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ORIGINAL ARTICLE

Acute effects of half-marathon and aronia juice on lipid and hematological parameters, muscle function and oxidative status in male runners

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Abstract

The present cross-over study investigated whether a half-marathon race might impose changes in lipid profile, muscle function, haematological parameters, and oxidative status in 10 amateur male runners, and whether polyphenol-rich aronia juice may counteract these potential alterations.

Subjects (age 30.8 ± 2.3 years) ran a simulated half-marathon race (21.1 km), after they had consumed breakfast with 200 mL of aronia juice/placebo at two occasions separated by one week. Blood samples were taken at baseline, 15 min, 1 h, and 24 h after the run.

Results revealed significant increase in the levels of total, LDL cholesterol, and triglycerides immediately after the run (by 6.97%, 9.23%, and 38.46%, respectively), which tended to return to the baseline values after 24 hours. The activity of lactate dehydrogenase increased significantly after the race and started decreasing 24h after the race, still being 16.18% higher compared with the baseline. The run induced a marked increase in total number of leukocytes, and granulocytes, with eventual return to the baseline levels.

The obtained results suggest that a half-marathon run is intense enough to cause lipid mobilization, muscle damage and compromise the immune response in recreational male runners. Acute aronia juice intake was not sufficient to attenuate the observed changes.

Keywords: half-marathon · aronia juice · runners · oxidative status · muscle function

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Introduction

Physical activity could be implemented as a tool in primary and secondary prevention of cardiovascular diseases (CVDs), but other chronic conditions as well (Duthie, Robertson, Maughan, & Morrice, 1990). Whether a type of physical activity imposes beneficial, or adverse health effects largely depends on its intensity and duration (Peake, Neubauer, Della Gatta, & Nosaka, 2017). High-intensity and prolonged exercise without the optimal recovery period, leads to an increase in the muscle glycogen breakdown and in the levels of calcium, catecholamines, growth hormone, and cortisol. The expression of cytokines is enhanced as well, followed by the overproduction of reactive oxygen species (ROS). Exhaustive physical exercise is followed by increase in oxygen consumption. Not all oxygen is reduced to water– 2-5% instead undergoes a univalent reduction which leads to the generation of ROS (Banerjee, Mandal, Chanda, & Chakraborti, 2003). Low amounts of ROS may act as cellular signals to enhance antioxidant defence. At higher exercise intensities and durations, the levels of ROS increase and may lead to deleterious consequences.

Running long distances lead to an increased fat oxidation, muscle damage, inflammation, and oxidative stress (Duthie et al., 1990; Smith, Garbutt, Lopes, & Pedoe, 2004). Prolonged physical activity promotes the mobilization of lipids from their storages into the blood, which enhances fat oxidation and affects the plasma lipid profile (Tambalis, Panagiotakos, Kavouras, & Sidossis 2009).

One of the most potent sources of polyphenols is aronia (Aronia melanocarpa), with beneficial effects on oxidative stress' status (Kardum et al., 2014). Analysis of 92 plant extracts showed remarkably high phenolic content and antioxidant capacity in berries, specifically in aronia (Kähkönen et al., 1999). Later, it has been shown that aronia has higher polyphenol content and antioxidant potential than other berries (Määttä-Riihinen et al., 2004). Antioxidant and anti-inflammatory effects of chronic aronia juice intake were reported in professional rowers (Skarpańska-Stejnborn, Basta, Sadowska, & Pilaczyńska-Szcześniak, 2014), as well as in handball players who consumed aronia extract for 12 weeks during the competition phase (Cikiriz et al., 2021). In a latter study, the index of lipid peroxidation and nitrites had linear decrease during the twelve-week treatment with aronia extract. As the authors suggested, aronia and its products could limit the amount of red blood cells damage induced by exercise (Cikiriz et al., 2021). Consumption of aronia-citrus juice in elite triathletes decreased concentration of lipid peroxidation metabolites from the central nervous system, suggesting potential beneficial effects on the nervous system during training (García-Flores et al., 2016). Recently, a group of authors has shown that aronia berry extract consumption may boost adaptive antioxidant defence against acute exercise-induced oxidative stress in healthy adults. The chronic supplementation enhanced the glutathione defence system manifested as an increase in glutathione availability and glutathione peroxidase activity immediately and half an hour post-exercise (Chung et al., 2023). Another study investigated the impact of one-month aronia juice consumption on exercise-induced changes in fatty acid and lipid profile, as well as lipid peroxidation, in elite handball players (Petrovic et al., 2016). The authors reported a decrease in lipid peroxidation with slight differences in fatty acid profile, suggesting a weak impact of aronia juice on attenuating the effects of training in elite handball players. In addition, it has been shown that aronia juice consumption might counteract the changes in platelet function induced by half-marathon race, while the underlying mechanisms need further investigation (Stevanovic et al., 2019).

Running longer distances can negatively impact the cardiovascular system and increase the risk of acute cardiac events, especially in less physically fit runners compared with professional athletes (Burkule, 2016). Although there is sound scientific background for introducing antioxidant supplementation to athletes, it has also been proposed that high doses of antioxidants may even blunt cellular signals that stimulate adaptations in the skeletal muscles after performing some kind of exercise (Myburgh, 2014).

Therefore, we aimed to investigate whether a half-marathon race is an intense physical activity that might impose changes in lipid profile, muscle function, haematological parameters, and oxidative status in 10 amateur male runners. In addition, we explored whether a potent antioxidant - polyphenol-rich Aronia juice - may counteract these potential alterations.

Method

Experimental design

We designed a cross-over single-blind placebo-controlled study to determine acute effects of running an outdoor half-marathon race with or

without prior consumption of aronia juice in healthy male recreational long-distance runners. Briefly, the participants came for the first visit and after a 10 min rest, we performed first baseline assessments (T0). They then received unified breakfast and aronia or placebo juice. Before the run, all subjects performed their own warm-up and stretch for 10min before the half marathon run (21.1km) on a flat, off-road track. The participants ran at their own pace, in pairs and in similar weather conditions (temperature: 8-14ºC; humidity 45-55%; wind: 2-4m/s). We took blood samples 15min (T1), 1h (T2) and 24h after the race (T3). The same protocol was repeated after 7 days for each of the participants. At this visit participants who first consumed aronia juice, were given placebo and vice versa.

Participants

The study subjects were ten apparently healthy recreational male runners, members of Belgrade Urban Running Team (mean (SD) age: 30.8 ± 2.3 years; hight: 185.3 ± 7.4 cm; body weight: 84.3 ± 13.5 kg).

Each participant voluntarily provided written informed consent before participating. The study was conducted according to the Declaration of Helsinki and the protocol was approved in advance by the Ethics Committee.

Breakfast

The non-vegan breakfast consisted of three slices of white bread, three slices of chicken breast, 10g of butter, and one banana (providing 478.50 kcal), while the vegan breakfast comprised of two slices of white bread, 75g of hummus and a banana (providing 480.55 kcal).

Aronia juice and Placebo

Aronia and Placebo juices were donated from Rheapharm d.o.o., Belgrade, Serbia. Total phenolic content in Aronia juice was 1.3g of gallic acid equivalents (GAE) per 200ml. The placebo juice was polyphenol-free, but had the same macro and micronutrient content, with the addition of artificial colours and flavours (Kardum et al., 2017).

Blood lipid and Haematological measurements

Blood lipids were analysed from serum samples using the clinical chemistry analyzer Cobas c111 and reagent kits (Roche Diagnostics, Basel, Switzerland), as recommended by the manufacturer. In the same samples we assessed the levels of creatine kinase (CK) and lactate dehydrogenase (LDH). Complete blood count was determined in samples collected into EDTA evacuated tubes using the automated haematological analyser ABX Micros 60 (Horiba, Kyoto, Japan).

Oxidative status analyses

The activities of antioxidant enzymes were determined in red blood cells. The activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured using commercially available kit (Randox-Ransod, Cat no. SD 125, UK, and Randox-Ransel, Cat no. RS 505, UK, respectively). The activity of CAT was measured according to the method developed by Aebi (1984). The activities of antioxidant enzymes were expressed in U/gHb.

The levels of thiobarbituric acid reactive substances (TBARS) were measured in plasma samples with the use of commercial kit (Cayman, Cat no. 10009055). Total antioxidant status (TAS) was determined in plasma samples by automated method with the application of commercially available kit (Sigma, Cat no.CS0790). Plasma levels of oxLDL-c were determined by employing a commercially available sandwich ELISA kit (Mercodia, Cat no. 10-1143-01).

Statistical Analysis

Before carrying out the analysis, all values of the dependent variables were expressed as their relative change from baseline values (T0) and expressed as percentages of the mean value at T0 according to the following formula: Tx/T0*100. All dependent variables were analysed using a 4 (Time variable: T0, T1, T2, T3) x 2 (Group variable: Aronia juice vs placebo consumption) analysis of variance (ANOVA) with repeated measures on both factors. Since some of the parameters violated the assumption of sphericity before carrying out the repeated measures ANOVA, we included the p-values obtained from the models adjusted by the Greenhouse-Geisser procedure, which are suitable for small sample sizes and that correct for this violation (Blanca et al., 2023). Significant changes in T1, T2 and T3 compared with T0 were explored with the post hoc tests using the Least Significant Difference (LSD) test. The significance threshold was set at $p<0.05$. All analysis were carried out in SPSS software, version 23.0 (SPSS, Chicago, USA).

Results

The effect on serum lipid profile

The half-marathon race significantly ($p=0.000$) increased triglycerides (TAG) levels by 38.46% (95% confidence interval (CI) [57.19, 19.73]) at T1 compared with T0, after which they reached the

baseline levels at T2 and declined at T3 by 33.04% (95%CI [22.37, 43.7]) (Table 1). The levels of total cholesterol (TC) significantly increased (p=0.003) at T1 and T2 by 6.97 (95%CI [11.72, 2.21]) and 4.02% (95%CI [6.12, 1.91]), respectively, and almost returned to the baseline levels at T3. Similarly, low-density lipoprotein cholesterol (LDL-c) significantly (p=0.020) increased at T1 and T2 (by 9.23, 95%CI [15.47, 2.99], and 7.38%, 95%CI [10.12, 4.64], respectively), and eventually returned to the baseline values. The concentration of high-density lipoprotein cholesterol (HDL-c) showed a significant (p=0.001) increase at all post-race points - the highest increase was observed at T3 (by 16.23%, 95%CI [27.97, 4.48]).

Aronia juice consumption did not significantly affect any of these parameters.

Table 1. Changes in serum lipid profile induced by the simulated half-marathon race

Variable	T0	T1	T2	T3	p-value
Total cholesterol (nmol/L)	4.471 (0.205)	4.780 (0.227)*	4.649 (0.223)*	4.362 (0.207)	0.003
LDL cholesterol (nmol/L)	1.397 (0.052)	1.541 (0.056)*	1.524 (0.057)*	1.618 (0.102)	0.020
HDL cholesterol (nmol/L)	2.544 (0.182)	2.774 (0.192)*	2.732 (0.194)*	2.498 (0.170)	0.001
Triglycerides (nmol/L)	0.895 (0.092)	1.098 (0.087)*	0.834 (0.083)	0.545 (0.064)*	0.000

Note: The values present mean (SE). p-values are based on the results of the main effects obtained after conducting two-way repeated measures ANOVA. * – denotes significant change compared with T0.

The effect on serum markers of skeletal muscle damage

The activity of LDH increased significantly (p=0.004) at both T1 and T2 (by 51.27, 95%CI [82.73, 19.81], and 52.88%, 95%CI [84.38, 21.39], respectively), and decreased at T3, still being 16.18% higher compared with the baseline. Interestingly, the levels of CK were not significantly (p=0.068) affected by the race (Table 2). Overall, both parameters were higher when the participants consumed placebo juice.

Table 2. Changes in serum markers of skeletal muscle damage induced by the simulated half-marathon race.

Variable	T0	T1	T2	T3	p-value
Creatine kinase (CK, U/L)	220.435 (61.024)	353.440 (96.304)	368.265 (91.424)	543.700 (126.695)	0.068
Lactate dehydrogenase (LDH, U/L)	155.660 (10.520)	223.855 (5.977)*	226.940 (8.643)*	176.625 (7.226)*	0.004

The effect on haematological parameters

The run induced a marked increase (p=0.001) in total number of leukocytes by 100.20 (95%CI [157.48, 42.92]) and 109.40% (95%CI [159.15, 59.65]) at T1 and T2, and they eventually returned to the baseline levels. The number of granulocytes significantly (p=0.000) increased after the race, and reached almost a 200% (95%CI [261.55, 87.66]) increase at T2, but it returned to the baseline levels at T3. On the contrary, number of lymphocytes decreased in all post-race time points (p=0.000), with the most pronounced decrease observed in T2 (Table 3). The race had no significant effects on other haematological indices.

Aronia juice had no significant effect on none of the analysed parameters.

The effect on oxidative stress parameters

We observed no significant effect of the race on any of the analysed oxidative status parameters (Table 4). The variations in the activities of all analysed parameters were negligible and were within the range plus/minus 10%.

Aronia juice consumption induced no effect on none of the oxidative stress markers.

Table 3. Changes in basic blood counts and selected circulating leukocyte subpopulations induced by the simulated half-marathon race

Variable	T0	T1	T2	T3	p-value
Hemoglobin (g/L)	145.600 (3.654)	146.700 (3.537)	147.050 (3.732)	149.100 (6.291)	0.636
Erythrocytes (10^{12}/L)	5.042 (0.139)	5.079 (0.121)	5.129 (0.135)	4.978 (0.106)	0.133
HCT (L/L)	0.436 (0.011)	0.440 (0.010)	0.438 (0.011)	0.431 (0.008)	0.324
Platelet count (10^9/L)	206.500 (13.772)	235.100 (16.873)	212.200 (13.926)	210.900 (14.232)	0.053
MCV (fL)	87.050 (1.076)	125.050 (38.395)	86.450 (0.953)	86.600 (0.999)	0.345
MCH (pg)	28.900 (0.407)	28.915 (0.353)	28.990 (0.374)	28.850 (0.389)	0.432
MCHC (g/L)	334.200 (1.775)	333.950 (1.119)	335.250 (1.665)	333.300 (1.191)	0.297
Leukocytes (10^9/L)	5.300 (0.342)	10.040 (1.079)*	10.665 (0.926)*	5.145 (0.501)	0.001
Lymphocytes (10^9/L)	1.975 (0.165)	1.725 (0.117)*	1.475 (0.105)*	1.735 (0.158)*	0.000
Monocytes (10^9/L)	0.200 (0.018)	0.230 (0.017)	0.235 (0.025)	0.210 (0.023)	0.108
Granulocytes (10^9/L)	3.125 (0.227)	8.085 (1.082)*	9.005 (0.936)*	3.205 (0.360)	0.000

Discussion

We observed significant increase in the levels of TC, LDL-c and TAG immediately after the half-marathon run, which tended to return to the baseline values after 24 hours. Only HDL-c increased at all time-points. These changes support the notion that lipids are the predominant fuel used during exercise. Lira et al. (2010) observed the same alterations in blood lipid profile (except for significant change in serum total cholesterol) under resistance exercise conditions. Immediate and delayed effects on blood cholesterol levels were recorded in female runners after a marathon race (Goodyear et al., 1990).

We observed no effect of Aronia juice consumption prior to the run on the lipid parameters. The results from other clinical trials with Aronia juice clearly pointed out a lack of effect in the healthy population, as opposed to those with some risk factors or disease. Four-weeks of Aronia juice consumption significantly decreased the levels of TAG in hypertensive patients (Kardum et al., 2015), while no significant effect on serum lipid profile was detected when healthy female participants consumed Aronia juice (Kardum et al., 2014).

The activity of LDH increased significantly after the race and started decreasing 24h after the race, still being by 16.18% higher compared with the baseline. Similar increase in LDH activity was observed in a study by Jürgenson et al. (2021) including well trained runners and recreational athletes. Furthermore, our results are in line with earlier studies including a half-marathon run (Lippi et al., 2011) and a marathon race (Smith et al., 2004), except for a non-significant effect of the run on CK that we observed.

Half-marathon race significantly enhanced the number of total white blood cells and granulocytes, and decreased the number of lymphocytes, with return to the pre-race values 24 hours after the race. Our results are in line with other trials, although we did not observe a significant effect of the race on the number of monocytes. Kratz et al. (2002) found enhanced levels of leukocytes, specifically monocytes and neutrophils, and a decrease in lymphocytes after a marathon race. In another study, ultramarathon stimulated leukocytosis, however, by increasing the number of lymphocytes (Kłapcińska et al., 2013),

Increase in white blood cells in recreational athletes, because of half-marathon race, may represent an adaptation to a regular chronic endurance exercising (Vassale et al.., 2020). Accordingly, run-induced leukocytosis can be explained by the hormonal response of the body to stressful conditions. Exercise bouts enhance catecholamine release, which further increases the circulating levels of epinephrine and norepinephrine

and promotes mobilization of white immune cells (Natale et al., 2003).

We found no effects on oxidative stress' markers. Our results are in line with the previously published studies evaluating the effects of half-marathon (Duthie et al., 1990) and an ultra-endurance race (Kłapcinska et al., 2013). Jürgenson et al. (2021) reported no post-race significant changes in markers of oxidative stress in both high-level well-trained runners and recreational athletes. Long-term moderate exercise might increase antioxidant defence, and thus, help maintaining redox homeostasis. Spanidis at al. (2017) observed no changes in TBARS nor TAS over three days follow-up in ultramarathon runners suggesting that these outcomes depend on training status of the participants. The measurement of oxidative stress induced by exercise has limitations in terms of selecting the biomarkers that could reflect the exact status in the cells (Thirupathi et al., 2021).

Different biomarkers of oxidative stress are not likely to measure identical aspects of redox status. Thus, the use of more sensitive biomarkers could give clearer inputs in redox status changes during half-marathon race. More specific biomarkers would include two largely applied indices of oxidative stress: reactive oxygen metabolites, and the total antioxidant capacity, as measure of the oxidant and antioxidant counterpart, with the calculation of oxidative stress index (Oxidative-INDEX) (Vassale et al., 2020). The most oxidative stress data are focused on changes that occur after long distance run, such are marathon or ultra-marathon, while half-marathon run is less demanding as it does not require the same level of training. Changes in oxidative related biomarkers, and physical adaptations observed in more studied marathon runners, could differ from those found in the half-marathon runners (Vassale et al., 2014). Production of ROS is not necessarily harmful in athletes, as ROS can modulate physiological processes such as muscle adaptation to training. In a recent trial, the possible differences in the training-induced adaptations depending on the type of training program have been tested. The acute response to the half-marathon induced a temporary alteration in blood markers of oxidative stress, inflammation, and muscle damage depending on the training adopted, including the training volume, and the running duration (Bonet et al., 2022).

Clinical trials regarding the effects of Aronia products on exercise-induced stress reported inconsistent results. One-month long Aronia juice consumption managed to decrease TBARS, GPx and SOD levels (Pilaczynska-Szczesniak, Skarpanska-Steinborn, Deskur, Basta, & Horoszkiewicz-Hassan, 2005), while after 8 weeks it significantly enhanced the levels of TAS in professional rowers (Skarpańska-Stejnborn et al., 2014). Cikiriz et al. (2021) reported significant decrease in TBARS levels and increase in CAT and SOD activities after 12 weeks of chokeberry extract consumption in active handball players. Also, eight-week long supplementation with Aronia berry extract in healthy middle aged adults increased glutathione availability and GPx activity immediately and 30 minutes post treadmill exercise (Chung et al., 2023). On the contrary, in a placebo-controlled trial, seven weeks of regular Aronia juice consumption did not affect TAS and TBARS levels following beep test in football players (Stankiewicz, et al., 2021).

Acute trials which investigated polyphenol-rich functional foods used only cocoa so far. Cocoa polyphenols increased plasma TAS levels after a bicycle ergometer test, with no effects on exercise-induced lipid peroxidation (Decroix et al., 2017). Acute pre-exercise dark chocolate consumption also had positive effects on plasma antioxidant status (measured as TAS; Davison, Callister, Williamson, Cooper, & Gleeson,2012).

In our study, acute intake of Aronia juice polyphenols (in a dose of 1.3g of GAE) was not sufficient to induce an effect on the lipid metabolism, muscle damage, leukocytosis or oxidative stress. The lack of a significant effect might be due to inclusion of young, healthy, physically active male participants who maintain homeostasis while performing exercise.

There are other limitations, such as the single blind approach. Although placebo design presents a major obstacle in food trials (Staudacher et al., 2017), we have incorporated placebo in our study. However, designing a placebo for aronia juice that has specific astringent juice was quite challenging (Kardum et al., 2017). Thus, some participants were able to distinguish placebo from aronia drink. Still, the researchers responsible for the outcome measurements and the data analysis remained blind throughout the study period. Another limitation is the small sample size. However, we applied the cross-over design (each subject served as his own control) and recruited healthy male volunteers from the same running team following the same training regime to counteract these limitations. We tested only acute intake of aronia juice in specifically targeted participants, which limits the generalizability of our findings to other populations.

Future investigations are needed with more participants enrolled (including diverse populations), extended period of consumption, and more direct measures of oxidative stress and antioxidant activity to validate and expand our findings. Evaluation of other biomarkers like indicators of inflammation and mitochondrial function, and application of higher doses of polyphenols would be a valuable addition to the obtained results.

Conflict of interest

All the authors contributed to the: conception and design of the study, data collection and interpretation, and manuscript preparation. Accordingly, they all approved the final version of the paper. None of the authors declare a conflict of interest.

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