

Effects of supplementation with acai (*Euterpe oleracea* Mart.) berry-based juice blend on the blood antioxidant defence capacity and lipid profile in junior hurdlers. A pilot study

AUTHORS: Sadowska-Krepa E¹, Kłapcińska B¹, Podgórski T², Szade B³, Tyl K¹, Hadzik A⁴

¹ Department of Physiological and Medical Sciences, the Jerzy Kukuczka Academy of Physical Education, Katowice, Poland

² Department of Biochemistry, University School of Physical Education, Poznań, Poland

³ Department of Individual Sports, the Jerzy Kukuczka Academy of Physical Education, Katowice, Poland

⁴ Department of Sport and Tourism Management, the Jerzy Kukuczka Academy of Physical Education, Katowice, Poland

ABSTRACT: The purpose of this pilot study was to examine whether regular consumption of an acai berry-based juice blend would affect sprint performance and improve blood antioxidant status and lipid profile in junior athletes. Seven junior hurdlers (17.5 ± 1.2 years) taking part in a pre-season conditioning camp were supplemented once a day, for six weeks, with 100 ml of the juice blend. At the start and the end of the camp the athletes performed a 300-m sprint running test on an outdoor track. Blood samples were taken before and immediately after the test and after 1 h of recovery. Blood antioxidant status was evaluated based on activities of antioxidant enzymes (superoxide dismutase [SOD], catalase [CAT], glutathione peroxidase [GSH-Px], glutathione reductase [GR]), concentrations of non-enzymatic antioxidants (reduced glutathione [GSH], uric acid), total plasma polyphenols, ferric reducing ability of plasma (FRAP), thiobarbituric acid reactive substances (TBARS) and activities of creatine kinase (CK) and lactate dehydrogenase (LDH) as muscle damage markers. In order to evaluate potential health benefits of the acai berry, the post-treatment changes in lipid profile parameters (triglycerides, cholesterol and its fractions) were analysed. Six weeks' consumption of acai berry-based juice blend had no effect on sprint performance, but it led to a marked increase in the total antioxidant capacity of plasma, attenuation of the exercise-induced muscle damage, and a substantial improvement of serum lipid profile. These findings strongly support the view of the health benefits of supplementation with the acai berry-based juice blend, mainly attributed to its high total polyphenol content and the related high *in vivo* antioxidant and hypocholesterolaemic activities of this supplement.

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Corresponding author:

Ewa Sadowska-Krepa

Department of Physiological and

Medical Sciences,

the Jerzy Kukuczka Academy of

Physical Education

40-065 Katowice, Poland

e.sadowska-krepa@awf.katowice.pl

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INTRODUCTION

It is well established that strenuous muscular exercise is associated with substantially enhanced production of free radicals and other reactive oxygen and nitrogen species (ROS/RNS) [1], widely regarded as factors that may contribute to exercise-induced muscle damage. This increases requirements for exogenous antioxidant nutrients to strengthen the antioxidant defence system in heavily exercising humans. Studies have generally shown that antioxidant supplements do not improve performance, but there is a lot of evidence that supplementation with natural antioxidants may limit or even prevent exercise-induced tissue injury, as well as help the athletes to recover from the oxidative damage induced by free radicals [2,3]. On the other hand, the reactive oxygen and nitrogen species (ROS/RNS) generated during muscle contraction play an important signalling role in muscle adaptation to exercise [1,4].

The plant polyphenolic compounds, containing a number of phenolic hydroxyl groups attached to ring structures, confer antioxidant properties through acting as hydrogen or electron-donating free radical and transition-metal (mainly Fe and Cu) chelators, which results in inhibition of the Fenton and Haber-Weiss reactions. Moreover, they inhibit the activity of enzymes responsible for ROS production, such as xanthine oxidase, cyclooxygenase and lipoxigenase [5].

Recent reports have indicated a relationship between the intake of flavonoids and polyphenolic-rich food or flavonoid-rich beverages and their direct or indirect antioxidant activities [6,7,8]. Direct antioxidant activities of dietary polyphenols *in vivo* are limited because they may exert their effects within the gastrointestinal tract, where they may come into direct contact with cells without being absorbed

and metabolized. Indirect antioxidant activities of dietary polyphenols are associated with activation of the nuclear factor erythroid-2-related factor 2 (Nrf2), which stimulates expression of antioxidant enzyme genes, such as those for superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) or glutathione S-transferase (GST), and haem oxygenase-1 (HO-1) [7].

Paradoxically, many polyphenols are known to have pro-oxidant activities both *in vitro* and *in vivo* [9]. As antioxidants, polyphenols enhance cell survival, while as pro-oxidants, they may induce apoptosis and prevent tumour growth. Among the most promising sources of natural antioxidants are fruits of the Amazonian palm tree *Euterpe oleracea* Mart., called acai, used to prepare acai pulp, then combined with different amounts of other fruit juices to produce several commercial beverages [10]. The product chosen for this study, mainly because of its proven non-toxicity, was the MonaVie Active juice blend [11]. MonaVie Active is a mixture of the Amazonian palm fruit acai (*Euterpe oleracea* Mart.), as a predominant ingredient, and of lesser amounts of some other processed fruits, including wolfberry, pomegranate, camu camu, passion fruit, aronia, acerola, bilberry, known for their antioxidant properties, and some common fruits, including white and purple grapes, nashi pear, cranberry, apricot, prune, kiwi, blueberry, lychee, banana and capuacu, and glucosamine hydrochloride as a functional ingredient, recognized as safe by the U.S. Food and Drug Administration [6,12]. The acai fruit is famous not only for its powerful antioxidant and anti-inflammatory properties [6,8,5,12], but also for its hypocholesterolaemic activity, evidenced by favourable changes in the cholesterol profile in the circulatory system [13,14,15,16]. To our knowledge, however, there is no information as to how consumption of the combination of these ingredients affects sports performance and metabolic response to exercise in junior athletes. Therefore, the aim of this pilot study was to examine whether 6 weeks' daily consumption of this acai berry-based juice blend would affect sprint performance and improve blood antioxidant status and lipid profile in junior hurdlers.

MATERIALS AND METHODS

Participants. Seven elite junior hurdlers (aged 17.5 ± 1.2 years, body height 187.5 ± 6.92 cm, body mass 76.4 ± 6.1 kg, $VO_2\max$ 59.9 ± 4.4 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$), all recent winners or finalists of the National Junior Indoor Championships in Athletics, volunteered to participate in this pilot study. None of the participants followed a special diet or were taking vitamins or mineral supplements that could affect the antioxidant status of the blood before or during the study. All subjects were informed of the purpose, possible risks and the benefits of the study before giving their written consent to participate. The study protocol conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki and was approved by the Ethics Committee of the Jerzy Kukuczka Academy of Physical Education in Katowice, Poland (certificate of approval no. 4/2011).

Study protocol

In this study, conducted during the early outdoor season, the athletes were tested before and after 6 weeks' training, during which they were supplemented daily with 100 ml of the MonaVie Active (MonaVie, Salt Lake City, Utah, USA) juice blend. During this time, all subjects were engaged in common hurdle training activities designed for the general preparation phase (GPP), including special endurance, technical and rhythm training. The weekly conditioning programme consisted of 6 to 7 training sessions, lasting approximately 90 minutes each. Before starting and after completion of a six-week training programme and daily supplementation with MonaVie Active, the athletes performed a 300-m sprint test, on an outdoor track, after an approximately 20-min warm-up. During this 300-m sprint run test, used for evaluation of the athletes' general endurance capacity during the GPP [17], blood samples were drawn from the antecubital vein into test tubes anticoagulated with heparin and/or into serum separator tubes (SST) at rest (before the warm-up), immediately following the run, and after 1 hour of passive recovery.

Biochemical analyses

Fresh whole blood samples were immediately assayed for reduced glutathione by a colorimetric method [18] with 5,5'-dithiobis-2-nitrobenzoic acid. The remaining blood was centrifuged for 10 min at 1,000g at 4°C to separate plasma and erythrocytes, which were then washed three times with cold (4°C) saline and kept frozen at -80°C until analysis for activities of antioxidant enzymes, i.e. superoxide dismutase (SOD, EC 1.15.1.1) using the commercially available RANSOD SD125 kit (Randox, UK); glutathione peroxidase (GSH-Px, EC 1.11.1.9) with the commercial RANSEL RS505 kit (Randox, UK), catalase (CAT, EC 1.11.1.6) by the method of Aebi [19] and glutathione reductase (GR, EC 1.6.4.2) according to Glatzle et al. [20]. The activities of all antioxidant enzymes were measured at 37°C and expressed per 1 g of haemoglobin as assayed by a standard cyanmethemoglobin method using a diagnostic kit (HG980, Randox, UK).

Fresh plasma samples were assayed for activities of creatine kinase (CK, EC 2.7.3.2) and lactate dehydrogenase (LDH, EC 1.1.1.27), and concentrations of uric acid (UA) and lactate (LA), using diagnostic kits from Randox Laboratories (CK522, LD3818 and UA230, LC2389, respectively).

Concentrations of serum total cholesterol (T-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were assessed by enzymatic methods using commercially available diagnostic kits (CH201, CH203, TR210, respectively) from Randox (UK). Concentrations of low-density lipoprotein cholesterol (LDL-C) were calculated using the Friedewald formula.

To evaluate risk for vascular disease, the lipid ratios (T-C/HDL-C, LDL-C/HDL-C, TG/HDL-C) and the atherogenic index of plasma [AIP = $\log_{10}(TG/HDL)$ with TG and HDL-C expressed in molar concentrations] [21] were calculated. Assessment of lipid peroxidation was done using the thiobarbituric acid (TBARS) reaction according to Buege and Aust [22] by reading the absorption at $\lambda = 532$ nm

using a multi-mode microplate reader (Synergy 2 SIAFRT, BioTek, USA). Standard curves were prepared using water solutions of 1,1,3,3-tetramethoxypropane (TMP) as standard.

The total antioxidant capacity of the MonaVie juice blend and plasma was assessed using the ferric reducing ability of plasma (FRAP) assay according to Benzie and Strain [23]. FRAP values were calculated from changes in absorbance at 593 nm at 37° to quantify the amount of coloured ferrous tripyridyltriazine complex formed as a result of ferric to ferrous ion reduction at pH=3.6. The final results were expressed in Trolox equivalents per ml of juice ($\mu\text{mol TE} \cdot \text{mL}^{-1}$) or per 1 L of plasma ($\mu\text{mol TE} \cdot \text{L}^{-1}$).

The total polyphenolic content of the MonaVie Active and plasma total polyphenols were determined using the colorimetric method with phosphomolybdic-phosphotungstic acid reagent (Folin-Ciocalteu) adapted from Singleton and Rossi [24]. The absorbance was read at 765 nm on a multi-mode microplate reader (Synergy 2 SIAFRT, BioTek, USA), and the results are expressed as mg gallic acid equivalents per ml of juice ($\text{mg GAE} \cdot \text{mL}^{-1}$) or per 1 L of plasma ($\text{mg GAE} \cdot \text{L}^{-1}$).

Total anthocyanin content was measured according to Niketic-Aleksic and Hrazdina [25]. Absorbance of juice dissolved in 0.1 N HCl was measured at 520 nm on a Marcel Media plus spectrophotometer (Marcel Sp. z o. o., Poland), and the results are expressed as equivalents of malvidin 3,5-diglucoside (Malvin), being the dominant pigment in blueberries, per ml of juice.

Colorimetric assays of glucose and total protein contents in the MonaVie juice blend were performed using commercial assay kits

from PZ Cormay S.A. (Poland). The absorbance was read on a multi-mode microplate reader (Synergy 2 SIAFRT, BioTek, USA) at 500 nm and 546 nm, respectively. The results of the phytochemical analysis of the MonaVie juice blend are presented in Table 1.

Statistical analysis

All data are reported as means (SD). A two-way ANOVA with exercise and supplementation as the main factors, followed, when appropriate, by the Newman-Keuls post hoc test, was used to test the significance of between-group differences. With the aim of interpreting the results by indicating the relative degree to which the variance found in the ANOVA is associated with each of the main effects and their interaction, the Eta squared (η^2), as a measure of the effect size in ANOVA, was calculated using the following formula: $\eta^2 = \text{SS}_{\text{effect}} / \text{SS}_{\text{total}}$, where $\text{SS}_{\text{effect}}$ is the sum of squares for whatever effect is of interest, and SS_{total} is the total sum of squares for all effects, interactions, and errors in the ANOVA [26,27]. Spearman's rank order correlation coefficients were computed to reveal relationships between the variables. In all cases, $P < 0.05$ was considered significant. All statistical analyses were performed with STATISTICA 9.0 (StatSoft, Inc. 2009, USA) software.

RESULTS

Mean time of running 300 m distance on an outdoor track achieved by the athletes at the start of the study (38.68 ± 0.88 s) was very close to that recorded after 6 weeks' consumption of acai berry-based juice blend (38.60 ± 0.36 s).

TABLE 1. Phytochemical analysis of the acai berry-based juice blend

Measurements	Units	
FRAP (Ferric reducing ability/antioxidant power)	$\mu\text{mol TE} \cdot \text{mL of juice}^{-1}$	15.78
Total polyphenols	$\text{mg GAE} \cdot \text{mL of juice}^{-1}$	1.98
Total anthocyanins	$\mu\text{g Malvin} \cdot \text{mL of juice}^{-1}$	31.30
Total proteins	$\text{mg} \cdot \text{mL}^{-1}$	6.44
Glucose	$\text{mg} \cdot \text{mL}^{-1}$	1.79

Note: TE – Trolox equivalents, GAE – gallic acid equivalents, Malvin – malvidin 3,5-diglucoside equivalents

TABLE 2. Changes in plasma lactate and activities of CK and LDH induced by the dietary treatment

Variable	Baseline			Post-treatment			Two-way ANOVA results
	Pre-exercise	Post exercise	After 1 hr recovery	Pre-exercise	Post exercise	After 1 hr recovery	
CK ($\text{U} \cdot \text{L}^{-1}$)	137.74 (34.60)	242.01* (38.69)	196.36 (75.64)	135.39 (47.61)	213.86 (72.83)	180.29 (50.06)	TE: $F=0.82, P=0.38, \eta^2=0.02$ EE: $F=9.54, P=0.0005, \eta^2=0.34$ INT: $F=0.19, P=0.83, \eta^2=0.01$
LDH ($\text{U} \cdot \text{L}^{-1}$)	323.42 (51.04)	434.43** (63.24)	391.71 (41.14)	325.15 (52.48)	365.62 (50.57)	375.07 (57.03)	TE: $F=2.91, P=0.09, \eta^2=0.05$ EE: $F=7.89, P=0.001, \eta^2=0.28$ INT: $F=1.67, P=0.20, \eta^2=0.06$
LA ($\text{mmol} \cdot \text{L}^{-1}$)	3.03 (0.32)	16.21** (1.97)	3.68 (0.55)	3.52 (0.20)	14.68**† (1.32)	3.63 (0.77)	TE: $F=3.48, P=0.07, \eta^2=0.01$ EE: $F=1093.7, P=0.000, \eta^2=0.98$ INT: $F=5.82, P=0.01, \eta^2=0.01$

Note: Data are means (SD). Significant differences: * $P < 0.05$, ** $P < 0.01$ vs. resting values; † $P < 0.05$ vs. respective values before treatment. TE – treatment effect; EE – exercise effect; INT – interaction

Acute exercise-induced changes in the activity of CK and LDH in plasma are presented in Table 2. In both trials, marked increases in the activities of CK and LDH, observed immediately post-run, were followed by a tendency toward lower levels after 1 h of recovery. What is noteworthy, as revealed by two-way ANOVA, the effect of exercise appeared to be significant, although significant ($P < 0.05$) post-run increases in CK and LDH activities were observed only in the first trial (baseline) performed before starting supplementation. Compared to the resting and post-recovery values, plasma lactate concentration was significantly higher immediately post-exercise (Table 2), but it was only moderately affected by supplementation.

In this study, neither exercise nor supplementation markedly affected the activity of SOD. More evident, but opposite in direction, were post-exercise changes in activities of GSH-Px and GR (Table 3).

Consumption of a diet supplemented with acai berry-based juice blend resulted in a borderline increase in activities of CAT and a significant rise in resting blood glutathione (GSH) level, followed by a marked post-exercise decline observed in both trials (Table 4). There was a significant increase in plasma UA level in response to exercise and during the recovery (Table 4), but obviously unaffected by supplementation. Six weeks' daily consumption of acai berry-based juice blend induced a slight enhancement of the total antioxidant potential of plasma determined by the FRAP assay, as well as an increase in plasma total polyphenol content and, as shown by 2-way ANOVA, in both cases the effect of supplementation appeared to be significant (Table 4). Post-exercise changes in plasma polyphenols were opposite in direction to those in FRAP, although repeated measures 2-way ANOVA revealed a significant effect of exercise only in

TABLE 3. Changes in activities of the blood antioxidant enzymes induced by the dietary treatment

Variable	Baseline			Post-treatment			Two-way ANOVA results
	Pre-exercise	Post exercise	After 1 hr recovery	Pre-exercise	Post exercise	After 1 hr recovery	
SOD (U · gHb ⁻¹)	1149.2 (268.2)	1109.8 (275.9)	1169.8 (191.9)	1184.2 (52.7)	1187.6 (117.2)	1380.7 (266.9)	TE: F=2.69, p=0.109, $\eta^2=0.07$ EE: F=1.44, P=0.25, $\eta^2=0.07$ INT: F=0.65, P=0.53, $\eta^2=0.03$
GSH-Px (U · gHb ⁻¹)	31.0 (3.5)	29.9 (2.9)	28.8 (4.9)	34.9 (3.9)	31.7 (2.7)	27.4* (4.0)	TE: F=1.56, P=0.22, $\eta^2=0.03$ EE: F=5.86, P=0.01, $\eta^2=0.23$ INT: F=1.78, P=0.18, $\eta^2=0.07$
CAT (k · gHb ⁻¹)	196.3 (68.7)	164.3 (41.5)	191.2 (34.7)	206.3 (14.6)	204.2 (43.9)	223.3 (37.5)	TE: F=4.21, P=0.05, $\eta^2=0.10$ EE: F=1.07, P=0.35, $\eta^2=0.05$ INT: F=0.45, P=0.64, $\eta^2=0.02$
GR (U · gHb ⁻¹)	31.1 (6.7)	37.2 (6.0)	35.2 (10.6)	31.1 (4.0)	36.9 (6.8)	48.9*#† (10.3)	TE: F=3.47, P=0.07, $\eta^2=0.06$ EE: F=7.00, P=0.001, $\eta^2=0.23$ INT: F=3.69, P=0.01, $\eta^2=0.13$

Note: Data are means (SD). Significant differences: * $P < 0.05$ vs. resting values; # $P < 0.05$ vs. post-exercise values; † $P < 0.05$ vs. respective values before treatment. TE – treatment effect; EE – exercise effect; INT – interaction

TABLE 4. Changes in non-enzymatic antioxidant status and antioxidant defence capacity induced by the dietary treatment

Variable	Baseline			Post-treatment			Two-way ANOVA results
	Pre-exercise	Post exercise	After 1 hr recovery	Pre-exercise	Post exercise	After 1 hr recovery	
GSH ($\mu\text{g} \cdot \text{gHb}^{-1}$)	2.79 (0.37)	2.21 (0.59)	2.04 (0.65)	3.57† (0.31)	2.61* (0.58)	2.30* (0.56)	TE: F=8.81, P=0.001, $\eta^2=0.12$ EE: F=14.16, P=0.000, $\eta^2=0.38$ INT: F=0.88, P=0.42, $\eta^2=0.03$
Uric acid (mg · dl ⁻¹)	6.16 (1.00)	8.53** (1.26)	10.89*** (0.53)	6.78 (0.51)	9.23** (1.03)	10.33** (1.11)	TE: F=0.72, P=0.40, $\eta^2=0.01$ EE: F=66.97, P=0.000, $\eta^2=0.77$ INT: F=1.93, P=0.16, $\eta^2=0.03$
Total plasma polyphenols (mg GAE · L ⁻¹)	1947.1 (85.2)	1826.7 (129.8)	1943.3 (56.2)	2121.7† (135.6)	1873.3** (93.7)	1930.0* (117.8)	TE: F=4.43, P=0.04, $\eta^2=0.07$ EE: F=10.47, P=0.001, $\eta^2=0.32$ INT: F=2.83, P=0.07, $\eta^2=0.09$
FRAP ($\mu\text{mol TE} \cdot \text{L}^{-1}$)	957.0 (49.7)	1004.2 (86.8)	1033.5 (74.2)	1091.7 (81.2)	1118.3 (85.6)	1164.2 (140.8)	TE: F=20.47, P=0.000, $\eta^2=0.34$ EE: F=2.37, P=0.11, $\eta^2=0.08$ INT: F=0.05, P=0.95, $\eta^2=0.01$
TBARS ($\mu\text{mol} \cdot \text{L}^{-1}$)	6.37 (1.32)	6.88 (1.07)	6.42 (1.68)	6.01 (0.40)	6.45 (0.69)	5.58 (1.14)	TE: F=2.44, P=0.13, $\eta^2=0.06$ EE: F=1.30, P=0.28, $\eta^2=0.07$ INT: F=0.8, P=0.84, $\eta^2=0.01$

Note: Data are means (SD). Significant differences: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. the resting values; † $P < 0.05$ vs. the respective values before treatment. TE - Treatment effect; EE - Exercise effect; INT - Interaction

the case of total plasma polyphenols. A slight decrease in pre- and post-exercise plasma TBARS levels was observed during the second trial performed after 6 weeks' consumption of acai juice blend (Table 4).

Importantly, this dietary intervention led to beneficial changes in lipid profile, such as a reduction in T-C, LDL-C and triglycerides, and an increase in HDL-C. This was associated with favourable changes in common lipid ratios (T-C/HDL-C, LDL-C/HDL-C, TG/HDL-C) and a significant decline in the atherogenic index of plasma (AIP), and in all these cases the effect of supplementation was significant (Table 5).

To identify the relationships between the biochemical variables, the Spearman rank correlation coefficients were computed, and the identified statistically significant associations are presented in Table 6. The most evident were negative associations of total plasma polyphenols with lipid profile measures, lipid ratios, muscle damage and lipid peroxidation markers, and a positive association with GSH.

DISCUSSION

This study attempted to evaluate whether 6 weeks' supplementation with acai berry-based juice blend (MonaVie Active) would enhance sprint performance, improve pro/antioxidant status and affect cholesterol homeostasis in junior hurdlers. We demonstrated that regu-

lar consumption of this flavonoid-rich juice blend at a daily dose of 100 ml had no effect on sprint performance of junior hurdlers, as evidenced by comparable 300 m running times recorded before and after this dietary treatment. The second objective of this study was

TABLE 6. Spearman's rank correlation coefficients between selected variables

Variables	R	P
LA & total plasma polyphenols	-0.43	<0.005
CK & total plasma polyphenols	-0.43	<0.004
GSH & total plasma polyphenols	0.47	<0.002
TBARS & total plasma polyphenols	-0.32	<0.05
T-C & total plasma polyphenols	-0.33	<0.05
LDL & total plasma polyphenols	-0.28	NS (P=0.07)
T-C/HDL-C & total plasma polyphenols	-0.26	NS (P=0.09)
TG/HDL-C & total plasma polyphenols	-0.42	<0.005
AIP & total plasma polyphenols	-0.42	<0.01
GSH & FRAP	0.34	<0.02
UA & FRAP	0.26	NS (P=0.09)
TG & FRAP	-0.31	<0.04
T-C/HDL-C & FRAP	-0.27	NS (P=0.08)
TG/HDL-C & FRAP	-0.33	<0.03
AIP & FRAP	-0.33	<0.05

TABLE 5. Changes in serum lipids and lipid ratios induced by the dietary treatment

Variable	Baseline			Post-treatment			Two-way ANOVA results
	Pre-exercise	Post exercise	After 1 hr recovery	Pre-exercise	Post exercise	After 1 hr recovery	
T-C (mg · dl ⁻¹)	159.2 (17.5)	167.7 (13.4)	171.1 (14.5)	133.7† (12.9)	157.4 (18.1)	139.8† (20.3)	TE: F=19.66, P=0.000, η ² =0.30 EE: F=3.40, P=0.05, η ² =0.11 INT: F=1.53, P=0.23, η ² =0.05
HDL-C (mg · dl ⁻¹)	50.0 (11.4)	52.1 (10.5)	50.5 (15.8)	59.9 (9.00)	64.9 (8.5)	57.6 (6.8)	TE: F=8.99, P=0.01, η ² =0.19 EE: F=0.69, P=0.51, η ² =0.03 INT: F=0.25, P=0.78, η ² =0.01
LDL-C (mg · dl ⁻¹)	90.4 (12.3)	91.4 (15.9)	98.9 (20.1)	59.6† (15.5)	74.0 (18.0)	65.7† (17.8)	TE: F=27.54, P=0.000, η ² =0.41 EE: F=0.93, P=0.40, η ² =0.03 INT: F=0.91, P=0.41, η ² =0.03
TG (mg · dl ⁻¹)	93.8 (30.4)	121.1 (39.2)	108.6 (35.4)	71.5 (10.6)	92.3 (13.2)	82.9 (18.3)	TE: F=9.49, P=0.01, η ² =0.19 EE: F=2.79, P=0.07, η ² =0.11 INT: F=0.05, P=0.95, η ² =0.01
T-C/HDL-C	3.32 (0.75)	3.41 (1.14)	3.80 (1.69)	2.27 (0.39)	2.45 (0.34)	2.44 (0.37)	TE: F=15.41, P=0.001, η ² =0.30 EE: F=0.45, P=0.64, η ² =0.02 INT: F=0.18, P=0.83, η ² =0.01
LDL-C/HDL-C	1.93 (0.66)	1.89 (0.81)	2.28 (1.26)	1.03† (0.37)	1.16 (0.34)	1.15 (0.34)	TE: F=17.32, P=0.001, η ² =0.32 EE: F=0.42, P=0.66, η ² =0.02 INT: F=0.28, P=0.76, η ² =0.01
TG/HDL-C	1.95 (0.72)	2.59 (1.75)	2.64 (2.27)	1.21 (0.19)	1.42 (0.13)	1.47 (0.40)	TE: F=7.39, P=0.01, η ² =0.17 EE: F=0.64, P=0.53, η ² =0.03 INT: F=0.14, P=0.87, η ² =0.01
AIP	-0.093 (0.15)	-0.003 (0.21)	-0.023 (0.26)	-0.283 (0.07)	-0.208 (0.04)	-0.207 (0.12)	TE: F=15.50, P=0.001, η ² =0.29 EE: F=1.13, P=0.33, η ² =0.05 INT: F=0.18, P=0.98, η ² =0.00

Note: Data are means (SD). Significant differences: †P<0.05 vs. the respective values before treatment. TE – treatment effect; EE – exercise effect; INT – interaction

to evaluate the effect of supplementation with MonaVie juice blend on the blood pro-antioxidant status, acute exercise-induced muscle damage and lipid profile in highly-trained elite junior athletes. Enhanced blood antioxidant capacity and attenuated exercise-induced oxidative stress have been clearly evidenced in adult athletes supplemented with antioxidants [2,3], but little is known about these aspects of adaptation in junior athletes. The main finding of our study is that dietary intervention in junior athletes, involving six weeks' supplementation with an acai pulp-fortified polyphenolic-rich fruit and berry juice blend, led to an increase in blood antioxidant capacity and to significant improvement of lipid profile, but had no effect on sprint performance.

First of all, we examined *in vitro* antioxidant properties of MonaVie Active, containing a mixture of 18 fruits and berries with acai (*Euterpe oleracea* Mart.), an Amazonian palm fruit, as the predominant ingredient. Several previous studies have provided evidence that acai berry-based juice blend is non-toxic, and no adverse events related to oral consumption of this beverage were reported [11]. Our results on the *in vitro* antioxidant activity of this juice measured by the FRAP method, as well as total polyphenol content (see Table 1), are very close to those reported previously by Jensen et al. [6], namely $22.2 \mu\text{mol TE} \cdot \text{mL}^{-1}$ as assessed by oxygen radical absorbance capacity (ORAC) and $1.48 \text{ mg GAE} \cdot \text{mL}^{-1}$ of juice, respectively. These and other authors [5] reported that the acai pulp, the main component of this juice blend, is a rich source of high concentrations of polyphenol compounds including anthocyanins (cyanidin 3-glucoside and cyanidin 3-rutinoside being the most predominant), proanthocyanidins, and phenolic acids, substances having a high antioxidant capacity, as well as high anti-inflammatory bioactivity [5,6].

It should be emphasized, however, that the bioefficacy of dietary polyphenolics depends on their bioavailability [28]. It has appeared that chronic or long-term ingestion of polyphenol-rich food does not always result in marked increases in their plasma concentrations. Intestinal absorption of polyphenols is highly variable, often slow and largely incomplete, as in the case of high-molecular-weight proanthocyanidins [29]. The maximal human plasma concentrations of them were reached usually between 0.75 and 4 hours after consumption of food rich in polyphenolic compounds [8,28]. Their half-lives in plasma are short, usually in the range of a few hours, which may restrict the capacity of dietary polyphenols to act as antioxidants in plasma *in vivo* [7]. In contrast to this view, our study showed that chronic consumption of acai juice blend resulted in a significant rise in plasma total polyphenols concentration and a significant tendency toward higher total plasma antioxidant capacity, assessed by the FRAP method. Interestingly, opposite tendencies toward lower plasma total polyphenols and higher FRAP scores were observed immediately after both 300-m sprint running tests. A post-exercise decline in plasma polyphenol content may imply that they have been used as scavengers of free radicals generated by contracting muscles [1], whereas a post-exercise rise in FRAP scores may, most likely, be attributed to concurrent increases in plasma urate con-

tent [30], although the association between FRAP and plasma urate in our study did not reach significance.

Several studies have reported that acute anaerobic exercise serves as a sufficient stimulus to elicit enhanced formation of ROS and RNS, and thus to increase oxidative stress [1,4,31]. However, unlike aerobic exercise, where mitochondrial leakage of electrons to molecular oxygen is the main source of ROS, the increased radical production during high intensity anaerobic exercise is mainly mediated by xanthine and NADPH oxidases [1,4]. The most widely used method for quantifying exercise-induced oxidative stress consists in measuring the concentration of thiobarbituric acid-reactive substances (TBARS), a commonly used marker reflecting the degree of free radical-induced oxidative damage to lipids [32]. There are no unequivocal reports of plasma TBARS concentrations after exercise. Some authors have reported an increase or no changes in post-exercise plasma TBARS level [1,3]. In the present study none of the factors tested, neither the supplementation nor acute physical load, had a significant impact on plasma TBARS concentration; however, a slight decrease in TBARS levels was evidenced during the second trial performed after 6 weeks' supplementation with acai juice blend. Moreover, plasma CK and LDH activities were significantly higher immediately after the 300-m sprint running test, but only when performed at baseline before starting supplementation. What is noteworthy, the relative post-exercise changes in CK and LDH activities, expressed as percentage increases, were markedly lower after 6 weeks of daily juice consumption, namely 175 vs. 158% and 134 vs. 112%, for CK and LDH respectively. We observed a significant inverse correlation between plasma TBARS and total polyphenols, as well as between CK and total polyphenols (see Table 6). It may be presumed that the trend toward lower TBARS and smaller post-exercise increases in CK and LDH activities after supplementation with acai juice blend might be related to the antioxidant properties of its constituents. What is more, Heim et al. [33] previously reported that a 3'4'-catechol structure in the B-ring of dietary flavonoids strongly enhances inhibition of lipid peroxidation. Several previous studies have demonstrated that consumption of polyphenol-rich foods and beverages may provide antioxidant and anti-inflammatory support to prevent exercise-induced muscle damage, slow the decline in force output, reduce pain and limit the decline in function associated with aging [2,4,6,7,8,28]; however, the results of these studies are equivocal.

The primary objective of the present study was to examine the effects of this dietary supplementation on anaerobic exercise-induced changes in the blood antioxidant defence system in junior hurdlers. Six weeks' daily consumption of this dietary supplement resulted in significant increases, compared to baseline levels, in plasma total polyphenols and blood GSH content. The latter observation is consistent with previous reports that dietary polyphenols can up-regulate expression of γ -glutamylcysteine synthetase (γ GCS), a rate-limiting enzyme involved in glutathione biosynthesis [34,35]. This presumption was additionally supported by our finding of a positive correlation

between GSH and total polyphenols. Interestingly, a two-way ANOVA with follow-up post hoc tests demonstrated medium effects of supplementation with the acai juice blend on GSH ($\eta^2=0.12$) or plasma polyphenols ($\eta^2=0.07$), and a larger effect on FRAP ($\eta^2=0.34$) [26]. Regarding the antioxidant enzymes, no significant changes in resting and post-exercise activities of SOD were recorded. Supplementation with acai berry-based juice blend resulted in moderate increases in the activities of CAT and GR, in the latter case this was associated with a significant increase in blood GSH contents. Opposite post-exercise changes were recorded in the activities of GSH-Px and CAT, which tended to lower levels, and GR, which tended to increase following the sprint running tests, although medium effects of exercise ($\eta^2 \geq 0.22$) were demonstrated only in the case of GSH-Px and GR. Significant post-exercise increases in plasma uric acid ($P < 0.0001$, $\eta^2 = 0.77$), a product of purine nucleotides degradation that functions as an antioxidant [1], were most likely consequent to a high rate of energy consumption which triggered activation of adenylate kinase and AMP signalling networks to ensure cellular energy homeostasis [31]. It cannot, however, be ruled out that the rise in plasma uric acid content may also derive from fructose metabolism, known to lead to a transient depletion of hepatic ATP and inorganic phosphate, thus stimulating enzymes involved in the degradation of purine nucleotides to urate [7]. However, given the non significant effect of supplementation with the acai juice blend on plasma urate ($P = 0.40$, $\eta^2 = 0.01$), one may presume that fructose metabolism had only a minor contribution to increased uric acid levels.

A number of studies have focused on other possible biological effects of dietary polyphenols and their importance in human health and disease. Besides antioxidant activity, polyphenols are considered to have cardio-protective properties, *inter alia*, due to their hypocholesterolaemic activities [15,28,36]. In most of these studies, polyphenol ingestion resulted in a decrease in T-C, LDL-C, triglycerides, apolipoprotein B or lipoprotein(a), and in an increase in the level of HDL-C, apolipoprotein A-I and up-regulation of LDL receptor activity.

In our study, a significant improvement, compared to baseline, in lipid profile parameters was observed in all athletes after six weeks' supplementation with acai-based juice blend. Taking into consideration the young age of our hurdlers, all lipid profile parameters and common lipid ratios were within the respective normal ranges already at the baseline, but their further improvement following supplementation was observed in each case (see Table 5). Of note, a two-way ANOVA with follow-up post hoc tests demonstrated larger effects of supplementation with the acai juice blend on all studied lipid profile markers ($0.20 \geq \eta^2 \leq 0.30$). In addition, the negative values of AIP recorded in all athletes at both endpoints, and especially at the end of the study (post-treatment), which was reported to be inversely correlated with particle size of LDL and HDL [7], and an inverse correlation of AIP with plasma total polyphenols, strongly support

the view of their high anti-atherogenic activity. The beneficial effect of polyphenolic compounds on lowering the incidence of coronary heart diseases is well documented in the literature [5,16,28]. Importantly, polyphenols are potent inhibitors of LDL oxidation, considered a key mechanism of atherosclerotic plaque formation [37].

On the other hand, it may be hypothesized that the observed improvement of lipid profile and higher blood antioxidant defence capacity at the endpoint of the study, compared to baseline, may also be due to the concurrent exercise training [38]. However, higher η^2 levels for supplementation-induced changes in individual lipid profile parameters, compared to those induced by 6 weeks of exercise training, indicate a larger impact of this dietary treatment on cholesterol homeostasis. Moreover, significant inverse correlations between plasma total polyphenols and total cholesterol, triglycerides, TG/HDL-C ratio and AIP, nearly significant negative associations with LDL-C and T-C/HDL-C, as well as inverse relationships between plasma total polyphenols and muscle damage (CK) and lipid peroxidation (TBARS) markers, seem to strongly support our presumption that the beneficial changes in lipid profile and the blood antioxidant defence status of our junior athletes were mostly due to 6 weeks' intake of the polyphenol-rich fruit and berry juice blend.

The main limitations of our study, designed to explore whether regular consumption of an acai berry-based juice blend would affect sprint performance and improve blood antioxidant status and lipid profile in junior athletes, is its open-label nature and the lack of a placebo-control group. This was due to the small number of participants recruited from the elite, national level junior hurdlers taking part in a pre-season training camp held in a special training centre, far away from their home locations.

CONCLUSIONS

Six weeks' consumption of the acai berry-based juice blend had no effect on sprint performance of junior hurdlers, but it caused a marked increase in the total antioxidant capacity of plasma, a substantial improvement of lipid profile, and moderate attenuation of the exercise-induced muscle damage. These findings strongly support the view of the health benefits of supplementation with acai berry-based juice blend, mainly attributed to its high total polyphenol content and the related high *in vivo* antioxidant and hypocholesterolaemic activities of this supplement.

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REFERENCES

- Fisher-Wellman K, Bloomer RJ. Acute exercise and oxidative stress: a 30 year history. *Dyn Med*. 2009;8:1.
- Peake JM, Suzuki K, Coombes JS. The influence of antioxidant supplementation on markers of inflammation and the relationship to oxidative stress after exercise. *J Nutr Biochem*. 2007;18(6):357-371.
- Skarpanska-Stejnborn A, Basta P, Pilaczynska-Szczesniak L, Horoszkiewicz-Hassan M. Black grape extract supplementation attenuates blood oxidative stress in response to acute exercise. *Biol Sport*. 2010;27(1):41-46.
- Powers SK, Nelson WB, Hudson MB. Exercise-induced oxidative stress in humans: cause and consequences. *Free Rad Biol Med*. 2011;51(5):942-950.
- Schauss AG, Wu X, Prior RL, Ou B, Huang D, Owens J, Agarwal A, Jensen GS, Hart AN, Shanbrom E. Antioxidant capacity and other bioactivities of the freeze – dried Amazonian palm berry, *Euterpe oleracea* Mart. (acai). *J Agric Food Chem*. 2006;54(22): 8604-8610.
- Jensen GS, Wu X, Patterson KM, Barnes J, Carter SG, Scherwitz L, Beaman R, Endres JR, Schauss AG. In vitro and in vivo antioxidant and anti-inflammatory capacities of an antioxidant-rich fruit and berry juice blend, placebo-controlled, crossover study. *J Agric Food Chem*. 2008;56(18):8326-8333.
- Lotito SB, Frei B. Consumption of flavonoid rich foods and increased plasma antioxidant capacity in humans: cause, consequence or epiphenomenon? *Free Rad Biol Med* 2006;41(12):1727-1746.
- Mertens-Talcott SU, Rios J, Jilma-Stohlawetz P, Pacheco-Palencia LA, Meibohm B, Talcott ST, Derendorf H. Pharmacokinetics of anthocyanins and antioxidant effects after consumption of anthocyanin-rich acai juice and pulp (*Euterpe oleracea* Mart.) in human healthy volunteers. *J Agric Food Chem*. 2008;56(17):7796-7802.
- Halliwell B. Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and in vivo studies? *Arch Biochem Biophys*. 2008;476(2):107-112.
- Lichtenthaler R, Rodrigues RB, Maia JG, Papagiannopoulos M, Fabricius H, Marx F. Total oxidant scavenging capacities of *Euterpe oleracea* Mart. (Acai) fruits. *Int J Food Sci Nutr*. 2005;56(1):53-64.
- Schauss AG, Clewell A, Balogh L, Szakonyi IP, Financsek I, Horvath J, Thuroczy J, Beres E, Vertesi A, Hirka G. Safety evaluation of an acai-fortified fruit and berry functional juice beverage (MonaVie Active). *Toxicology*. 2010;278(1):46-54.
- Jensen GS, Ager DM, Redman KA, Mitzner MA, Benson KF, Schauss AG. Pain reduction and improvement in range of motion after daily consumption of an acai (*Euterpe oleracea* Mart.) pulp-fortified polyphenolic-rich fruit and berry juice blend. *J Med Food*. 2011;4(7-8):702-711.
- de Souza MO, Silva M, Silva ME, Oliveira Rde P, Pedrosa ML. Diet supplementation with acai (*Euterpe oleracea* Mart.) pulp improves biomarkers of oxidative stress and the serum lipid profile in rats. *Nutrition*. 2010;26(7-8): 804-810.
- de Souza MO, Souza E Silva L, de Brito Magalhaes CL, de Figueiredo BB, Costa DC, Silva ME, Pedrosa ML. The hypocholesterolemic activity of acai (*Euterpe oleracea* Mart.) is mediated by the enhanced expression of the ATP-binding cassette, subfamily G transporters 5 and 8 and low-density lipoprotein receptor genes in the rat. *Nutr Res*. 2012;32(12):976-984.
- Udani JK, Singh BB, Singh VJ, Barrett ML. Effects of Acai (*Euterpe oleracea* Mart.) berry preparation on metabolic parameters in a healthy overweight population: a pilot study. *Nutr J*. 2011;10:45.
- Zern TL, Fernandez ML. Cardioprotective effects of dietary polyphenols. *J Nutr*. 2005;135(10):2291-2294.
- Klapińska B, Iskra J, Poprzecki S, Grzesiok K. The effects of sprint (300m) running on plasma lactate, uric acid, creatine kinase and lactate dehydrogenase in competitive hurdlers and untrained men. *J Sports Med Phys Fitness*. 2001;41(3):306-311.
- Beutler E, Duron O, Kelly B. Improved method for the determination of blood glutathione. *J Lab Clin Med*. 1963;61:882-888.
- Aebi H. Catalase in vitro. *Methods Enzymol*. 1984;105:121-126.
- Glatzle G, Korner WF, Christeller S, Wiss O. Method for the detection of biochemical riboflavin deficiency stimulation of NADPH2-dependent glutathione reductase from human erythrocytes by FAD in vitro. Investigations on the vitamin B2 status-in healthy people and geriatric patients. *Int Z Vitaminforsch*. 1970;40:166-183.
- Dobiasova M, Frohlich J. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FER(HDL)). *Clin Biochem*. 2001;34(7):583-588.
- Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol*. 1978;52:302-310.
- Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP Assay. *Anal Biochem*. 1996;239(1):70-76.
- Singleton VL, Rossi JA Jr. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic*. 1965;16:144-158.
- Niketic-Aleksic GK, Hrazdina G. Quantitative analysis of the anthocyanin content in grape juices and wines. *Lebensm Wiss Technol*. 1972;5:163-165.
- Kotlik JW, Williams HA. The incorporation of effect size in information technology, learning, and performance research. *Inf Technol Learn Perform J*. 2003;21(1):1-7.
- Vacha-Haase T. How to estimate and interpret various effect sizes. *J Counsel Psychol*. 2004;21(4):473-481.
- Manach C, Williamson G, Morand C, Scalbert A, Remesy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr*. 2005;81(1 Suppl):230S-242S.
- Scholz S, Williamson G. Interactions affecting the bioavailability of dietary polyphenols in vivo. *Int J Vitam Nutr Res*. 2007;77(3):224-235.
- Janaszewska A, Bartosz G. Assay of total antioxidant capacity: comparison of four methods as applied to human blood plasma. *Scand J Clin Lab Invest*. 2002;62(3):231-236.
- Dzeja P, Terzic A. Adenylate kinase and AMP signaling networks: Metabolic monitoring, signal communication and body energy sensing. *Int J Mol Sci*. 2009;10(4):1729-1772.
- Banfi G, Colombini A, Lombardi G, Lubkowska A. Metabolic markers in sports medicine. *Adv Clin Chem*. 2012;56:1-54.
- Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J Nutr Biochem*. 2002;13(10):572-584.
- Moskaug JO, Carlsen H, Myhrstad CW, Blomhoff R. Polyphenols and glutathione synthesis regulation. *Am J Clin Nutr*. 2005;81(1 Suppl):277S-283S.
- Guerra JF, Magalhaes CL, Costa DC, Silva ME, Pedrosa ML. Dietary acai modulates ROS production by neutrophils and gene expression of liver antioxidant enzymes in rats. *J Clin Biochem Nutr*. 2011;49(3):188-194.
- Wang Y, Chun OK, Song WO. Plasma and dietary antioxidant status as cardiovascular disease risk factors: a review of human studies. *Nutrients*. 2013;5(8):2969-3004.
- Liu LK, Lee HJ, Chyau CC, Wang CJ. Mulberry anthocyanin extracts inhibit LDL oxidation and macrophage – derived foam cell formation induced by oxidative LDL. *J Food Sci*. 2008;73(6):H113-H121.
- Armstrong N, Simons-Morton B. Physical activity and blood lipids in adolescents. *Pediatr Exerc Sci*. 1994;6:381-405.