

Effects of antioxidant supplementation on oxidative stress balance in young footballers: a randomized controlled trial

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Abstract

Background

The intensive physical exercise in which athletes take part in competitive sports can negatively affect the pro-oxidative-antioxidant balance. The use of compounds with high antioxidant potential, which certainly should include chokeberry, can prevent these adverse changes.

Methods

The study was conducted on a group of football players aged 16–17 years, who underwent 7 weeks of supplementation with 200 ml chokeberry juice per day. Before and after supplementation, the participants performed an intensive physical exercise test (beep test). At rest, immediately after exercise and after 3 and 24 h of rest, venous blood was taken from the subjects, in which levels of thiobarbituric acid reactive products (TBARS), hydroxy-2'-deoxyguanosine (8-OHdG), total antioxidant capacity (TAC), iron (Fe), hepcidin, ferritin, myoglobin, albumin and morphological parameters were examined.

Results

There was a significant impact of the intervention in response to the physical exercise test in the studied groups on parameter dynamics: 8-OHdG ($t = 3.56$, $p = 0.0005$), albumin ($t = 1.98$, $p = 0.049$), TBARS ($t = 4.33$, $p = 0.00003$), hepcidin ($t = 2.21$, $p = 0.03$), and Mono level ($t = 2.14$, $p = 0.04$) and percentage ($t = 2.27$, $p = 0.03$). The post-hoc test showed no effect of chokeberry juice supply on any of the morphological, biochemical or performance parameters analysed.

Conclusions

The supplementation applied to footballers showed no effects under the influence of the applied exercise stress test. Such results may be the result of both the players' adaptation to the applied exercise loads and the insufficient antioxidant capacity of the supplement used.

Background

Increased metabolism during physical exercise is accompanied by increased generation of reactive oxygen species, which may also cause disorders in the functioning of the immune system [1, 2]. This applies primarily to physical exercise of high intensity or long duration. The mechanism of this process is not fully understood. It is believed that excessive production of free oxygen radicals leads, among other things, to damage to erythrocytes (as a consequence of lipid peroxidation), thus increasing their sensitivity to degradation. As a result of increased haemolysis, there is a significant increase in redox-active iron in the circulation. Fenton's reaction under the influence of iron is the main cause of Fe toxicity in the body. High reactivity and low specificity $\cdot\text{OH}$ might facilitate destructive process of cell components and body fluids Under conditions of oxidative stress, activation of the immune system and inflammation are also observed, which is an early defence response of the body. Probably in this way, oxidative stress is 'sustained' also during post-workout restitution. The increase in ionized iron concentration in the blood serum, which is a consequence of this process, can contribute both to the intensification of free radical reactions on the one hand [3], and to weakening of the immune system on the other, thereby increasing susceptibility to infection [4–6]. Acute post-exercise depression of the immune system may result not only in increased frequency of infections in competitors, but also a higher percentage of cases (especially for upper respiratory tract diseases – URTI) and a much longer duration of URTI [7].

Chokeberry contains a wide range of biologically active compounds, including polyphenols such as anthocyanin, flavonoids and phenolic acids [8, 9]. Analysis of literature data indicates that compounds contained in chokeberry can have a positive effect on health [10]. Particular importance is attributed to anthocyanins, in which chokeberry fruit is rich. The advantage of these compounds is their comprehensive impact on both the immune system and reduction of oxidative stress, including the ability to chelate iron ions, which seems to be a key element not only for iron management. Anthocyanin supplementation might lead to reduced post-exercise muscle soreness [11] and improvement of performance parameters.

Analysis of the results available in the scientific databases conducted on athletes, as well as the results of numerous scientific reports based on research conducted on non-training people and animals, leads to the conclusion that the endogenous defence of an organism subjected to intense exercise load is insufficient. It seems that preparations rich in anthocyanins may be an important factor in alleviating the adverse effects of extreme exercise loads. Thus, it seems advisable to introduce to the diet of competitors plants rich in anthocyanins, which not only show the ability to form stable complexes with transition metals, but also increase the body's antioxidant potential. Such supplementation can both reduce oxidative stress, significantly reducing post-exercise inflammatory processes, and contribute to an increase in ergogenic potential.

The aim of the study was to analyse the effect of 7-week supplementation with chokeberry juice on the parameters of pro-oxidative–antioxidant balance and iron levels in footballers during the football season.

Methods

Research material

Twenty footballers from the MUKS Zawisza Bydgoszcz club participating in the Central Junior League competitions took part in the research. Basic data regarding the study group are included in Table 1. Football players receiving chokeberry juice and a placebo implemented a uniform training load scheme. Training loads in the week preceding and ending the experiment are shown in Table 2. All subjects were informed about the purpose of the research and the procedures used, and voluntarily agreed to participate in the experiment. The research received a positive opinion from the Bioethics Committee at Collegium Medicum in Bydgoszcz (consent No. KB 382/2017).

Table 1
Characteristics of the examined group

N = 20	Age [years]	Training internship [years]	Height [cm]	Weight [kg]	BMI	Body fat [%]
	17.6	6.5	182.9	72.4	21.6	13.2
Min.	16	5	168	61.7	18.8	10.6
Max.	18.7	8	190	80.8	24.1	16.8
σ	0.7	0.8	5	5.6	1.3	1.8
V	4	13.6	2.7	7.7	6.1	13.3

Legend: N – population, cm – centimetre, kg – kilogram, % – percent, – arithmetic average, min. – minimum value, max. – maximum value, σ – standard deviation, V – coefficient of variation

Table 2
Training loads in microcycles preceding exercise tests

Training	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Training time	45–60 min	90 min	90 min	90 min	45–60 min		
Exercise time	Short 2–4 min	Medium 4–8 min	Long 8–20 min	Very short 1–2 min	Short 2–5 min		
Training loads, scale 1–10*	1–2	4–6	5–8	2–4	3–5	8–10	1–2
Training content	Active regeneration. Large forms of tactics with technique breaks.	Elements of the game in attack and defence in a limited field of play.	Maximum intensity. Endurance. Small and large games.	Game speed without much resistance. Defence against counterattack.	Force-speed stimulation. Tactical games and exercises with an accent of speed.	Control/championship match.	Regeneration.

Legend: min – minutes, * – training load scale where 1 means training at the lowest intensity and 10 means training at the highest intensity

Experiment/supplementation

The participants were randomly divided into two groups: supplemented ($N = 12$), which received 200 ml of chokeberry juice (100 ml twice a day in the morning and evening) for 7 weeks, and control ($n = 8$) which received a placebo at the same time. The placebo was identical to chokeberry juice in appearance and taste. Both the chokeberry juice and placebo were produced by MLB Biotrade Sp. z o.o., Poland. Juice and placebo were placed in identical dark coded bottles. The label codes were decoded after the test. In addition, the antioxidant capacity of chokeberry juice was determined using the DPPH and ABTS methods, which amounted to 8.83 and 7.62 mg/ml, respectively (tests were carried out by the Lubuskie Centre for Innovation and Agricultural Implementation of the University of Zielona Góra). The players participating in the study were informed during the experiment not to take any additional supplements or medications or to change their daily diet. Before and after supplementation, all competitors performed the Maximal Multistage 20 m Shuttle Run Test [12]. The exercise test ('beep test') was carried out in a full-size sports hall with a classic surface. During the test, the air temperature was 19.1 °C and humidity was 51%. All tested players were informed about the test procedures and additionally motivated by the trainer to make maximum effort.

Material Collection And Examination

During the control exercise tests (beep test) carried out at the beginning and end of the experiment, blood was taken from the competitors four times. Blood was drawn before the exercise test, immediately after the end of the exercise test, and 3 and 24 h after the end of the exercise. Blood was drawn from the vein of the elbow into 9 ml polyethylene tubes (to obtain serum). The resulting material was then centrifuged (3000 rpm/10 min) and stored at -80 °C until further examination. In addition, venous blood was also collected from the competitors in 5 ml tubes that contained EDTAK2 anticoagulant. This blood was used to determine the morphological parameters of the blood (RBC, HGB, HCT, MCV, MCH, MCHC). The examinations were made using flow cytometry using Sysmex XS-1000i apparatus.

The iron (Fe) level was determined in plasma taken from lithium heparin and determined by in vitro IRON 2 test for the quantitative determination of iron in human serum and plasma in a Roche/Hitachi Cobas c. system using a Cobas c 501 analyser.

Lactic acid (LA) was measured in capillary blood collected from the earlobe before and immediately after the test. The measurement was made using a Dr. Lange Plus LP20 biochemical analyser.

For detailed analysis of changes in iron management, total antioxidant levels and inflammatory cell response, the following tests were used: ferritin ELISA EIA-1872, IL-6 ELISA EIA-4640, myoglobin ELISA EIA-3955 and hepcidin 25 (bioactive) HS ELISA EIA-5782 from DRG International, Inc., USA; human (TBARS) ELISA kit (catalogue no.: 201-12-7298) and human (8-OHdG) ELISA kit (catalogue no.: 201-12-1437) from Shanghai SunRed Biological Technology Co. Ltd.; human albumin ELISA kit from Assaypro LLC, St. Charles, MO, USA; and TAC Fast Track DM P-4100 from Omnisignostica Forschungs GmbH, Austria. A Thermo Scientific Multiscan GO Microplate Spectrophotometer produced by Fisher Scientific Finland was used for the material examination.

Statistical analysis

Sample size calculation was done based on previous results on the effects of supplementation on TBARS in males [13], as the variable of primary interest in the above study, using a calculator available online [<http://powerandsamplesize.com/Calculators/Compare-2-Means/2-Sample-1-Sided>]. As in previous research [13], it was decided to increase the sample size in the intervention group by setting the sampling ratio as 1.5. Power was set to 0.8, while the Type I error rate was 5%. The calculated sample size in intervention group was $n = 12$. The Shapiro–Wilk W test was used to test the assumption of normality.

Two-factor ANOVA with group coefficient (supplemented group/placebo group) and time (trial/trial II) was selected for the analysis of physical fitness variables when all assumptions were met. In the case where the normality assumption was violated, aligned rank transform for nonparametric factorial ANOVA was used, analysed using R [14]. The post-hoc test for differences of differences was done using the R package phia [15]. To assess dynamics of biochemical parameters in response to the physical exercise test, a linear mixed model fit by REML with *t*-tests using Satterthwaite's method was applied with the R statistical packages lme4 and lmerTest. Subject and time (before vs just after vs 3 h after vs 24 h after physical exercise test in the case of biochemical parameters; and before vs 3 h after in the case of blood morphometry parameters) factors were set as random effects. Group (placebo vs supplemented) and intervention (before vs after physical exercise programme) were set as fixed effects. Interaction between fixed effects was calculated as well as the confidence interval (95%) for estimating interaction. As post-hoc tests, a series of *t*-tests with Benjamini–Hochberg adjusted p value was applied to control for false discovery rate (FDR) using an online calculator (<https://tools.carbocation.com/FDR>). Mean value and standard deviation (SD) are reported and the alpha level was set to 0.05.

Results

There was no significant interaction between time × group and VO₂max (58.82 ml/kg/min before vs 60.35 after in the juice group, 58.48 ml/kg/min before vs 60.36 after in the placebo group) ($F = 0.19$, $p = 0.66$). In addition, there was no significant interaction of time × group in the distance covered in the 20 m shuttle run test (before supplementation 2509.09 m – level 13, interval 8 vs 2623.64 m – level 13, interval 13 and after supplementation in the juice group 2482.22 m – level 13, interval 7 vs 262.22 m – level 13, interval 13 after in the placebo group; $F = 0.02$, $p = 0.88$).

A significant impact of the intervention on albumin dynamics was observed under the influence of the physical effort test in the studied groups (estimate = 0.58 (CI of estimate = 0.02; 1.15), $t = 1.98$, $p = 0.049$ (Table 3a)). The results of post-hoc tests were statistically insignificant.

Table 3

a. The impact of chokeberry supplementation on selected parameters of inflammation and iron management

Parameters	Supplemented group		Placebo group	
	Trial I Mean (SD)	Trial II Mean (SD)	Trial I Mean (SD)	Trial II Mean (SD)
Albumin [µg/ml]				
Before	4.55 (1.1)	3.55 (0.7)	4.65 (0.9)	4.02 (1.0)
After	4.85 (0.6)	3.79 (0.6)	5.34 (1.7)	3.69 (0.6)
3 h after	4.46 (0.8)	3.31 (0.5)	5.57 (1.8)	3.63 (0.8)
24 h after	4.33 (1.2)	3.73 (0.9)	5.15 (1.8)	3.23 (0.4)
Myoglobin [ng/ml]				
Before	15.23 (7.5)	14.11 (4.3)	19.35 (15.6)	17.64 (8.9)
After	17.50 (5.8)	17.77 (6.9)	21.21 (17.9)	22.82 (11.0)
3 h after	19.17 (8.3)	14.98 (6.1)	24.16 (14.7)	24.18 (12.3)
24 h after	28.85 (13.6)	17.60 (13.9)	28.25 (16.4)	16.30 (7.5)
IL-6 [pg/ml]				
Before	47.44 (13.1)	48.42 (18.8)	42.80 (7.0)	44.51 (4.2)
After	49.97 (12.7)	54.25 (24.5)	43.83 (2.3)	49.11 (4.8)
3 h after	51.35 (23.1)	47.47 (10.4)	45.75 (11.9)	45.33 (4.0)
24 h after	46.84 (13.7)	46.98 (10.8)	43.97 (5.0)	45.18 (3.9)
Hepcidin [ng/ml]				
Before	6.99 (3.5)	9.31 (12.9)	7.34 (8.6)	4.74 (1.6)
After	7.24 (4.3)	11.29 (16.5)	7.55 (8.8)	4.53 (2.2)
3 h after	7.56 (4.9)	12.42 (15.4)	8.96 (9.1)	7.04 (4.1)
24 h after	8.27 (5.6)	8.69 (11.5)	4.39 (2.8)	4.05 (1.4)
Ferritin [ng/ml]				
Before	12.11 (7.2)	13.08 (8.6)	10.09 (4.8)	11.55 (4.3)
After	12.46 (8.3)	14.84 (9.5)	11.14 (5.7)	13.12 (5.9)
3 h after	11.17 (6.2)	12.76 (8.8)	10.72 (6.0)	10.68 (4.3)
24 h after	11.79 (7.2)	13.14 (9.3)	10.78 (5.4)	11.04 (6.8)
Iron [µg/ml]				
Before	97.12 (19.8)	104.67 (43.3)	114.74 (32.5)	120.19 (23.8)
3 h after	78.35 (20.1)	81.37 (35.2)	88.71 (24.4)	125 (16.7)

Legend: (SD) – standard deviation, µg/ml – micrograms/millilitre, ng/ml – nanograms/millilitre, pg/ml – picograms/millilitre, before – before the test, after – after the test, 3 h after – 3 h after the test, 24 h after – 24 h after the test

Table 3

b. Influence of chokeberry supplementation on selected parameters of pro-oxidative-antioxidant balance

Parameters	Supplemented group		Placebo group	
	Trial I Mean (SD)	Trial II Mean (SD)	Trial I Mean (SD)	Trial II Mean (SD)
TAC [mmol/l]				
Before	1.36 (0.3)	1.94 (0.7)	1.03 (0.3)	1.76 (0.5)
After	0.97 (0.3)	0.74 (0.4)	0.81 (0.3)	0.97 (0.3)
3 h after	1.18 (0.3)	1.52 (0.4)	1.00 (0.3)	1.45 (0.2)
24 h after	1.13 (0.3)	1.77 (0.3)	1.25 (0.5)	1.75 (0.5)
TBARS [nmol/ml]				
Before	22.45 (1.9)	17.11 (5.3)	23.92 (3.5)	14.69 (4.6)
After	22.26 (3.1)	18.58 (7.8)	23.37 (3.9)	14.36 (3.6)
3 h after	21.01 (2.9)	18.53 (6.8)	24.09 (3.6)	16.18 (3.6)
24 h after	21.47 (3.2)	16.85 (5.7)	24.24 (6.2)	15.18 (5.1)
8-OHdG [ng/ml]				
Before	3.2 (0.8)	2.04 (0.5)	4.02 (1.4)	1.87 (0.3)
After	3.01 (0.8)	2.28 (0.7)	4.13 (1.6)	2.09 (0.4)
3 h after	3.04 (0.9)	2.12 (0.5)	3.28 (1.0)	1.98 (0.3)
24 h after	3.12 (0.6)	1.99 (0.8)	3.35 (1.0)	1.73 (0.2)

Legend: SD – standard deviation, mmol/l – millimoles/litre, nmol/ml – nanomoles/millilitre, ng/ml – nanograms/millilitre, TBARS – thiobarbituric acid reactive substances, TAC – total antioxidant capacity, 8-OHdG – 8-oxo-2'-deoxyguanosine, before – before the test, after – after the test, 3 h after – 3 h after the test, 24 h after – 24 h after the test

A significant impact of the intervention period on hepcidin dynamics was observed under the influence of the physical exercise test in the studied groups (estimate = 4.88 (CI of estimate = 0.56; 9.21), t = 2.21, p = 0.03 (Table 3a)). The results of post-hoc tests were statistically insignificant.

Biochemical analysis of the remaining selected parameters of inflammation did not show a significant interaction in the supplemented or placebo groups (Table 3a).

A significant effect of the intervention period on TBARS dynamics was observed under the influence of the physical exercise test in the studied groups (estimate = 4.77 (CI of estimate = 2.62; 6.92), t = 4.33, p = 0.00003 (Table 3b)). The results of post-hoc tests were statistically insignificant.

A significant effect of the intervention period on 8-OHdG dynamics was observed under the influence of the physical exercise test in the studied groups (estimate = 0.79 (CI of estimate = 0.36; 1.22), t = 3.56, p = 0.0005 (Table 3b)). The results of post-hoc tests were statistically insignificant.

Biochemical analysis of the remaining selected parameters of pro-oxidative-antioxidant balance did not show any significant interaction in the supplemented group or the placebo group (Table 3b).

A significant impact of the intervention period on Mono dynamics was observed under the influence of the physical effort test in the studied groups (estimate = 0.1 (CI of estimate = 0.01; 0.2), t = 2.14, p = 0.04). The results of post-hoc tests were statistically insignificant.

A significant impact of the intervention period on the dynamics of Mono percentage was observed under the influence of the physical effort test in the studied groups (estimate = 1.68 (CI of estimate = 0.23; 3.12), t = 2.27, p = 0.03). The results of post-hoc tests were statistically insignificant.

No significant effect of the chokeberry supplementation period was observed on the blood morphotic elements tested (Table 4).

Table 4

Effects of periods of antioxidant supplementation on blood morphology before and after physical exercise test

Parameters	Supplemented group		Placebo group	
	Trial I Mean (SD)	Trial II Mean (SD)	Trial I Mean (SD)	Trial II Mean (SD)
WBC before	7.80 (1.5)	7.05 (1.7)	7.57 (2.7)	6.79 (2.6)
WBC 3 h after	9.75 (2.2)	9.13 (1.5)	9.95 (4.2)	9.61 (3.0)
RBC before	4.89 (0.2)	5.07 (0.3)	4.75 (0.2)	4.98 (0.3)
RBC 3 h after	4.86 (0.2)	4.87 (0.3)	4.68 (0.3)	4.77 (0.2)
Hb before	14.45 (0.6)	14.86 (0.9)	14.21 (0.5)	14.93 (0.8)
Hb 3 h after	14.37 (0.5)	14.34 (0.9)	14.01 (0.7)	14.23 (0.8)
Hct before	40.76 (1.6)	42.48 (2.2)	39.70 (1.5)	42.03 (2.4)
Hct 3 h after	40.26 (1.5)	40.40 (2.1)	38.91 (2.3)	39.43 (2.5)
Fe before	97.12 (19.8)	104.67 (43.3)	114.74 (32.5)	120.19 (23.8)
Fe 3 h after	78.35 (20.1)	81.37 (35.2)	88.71 (24.4)	125 (16.7)
LA before	1.45 (0.3)	1.60 (0.3)	1.56 (0.3)	1.48 (0.2)
LA after	9.85 (2.4)	10.58 (1.8)	9.62 (1.9)	10.56 (1.8)

Legend: SD – standard deviation, WBC – white blood cells, RBC – red blood cells, Hb – haemoglobin, Hct –haematocrit, Fe – iron, LA – lactate acid, before – before the test, after – 180 s after the test, 3 h after – 3 h after the test

As Table 5 shows, no significant changes in body weight, BMI or adipose tissue were observed after supplementation in any of the groups.

Table 5
Body mass, body mass index and body fat level changes

Parameters	Supplemented group		Placebo group	
	Trial I	Trial II	Trial I	Trial II
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Weight [kg]	68.42 (6.7)	69.4 (6.4)	63.66 (5.6)	64.44 (5.5)
BMI	21.1 (1.9)	22.1 (1.7)	20.51 (1.4)	20.75 (1.3)
Body fat [%]	12.9 (1.6)	10.8 (1.9)	13.8 (1.9)	11.7 (0.6)

Legend: SD – standard deviation, BMI – body mass index, body fat – percentage body fat

Discussion

The physical exercise in which athletes take part in competitive sport may result in a disturbance of homeostasis of the body, which in turn leads to much worse sports performance, as well as a deterioration of health. Analysis of the available literature indicates that the compounds contained in chokeberry have strong antioxidant activity. In this respect, a key role is attributed to anthocyanins, which prevent the excessive formation of free radicals, namely superoxide, hydroxyl, nitrite and chlorine radicals [16, 17]. The anti-radical activity of these compounds is increased by the number of hydroxyl groups on the B ring and the arylation of sugar residues with phenolic acids. As demonstrated in the studies of van Acker et al., anthocyanins have 100 times higher activity in nitric oxide radical ($\bullet\text{NO}$) removal than the endogenous antioxidant glutathione [18]. Due to the presence of hydroxyl groups in the C ring, these compounds are able to chelate transition metal ions (e.g. iron and copper) [19]. Another important feature of anthocyanins from the point of view of health is their ability to inhibit lipid peroxidation [20], which can be of great importance in reducing haemolysis induced by intense physical exertion.

Research shows that the antioxidant capacity of chokeberry measured by total antioxidant capacity (TAC) as well as the strength of reduction of Fe^{3+} to Fe^{2+} is very high and depends on weather conditions; the presence of active compounds is influenced by both the average air temperature and hours of sunshine from May to September [21].

The antioxidant potential of the chokeberry juice given to football players was measured using two methods, DPPH and ABTS, as 8.83 mg/ml and 7.62 mg/ml, respectively (relative to the activity of the Trolox reference compound), which indicates that it was relatively low. This can probably be explained by the lack of statistically significant differences demonstrated not only in the level of TAC, but also in other biochemical, morphological and performance parameters in football players (Tables 3a, 3b, 4 and 5).

Petrovic et al. used chokeberry juice supplementation in handball players and showed that 100 ml/day supplementation of chokeberry contributed to small changes in the lipid profile and reduced TBARS levels; however, these changes were observed only in men [22]. In our research, the chokeberry juice dose was twice as high, which had no effect on the reduction of free radical damage measured with both TBARS and 8-OHdG levels (Table 3b). García-Flores et al. combined chokeberry extract with citrus juice (200 ml of drink was 95% fresh citrus juice and 5% chokeberry extract); this combination of ingredients significantly reduced post-exercise changes in the level of DNA damage markers measured in both the plasma and urine of triathlon riders [23].

Analysis of the available literature indicates that the advantage of compounds derived from chokeberry is their comprehensive effect on both the immune system and reduction of oxidative stress, including the ability to chelate iron ions, which seems to be a key element not only for iron management. For this reason, we expected it to reduce markers of oxidative stress. However, the lower (statistically insignificant) average values of the tested markers of oxidative stress, obtained in the second test period (after supplementation), concerned both the supplemented and control groups, which

may be a result of the players' adaptation to the applied exercise loads. Zügel et al. analysed the cumulative effect of training stress in highly qualified athletes practising rowing on the level of hepcidin and its impact on parameters related to iron management. They showed that the levels of hepcidin and ferritin as acute-phase proteins were a sensitive indicator of changes in training loads (increase in volume and intensity of exercise). In their own research, football players were subjected to the same training loads throughout the entire study period (Table 2), which probably explains the lack of statistically significant differences in the levels of hepcidin and ferritin (Table 3a) [24]. In other studies conducted by the team of Villaño et al., the effect of physical exercise and supplementation with juice high in polyphenols (the juice also contained chokeberry extract) on the level of hepcidin was analysed in a group of triathletes of both sexes. The study did not show a significant impact of the supplement on this parameter, while its reduction was associated with adaptation of the players' bodies to the applied exercise loads [25].

In our study of footballers, interesting trends related to iron levels were observed in the second study period; namely, the level of this parameter after 3 h of rest in the supplemented group decreased, while in the control group it increased (Tables 3a and 4). Similar changes in iron levels were observed by Punduk et al. in volunteers who received intensive platelet-rich plasma therapy during intense exercise. This therapy aimed to improve muscle regeneration, which was damaged by the use of high-intensity exercises (exercise-induced muscle damage, EIMD) [26]. It can be assumed that the ability to chelate iron ions, through the active compounds contained in chokeberry, can also counteract damage to muscle fibres. Confirmation of this thesis can be observed from the changes in myoglobin level in subjects, although they were not statistically significant (Table 3a). Myoglobin is a marker of muscle fibre damage; in the group supplemented with chokeberry it showed a downward trend, while in the control group the level of this parameter increased.

In the inflammatory process, the role of anthocyanins can result from both the ability to sequestrate iron [27] and from their regulatory action on various components of the immune system involved in the development of inflammation [28]. Research conducted by Ohgami et al. on animal models has shown that chokeberry extract has a strong anti-inflammatory effect on endotoxin-induced uveitis in rats. The authors observed that the number of inflammatory cells, the protein concentration, and the levels of NO, pyrogenic prostaglandin E2 (PGE2) and TNF α in the aqueous humour in the groups treated with aronia crude extract were significantly reduced, and effect strength depends on the dose used [29]. For this reason, the standardization of chokeberry products for the presence of anthocyanin compounds, which play a key role in health protection, may be of great importance.

Summing up the research results presented above, it can be stated that the use of chokeberry products in the diet did not cause significant changes in the parameters analysed. The reason could be both good adaptation of the examined players to their physical exercise, and the use of juice with low antioxidant capacity. Therefore, it seems reasonable to consider the use of chokeberry extracts standardized for the presence of anthocyanins. Another issue that should be explored is to understand the mechanisms of how and what compounds contained in chokeberry may be responsible for improving the parameters studied.

Conclusions

Chokeberry juice supplementation applied to footballers did not affect the changes observed in players under the influence of the applied stress test. Such results could be the result of both good adaptation of athletes to the applied exercise loads, and the insufficient antioxidant capacity of the chokeberry juice used. Further research should consider the supply of chokeberry in a more concentrated form, e.g. a concentrate or lyophilisate, which would have a much higher antioxidant potential.

Abbreviations

ANOVA analysis of variance

n	sample size
F	result of variance analysis
t	ratio of the departure of the estimated value of a parameter from its hypothesized value to its standard error/result of Satterthwaite's method
p	p value
%	percent
σ	standard deviation
SD	standard deviation
\bar{x}	arithmetic average
V	coefficient of variation
min	minimum
max	maximum
R	language and environment for statistical computing and graphics
phia	post-hoc interaction analysis R package
ggplot2	data visualization package for the statistical programming language R
REML	restricted maximum likelihood
FDR	false discovery rate
CI	<i>confidence interval</i>
DPPH	2,2-diphenyl-1-picrylhydrazyl
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
UV-VIS	<i>ultraviolet-visible</i>
$\cdot\text{OH}$	hydroxyl radical
URTI	<i>upper respiratory tract infection</i>
vs	<i>versus</i>
mg	milligram
ml	millilitre
ng	nanogram
pg	picogram
μg	microgram

°C degrees Celsius

VO₂max maximal oxygen consumption

rpm revolutions per minute

s second

min minute

h hour

Fe serum iron

WBC white blood cells

RBC *red blood cells*

HGB haemoglobin

HCT haematocrit

MCV *corpuscular volume*

MCH corpuscular haemoglobin

MCHC corpuscular haemoglobin concentration

BMI body mass index

IL-6 interleukin 6

TAC total antioxidant capacity

TBARS thiobarbituric acid reactive substances

8-OHdG hydroxy-2'-deoxyguanosine

Nrf2 nuclear factor erythroid 2-related factor 2

ARE antioxidative response

RNA ribonucleic acid

DNA deoxyribonucleic acid

LDL low-density lipoprotein

kg kilogram

mmol millimole

Cd cadmium

MAP mitogen-activated protein

NF-κB	nuclear factor kappa B
ROS	reactive oxygen species
•NO	nitric oxide radical
eNOS	endothelial nitric oxide synthase
iNOS	<i>inducible nitric oxide synthase</i>
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator 1-alpha
mtTFA	mitochondrial transcription factor A
LOOH	lipid hydroperoxides
EIMD	exercise-induced muscle damage
PGE2	prostaglandin E2
TNF α	<i>tumour necrosis factor α</i>
et al.	and others
KB	bioethics committee

Declarations

Ethics approval and consent to participate

Experimental procedures and potential risks were discussed with the participants, and informed consent forms were provided and signed prior to inclusion in the study. The study was conducted in accordance with the Declaration of Helsinki, and its protocol was approved by the Bioethics Commission at the Collegium Medicum in Bydgoszcz (decision no. KB 382/2017 of 22.05.2018).

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Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on a reasonable request.

Competing interests

The authors declare no conflict of interest, financial or otherwise. The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation.

Author contributions

BS, MC and ASS designed the study; BS, MC, EP and TK collected the data; BS, SK and ASS interpreted the results and drafted the manuscript. All authors approved the final version of the paper.

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