek Co, Bellefonte, PA, USA) was applied to separate fatty acids
161 methyl esters. Individual FA methyl esters in the samples were identified by comparing
162 sample peak retention times with authentic standards (Sigma Chemical Co, St Louis, MO,
163 USA) and/or (PUFA)-2 standard mixture (Restek Co, Bellefonte, PA, USA). Phospholipids
164 FA profiles were expressed as the relative percentage areas of total FA.

165 The activities of enzymes involved in FA biosynthesis, desaturases and elongases,
166 were estimated as the product-to-precursor ratios, as previously described (Petrovic et al.
167 2014). Estimate of Δ 5-desaturase was computed using the 20:4n-6/20:3n-6 ratio, while the
168 20:3n-6/18:2n-6 ratio was used as a measure of Δ 6-desaturase and elongase activities. The
169 estimated Δ 9-desaturase and elongase activities were derived from 18:1/18:0 and 18:0/16:0
170 ratios, respectively.

171

172 2.6. *Statistical analysis*

173 The data are presented as mean values \pm standard deviation. Normal distribution was
174 evaluated using Shapiro-Wilk test prior to statistical analysis. For non-normally distributed
175 variables (18:3n-3, 22:4n-6 and MUFA), a logarithmic transformation was performed before
176 comparison was made. Paired Student t-test was applied for the start vs. end of the study, and
177 independent samples t-test for aronia vs. placebo group at the same time point for normally
178 distributed variables. The statistical significance was defined as $p \leq 0.05$.

179

180 **3. Results**

181 *3.1. Anthropometric and biochemical parameters of male and female handball players treated*
182 *with chokeberry juice and placebo*

183 As it can be seen from the Table 1, there were no differences in the anthropometric
184 parameters both before and after the study in the same group, nor between chokeberry and
185 placebo groups.

186 In both male and female athletes, there was no difference in serum glucose and total
187 cholesterol concentration before and after the intervention with chokeberry juice or placebo
188 (Table 2). However, triacylglycerol (TAG) concentration significantly decreased in male and
189 increased in female handball players after the treatment with chokeberry juice or placebo ($p <$
190 0.05).

191 Lipid peroxidation was decreased in male players who consumed chokeberry juice, but
192 not in the placebo controlled group nor in the female players.

193 *3.2. Fatty acid profile of plasma phospholipids in placebo and chokeberry juice-treated male*
194 *and female handball players*

195 As presented in Table 3, consumption of chokeberry juice (100 ml/ day) in parallel with
196 intense and regular physical training during 4 weeks, induced slight changes in FA profile of

197 plasma phospholipids in male handball players. The significant decrease was observed only in
198 oleic acid (18:1 n-9) and α -linolenic acid (18:3 n-3, ALA) after the treatment. However,
199 consumption of placebo with the same nutritional and training regimes led to significant
200 decreases of palmitoleic acid (16:1 n-7), vaccenic acid (18:1 n-7), ALA, eicosapentaenoic
201 acid (20:5 n-3, EPA) and docosatetraenoic acid (22:4 n-6) (Table 3).

202 Although the female players were subjected to the same intervention, the obtained
203 results are different. While chokeberry juice induced no changes after 4 weeks, n-6 PUFA and
204 consequently total PUFA were reduced in the placebo controlled group (Table 4).
205 Interestingly, estimated desaturase and elongase activities were not amended in both male and
206 female handball players after the treatment with chokeberry juice or placebo (Table 5).

207

208 **4. Discussion**

209 In elite athletes chronic intensive training leads to oxidative stress and metabolic changes of
210 lipid and FA profiles. Since lipids, especially PUFA, are the most susceptible targets for free
211 radicals induced damages, polyphenol rich chokeberry juice could protect membrane
212 phospholipids from oxidative damage. However, in this study no significant increase in PUFA
213 was found in male and female handball players after 4 weeks of chokeberry juice
214 consumption.

215 All study participants had biochemical parameters within the normal range, including serum
216 TAG levels. After 4 weeks of preparatory training in the male players serum TAG
217 concentration was further reduced. In accordance, reduced plasma TAG concentration was
218 reported in sportsmen participating in other sports, as in long distance runners, cyclists, soccer
219 players and swimmers (Kelley and Kelley 2009; Lira et al. 2010; Rahnama et al. 2009;
220 Saldana et al. 1995). This can be explained by the increased metabolism and utilization of
221 TAG to meet higher energy demands in endurance exercise (Kelley and Kelley 2009). On the

222 other hand, in female players TAG concentration increased in both placebo and chokeberry
223 groups. Although previous studies reported lipid-lowering effect of chokeberry extracts
224 (Sikora et al. 2014; Skoczynska et al. 2007) these studies have been conducted in subjects
225 with hyperlipidemia and/or metabolic syndrome. Elevated levels of TAG in female players
226 may be attributed to enhanced lipid mobilization from adipose tissue during intensified
227 trainings in the campus. In addition, the observed differences between males and females
228 could be explained by sex differences in exercise metabolism (Tarnopolsky 2008; Wu and
229 O'Sullivan 2011). Women use fat as a main energy source for endurance exercise, mostly due
230 to the higher fat mass and greater adipocyte lipolysis. Compared with men, they have less
231 muscle glycogen utilization and higher plasma free fatty acid during endurance exercise
232 (Hamadeh et al. 2005; Roepstorff et al. 2002). These sex differences are due to differences in
233 estrogen concentration and/or activity (Mittendorfer et al. 2002). This could explain transient
234 elevation in TAG concentration in female players after intensive trainings in campus.

235 Plasma TBARS levels are the most often used marker of lipid peroxidation and
236 oxidative damage in general. Thus we determined TBARS at the beginning and at the end of
237 the study. Male players who consumed chokeberry juice showed decreased levels of TBARS
238 after the study period, unlikely those who consumed placebo. These results are in accordance
239 with previous studies (Pilaczynska-Szczesniak et al. 2005). Since both the juice and placebo
240 contained vitamin C (around 29 mg), which is well known antioxidant, the observed
241 differences occurred due to polyphenols and their strong antioxidative capacity. However, in
242 female athletes we found no changes in TBARS level in both groups, although Kardum et al.
243 (2014b) have recently reported reduced TBARS in healthy sedentary women after
244 consumption of chokeberry juice. This suggests that the effect of intensive training, which
245 induces increased lipid peroxidation, is stronger than the effect of chokeberry juice on

246 TBARS in females. Previous studies have also shown that lipid peroxidation during exercise
247 is higher in women than in men due to effect of estrogen (Devries et al. 2007).

248 The fatty acid composition of plasma phospholipids followed in this study, showed
249 some treatment- and sex-dependent alternations. Total SFA, MUFA n-3 and n-6 PUFA
250 (except n-6 PFA in the female placebo group) remained unchanged after the training cycle in
251 both males and females and regardless of the juice consumption. Similarly, no differences
252 were found in the activities of enzymes responsible for FA metabolism. Moderate differences
253 were found in specific FA. In placebo-treated male handball players significant reduction of
254 MUFA: palmitoleic and vaccenic acid, and anti-inflammatory PUFA: ALA (n-3), EPA (n-3)
255 and docosatetraenoic acid (n-6) was found. These effects were mostly attenuated by the intake
256 of chokeberry juice and these sportsmen had lower levels of ALA and oleic acid after the
257 treatment. At the same time, chokeberry juice intake produced no significant alternations in
258 proportion of individual fatty acids in female athletes, while in placebo group a decrease of n-
259 6 and consequently total PUFA was observed.

260 Interestingly, all FA which proportion was reduced in male handball players at the end
261 of the study have beneficial effects on health. Palmitoleic acid plays roles in different
262 physiological processes from cell growth and proliferation, to *de novo* fat synthesis and
263 storage (De Fabiani 2011). It exerts anti-apoptotic activity, increases insulin sensitivity by
264 suppressing inflammation, and inhibits the destruction of insulin-secreting pancreatic β -cells
265 (Chajes et al. 2011). The pro-lipogenic activity of palmitoleic acid in the adipose tissue
266 protects other tissues and organs from TAG accumulation and lipotoxicity (Cao et al. 2008;
267 Scherer et al. 2011). The other two MUFAs, vaccenic acid (18:1n-7, VV) and oleic acid (18:1,
268 n-9), declined in sportsmen treated with placebo and chokeberry, respectively. These FA are
269 both associated with improvement of cell membrane fluidity and with the lower risk of
270 coronary heart disease (Djoussé et al. 2012; Haug et al. 2007). Furthermore, both groups of

271 male players had lower level of ALA after the study. ALA is an essential FA, precursor of
272 long chain n-3 PUFAs and its decrease could be a consequence of intensive training or
273 different intake in the campus. Together with EPA (which level was reduced in the placebo
274 group but not in the chokeberry treated sportsmen), ALA has important role in prevention of
275 different physiological disorders, including hyperlipidemia, inflammation, loss of bone
276 mineral and coronary heart disease (Vucic and Ristic-Medic 2012). The decrease of n-6
277 docosatetraenoic acid can affect prostaglandin and thromboxane synthesis, having an impact
278 on aortic endothelial cells and platelet function (Campbell et al. 1985). However, the results
279 of the same study in female players are different. Chokeberry consumption induced no effect
280 in serum FA profiles, while in placebo controlled group a decrease of n-6 and total PUFA,
281 which could be mainly attributed to a decrease of linoleic acid, has been found. n-6 PUFA are
282 generally known to give rise to pro-inflammatory eicosanoids (prostaglandin E₂ and
283 leukotriene B₄), potent lipid signaling molecules which are the mediators of various
284 pathophysiological processes as asthma, obesity, rheumatoid arthritis, atherosclerosis, and
285 cardiovascular disease (Burtscher and Gnaiger 2013; Grimble 2012). A possible reason for
286 decreased PUFA in female athletes, could be lipid peroxidation induced by the oxidative
287 stress (Dunford and Doyle 2015). The results previously published from our laboratory
288 indicated a significant increase of the antioxidant enzymes activity (superoxide dismutase and
289 glutathione peroxidase) and the decrease of pro-oxidant-antioxidant balance and lipid
290 peroxidation in apparently healthy subject after the long-term (12 weeks) chokeberry juice
291 consumption (Kardum et al. 2014a, b). Nevertheless, we did not find these effects in active
292 handball players. When compared the FA profiles of the chokeberry consuming groups at the
293 beginning and at the end of the study, we found slight differences in male and no differences
294 in female players. When compared chokeberry vs placebo group, there was no significant
295 changes in any FA. In line with this is the result on the estimated desaturase and elongase

296 activities which remained unaltered in all study groups, suggesting that the observed changes
297 in FA profiles are not due to amended metabolism of FA.

298 The weakness of this study is relatively small number of study participants per study group,
299 which is conditioned by the limited number of players chosen for the National team and/or
300 elite clubs. It is particularly important since most changes in FA profiles are found in those
301 FA that are presented in small amounts. Another limitation is the lack of the data on hormonal
302 status of the study participants (progesterone/estradiol ratio) which may affect redox status
303 (Devries et al. 2007). Although we can assume that female players were not in the same phase
304 of menstrual cycle, we did not find striking differences in the studied parameters among them,
305 which could be attributed to the differences in hormonal status. Furthermore, longer treatment
306 with a greater amount of chokeberry juice might lead to desirable changes in FA profiles.
307 According to our results we can conclude that handball players during the period of
308 preparatory training should have nutritional intervention and/or supplementation primarily
309 with n-3 PUFA, while chokeberry juice had weak beneficial effects in these athletes.

310

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315

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441 in blueberries, cranberries, chokeberries, and lingonberries. *J. Agric. Food Chem.* **51**(2):502-
442 509.

443 Table 1. The anthropometric characteristics of male and female handball players and
 444 responsiveness to chokeberry juice and placebo

	Chokeberry juice (n= 8)		Placebo (n=7)	
Men	Before treatment	After treatment	Before treatment	After treatment
Age	18.57±0.53	18.80±0.58	18.38±1.41	18.57±0.53
Height (cm)	192.86±6.91	192.86±6.91	192.63±6.23	195.33±4.37
Weight (kg)	89.81±12.02	90.27±12.32	91.40±17.95	97.13±18.77
WHR	0.78±0.04	0.76±0.03	0.84±0.09	0.85±0.10
BMI (kg/m ²)	24.10±2.63	23.63±2.72	24.61±4.78	25.62±5.67
Basal metabolism (kcal)	2163±232	2212±230	2088±186	2188±160
Body fat (%)	7.43±2.47	5.34±2.57	8.40±2.78	6.63±3.21
Free fat mass (kg)	6.82±4.54	5.04±3.66	7.75±3.45	6.35±3.15
Total body water (kg)	60.93±7.96	62.56±7.91	58.40±6.06	61.73±5.34
	Chokeberry juice (n=10)		Placebo (n=7)	
Women	Before	After	Before treatment	After treatment

	treatment	treatment		
Age	16.8±0.6	17.1±0.7	17.3±1.3	17.5±1.7
Height (cm)	172.8±6.5	172.2±7.3	168.3±8.8	169.4±8.7
Weight (kg)	65.8±7.8	66.3±7.7	64.0±11.4	66.2±11.8
WHR	0.84±0.02	0.85±0.04	0.84±0.05	0.85±0.05
BMI (kg/m ²)	21.5±5.4	22.3±2.5	22.8±2.0	22.9±2.0
Basal metabolism (kcal)	1489±138	1498±150	1394±175	1426±167
Body fat (%)	21.06±5.41	21.02±6.86	25.80±1.70	25.96±3.45
Free fat mass(kg)	14.33±3.93	14.81±6.96	15.18±3.95	14.80±4.86
Total body water (kg)	38.05±4.69	38.24±5.11	34.75±5.95	35.78±5.69

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446 Data are presented as a mean ± SD.

447 BMI, body mass index.

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453 Table 2. Serum lipid status and glucose of handball players (male – M and female – F) before
 454 and after the treatment with chokeberry juice or placebo.
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Parameter (mmol/L)	Chokeberry juice		Placebo	
	Before treatment	After treatment	Before treatment	After treatment
Glucose				
M	4.47 ± 0.29	4.27 ± 0.30	4.84 ± 0.30	4.33 ± 0.47
F	4.42 ± 0.26	4.32 ± 0.41	4.61 ± 0.29	4.41 ± 0.41
Triacylglycerol				
M	0.87 ± 0.19	0.64 ± 0.22*	0.96 ± 0.28	0.64 ± 0.13*
F	0.47 ± 0.12	0.67 ± 0.17*	0.57 ± 0.13	0.73 ± 0.12*
Total cholesterol				
M	4.06 ± 0.61	3.72 ± 0.46	4.08 ± 0.45	3.90 ± 0.51
F	3.94 ± 0.93	4.38 ± 0.46	3.93 ± 0.47	4.14 ± 0.45
TBARS				
M	0.63 ± 0.21	0.33 ± 0.25*	0.58 ± 0.38	0.56 ± 0.24
F	6.68 ± 0.35	7.71 ± 1.05	6.58 ± 0.22	7.02 ± 1.04

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457 Data are presented as a mean ± SD. * $p < 0.05$ compared to baseline (before treatment).

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462 Table 3. Plasma phospholipids fatty acid composition in male handball players before and
 463 after the treatment with chokeberry juice and placebo

Fatty acid (%)	Chokeberry juice		Placebo	
	Before treatment	After treatment	Before treatment	After treatment
16:0	26.59 ± 2.06	28.07 ± 1.60	26.20 ± 2.51	27.53 ± 1.76
18:0	14.79 ± 0.82	14.46 ± 1.27	15.68 ± 1.46	15.12 ± 1.02
SFA	41.38 ± 1.68	42.53 ± 1.82	41.88 ± 1.22	42.65 ± 1.39
16:1n-7	0.54 ± 0.29	0.38 ± 0.14	0.60 ± 0.17	0.38 ± 0.12*
18:1n-9	9.31 ± 1.34	8.23 ± 0.71*	8.49 ± 0.97	8.10 ± 0.70
18:1n-7	1.60 ± 0.24	1.62 ± 0.15	1.53 ± 0.22	1.31 ± 0.21*
MUFA	11.44 ± 1.72	10.23 ± 0.69	10.62 ± 0.97	9.79 ± 0.69
18:2n-6	26.96 ± 2.66	27.20 ± 2.41	26.03 ± 2.79	27.46 ± 3.33
20:3n-6	3.40 ± 0.76	3.48 ± 0.53	3.22 ± 0.41	3.22 ± 1.03
20:4n-6	12.44 ± 2.07	12.19 ± 1.41	13.79 ± 2.73	12.85 ± 2.35
22:4n-6	0.61 ± 0.08	0.57 ± 0.10	0.78 ± 0.21	0.62 ± 0.12*
n-6 PUFA	43.42 ± 2.67	43.43 ± 2.40	43.82 ± 1.72	44.15 ± 1.41
18:3n-3	0.26 ± 0.15	0.13 ± 0.02*	0.41 ± 0.17	0.13 ± 0.05***
20:5n-3	0.38 ± 0.07	0.30 ± 0.10	0.38 ± 0.09	0.24 ± 0.10**
22:5n-3	0.70 ± 0.15	0.75 ± 0.09	0.62 ± 0.16	0.64 ± 0.10

22:6n-3	2.41 ± 0.77	2.56 ± 0.56	2.31 ± 0.17	2.41 ± 0.39
n-3 PUFA	3.75 ± 0.96	3.72 ± 0.66	3.66 ± 0.41	3.41 ± 0.36
n-6/n-3	47.18 ± 3.36	47.16 ± 2.31	47.49 ± 1.60	47.56 ± 1.44
ratio	12.10 ± 2.47	11.97 ± 2.15	12.11 ± 1.61	13.06 ± 1.36
PUFA/SFA	1.13 ± 0.07	1.12 ± 0.07	1.14 ± 0.13	1.11 ± 0.10
ratio				

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465 Data are presented as a mean ± SD.

466 SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated
 467 fatty acids. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to baseline (before treatment).

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481 Table 4. Serum phospholipids fatty acid composition in female handball players before and
 482 after the treatment with chokeberry juice and placebo

Fatty acid (%)	Chokeberry juice		Placebo	
	Before treatment	After treatment	Before treatment	After treatment
16:0	28.07 ± 1.32	28.44 ± 1.94	27.90 ± 1.89	28.08 ± 0.64
18:0	13.93 ± 1.17	14.47 ± 1.52	14.76 ± 0.94	15.47 ± 0.80
SFA	42.00 ± 1.23	42.91 ± 0.96	42.67 ± 1.35	43.55 ± 0.73
16:1n-7	0.46 ± 0.06	0.42 ± 0.12	0.49 ± 0.12	0.56 ± 0.13
18:1n-9	8.52 ± 0.91	8.85 ± 0.85	7.81 ± 0.49	8.55 ± 0.78
18:1n-7	1.45 ± 0.17	1.40 ± 0.23	1.34 ± 0.18	1.39 ± 0.19
MUFA	10.42 ± 0.91	10.67 ± 0.99	9.65 ± 0.49	10.50 ± 0.71
18:2n-6	29.51 ± 2.66	29.46 ± 2.35	29.85 ± 1.64	27.11 ± 1.78
20:3n-6	2.74 ± 0.75	2.71 ± 0.69	2.69 ± 0.77	3.34 ± 0.62
20:4n-6	11.15 ± 1.63	10.56 ± 1.63	11.10 ± 1.47	11.32 ± 1.95
22:4n-6	0.51 ± 0.06	0.46 ± 0.12	0.50 ± 0.19	0.61 ± 0.11
n-6 PUFA	43.92 ± 1.57	43.20 ± 1.12	44.14 ± 1.03	42.37 ± 0.57*
18:3n-3	0.26 ± 0.17	0.19 ± 0.12	0.17 ± 0.15	0.21 ± 0.16
20:5n-3	0.25 ± 0.09	0.23 ± 0.12	0.19 ± 0.12	0.24 ± 0.08
22:5n-3	0.52 ± 0.11	0.50 ± 0.13	0.54 ± 0.22	0.53 ± 0.15

22:6n-3	2.63 ± 0.49	2.29 ± 0.63	2.64 ± 0.75	2.59 ± 0.39
n-3 PUFA	3.66 ± 0.52	3.22 ± 0.74	3.54 ± 0.89	3.58 ± 0.46
PUFA	47.58 ± 1.51	46.42 ± 0.92	47.69 ± 1.10	45.95 ± 0.85*
n-6/n-3 ratio	12.25 ± 2.02	14.08 ± 3.14	13.32 ± 4.15	12.05 ± 1.89
PUFA/SFA ratio	1.13 ± 0.07	1.08 ± 0.04	1.12 ± 0.06	1.06 ± 0.03

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484 Data are presented as a mean ± SD.

485 SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated
 486 fatty acids. * $p < 0.05$ compared to baseline (before treatment).

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501 Table 5. The estimated plasma desaturase and elongase activities in male (M) and female (F)
 502 handball players before and after the treatment with chokeberry juice and placebo

Desaturase and elongase	Chokeberry juice		Placebo	
	Before treatment	After treatment	Before treatment	After treatment
20:4n-6/20:3n-6 ($\Delta 5$)				
M	3.82 \pm 1.06	3.56 \pm 0.55	4.35 \pm 0.97	4.38 \pm 1.58
F	4.34 \pm 1.24	4.17 \pm 1.44	4.39 \pm 1.37	3.50 \pm 0.91
20:3n-6/18:2n-6 ($\Delta 6$)				
M	0.13 \pm 0.04	0.13 \pm 0.02	0.13 \pm 0.02	0.12 \pm 0.05
F	0.09 \pm 0.03	0.09 \pm 0.03	0.09 \pm 0.03	0.12 \pm 0.03
18:1/18:0 ($\Delta 9$)				
M	0.63 \pm 0.11	0.57 \pm 0.07	0.55 \pm 0.09	0.54 \pm 0.05
F	0.62 \pm 0.11	0.62 \pm 0.10	0.53 \pm 0.05	0.55 \pm 0.06
18:0/16:0 (elongase)				
M	0.56 \pm 0.06	0.52 \pm 0.06	0.61 \pm 0.12	0.55 \pm 0.06
F	0.50 \pm 0.06	0.51 \pm 0.08	0.53 \pm 0.07	0.55 \pm 0.04

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504 Data are presented as a mean \pm SD.

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