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ARTICLE

Consumption of açai (*Euterpe oleracea* Mart.) functional beverage reduces muscle stress and improves effort tolerance in elite athletes: a randomized controlled intervention study

Jacqueline Carvalho-Peixoto, Mirian Ribeiro Leite Moura, Felipe Amorim Cunha, Pablo Christiano B. Lollo, Walace David Monteiro, Lucia Maria Jaeger de Carvalho, and Paulo de Tarso Veras Farinatti

Abstract: The study analyzed the effect of an açai (*Euterpe oleracea* Mart.) functional beverage (AB) on muscle and oxidative stress markers, cardiorespiratory responses, perceived exertion, and time-to-exhaustion during maximal treadmill running. The beverage was developed as an ergogenic aid for athletes and contained 27.6 mg of anthocyanins per dose. Fourteen athletes performed 3 exercise tests: a ramp-incremental maximal exercise test and 2 maximal exercise bouts performed in 2 conditions (AB and without AB (control)) at 90% maximal oxygen uptake. Blood was collected at baseline and after maximal exercise in both conditions to determine biomarkers. AB increased time to exhaustion during short-term high-intensity exercise (mean difference: 69 s, 95% confidence interval = −296 s to 159 s, $t = 2.2$, $p = 0.045$), attenuating the metabolic stress induced by exercise ($p < 0.05$). AB also reduced perceived exertion and enhanced cardiorespiratory responses ($p < 0.05$). The AB may be a useful and practical ergogenic aid to enhance performance during high-intensity training.

Key words: oxidative stress, muscle damage, assai, running, performance, pentathlon athletes.

Résumé : Cette étude analyse l'effet de la boisson fonctionnelle d'açai (« AB ») sur le muscle et les marqueurs de stress oxydatif, les réponses cardiorespiratoires, l'intensité de l'effort perçu et le temps jusqu'à épuisement au cours d'une course maximale sur un tapis roulant. La boisson conçue en tant que facteur ergogène contient 27,6 mg d'anthocyanines par dose. Quatorze athlètes effectuent trois tests à l'effort : un test maximal d'effort progressif et deux séances d'effort maximal dans deux conditions (avec AB et sans AB (contrôle)) à 90 % de la consommation maximale d'oxygène. On prélève des échantillons de sang pour l'analyse des biomarqueurs au début et après l'effort maximal dans les deux conditions. AB accroit le temps jusqu'à épuisement au cours de l'effort d'intensité élevée à court terme (différence moyenne : 69 s, intervalle de confiance 95 % = de −296 s à 159 s, $t = 2,2$, $p = 0,045$), diminuant ainsi le stress métabolique causé par l'exercice ($p < 0,05$). AB diminue aussi l'intensité de l'effort perçu et améliore les réponses cardiorespiratoires ($p < 0,05$). AB pourrait s'avérer un facteur ergogène utile et pratique pour améliorer la performance au cours d'une séance d.entraînement très intense. [Traduit par la Rédaction]

Mots-clés : stress oxydatif, lésion musculaire, açai, course, performance, pentathloniens.

Introduction

The consumption of tropical fruits has increased due to their nutritional benefits. Among them, the açai (*Euterpe oleracea* Mart.) has drawn much attention because of its great antioxidant capacity (Heinrich et al. 2011). The fruit has been extensively studied and can counterbalance the negative effects of pro-oxidant diets, thus improving health and immune function and lowering the risk of injury and oxidative stress (Margaritis and Rousseau 2008). These positive effects can help in preventing muscle damage and the onset of chronic diseases, since the açai contains a high content of phenolic compounds, which are antioxidant, anti-inflammatory, and cardioprotective (Xie et al. 2012). The interest of the food and drink industry in using açai as a functional ingredient has grown increasingly and the freeze-dried açai has been used as a food ingredient because of its antioxidant activity, which has been demonstrated by in vitro and in vivo assays in animals and in human cell culture models (Jensen et al. 2008).

Anthocyanins and other phenolic compounds present in açai have demonstrated the capacity for superoxide and peroxyl radical scavenging, antiproliferative activity against many types of cancer cells, reducing DNA oxidative damage, and also have been

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shown to be effective in pain control (Silva et al. 2014). Vigorous physical activity has been shown to generate reactive oxygen species (ROS), resulting in oxidative stress and muscle injury (Jackson 2008). During exercise, inflammation and oxidative stress occur via muscle metabolism and muscle damage, which can induce fatigue and impair recovery from exercise (Peake et al. 2007). Previous studies also verified that the ROS effects and oxidative stress may influence ventilatory control by altering not only central chemoreceptors, but many elements of the respiratory control system, including nonrespiratory systems that modulate the chemoreflexes that lead to respiratory muscle fatigue (Danson and Paterson 2006). Hence sport beverages that contain açai could help athletes by increasing the energy and antioxidant content in the usual diet, improving cardiorespiratory responses and reducing the risk of muscle injury during strenuous exercise (Peake et al. 2007). The use of açai-containing beverages by athletes has not been specifically investigated despite the interest in its properties as a functional food (Carvalho-Peixoto et al. 2010). Previous studies reported the effects of beverages and foods rich in antioxidant phytochemicals in reducing markers of oxidative stress induced by exercise (Muñoz et al. 2010). Morillas-Ruiz et al. (2006) observed that vigorous-intensity training performed at 70% of the maximal oxygen uptake ($\dot{V}O_{2max}$) for 90 min increased oxidative stress, and that the use of an antioxidant beverage reduced protein oxidation damage in a group of athletes. Muñoz et al. (2010) showed that the daily intake of an antioxidant functional beverage counteracted the exercise-induced oxidative stress in free-living older subjects. Questions remain with regard to the effectiveness of an açai functional beverage (AB) for athletes as an antioxidant drink able to attenuate injury muscle markers, cardiorespiratory responses, perceived exertion, and fatigue in high-intensity training. Based on a previous study (Xie et al. 2012) that showed acai to have a high antioxidant capacity, we decided to develop an AB (Patent Protocol 020110042177, Federal University of Rio de Janeiro, Brazil) and investigate its efficacy using a factorial experimental design.

Hence, the main purpose of the present study was to analyze the efficacy of AB consumption for controlling muscle and oxidative stress biomarkers, cardiorespiratory responses, and perceived exertion in elite male athletes during continuous maximal treadmill running bouts performed at 90% $\dot{V}O_{2max}$. It was hypothesized that the AB would improve effort tolerance and, therefore, time-to-exhaustion during high-intensity exercise and that AB also would reduce the muscle and oxidative stress biomarkers after the maximal exercise.

Materials and methods

The clinical intervention study was designed to determine the effects of supplementation with an AB on stress biomarkers and performance in elite athletes. The athletes were monitored before and after the supplementation period (Fig. 1).

Development and analysis of the AB

Factorial design and nutritional content

For the clinical intervention a functional drink for athletes was developed based on lyophilized acai from a fractional factorial design (2^{4-1}) with 4 components, totalling 8 drinks for the study of antioxidant activity. The main ingredients of the drink selection (carbohydrates, freeze-dried acai, glutamine, and lemon juice) were defined as independent variables in the experimental design and the interactions between the components were related to the response variable, based on the oxygen radical absorbance capacity-fluorescein (ORAC-FL) assay, to select the drink highest in antioxidant activity.

Proximate analysis (total protein, fats, carbohydrate, ash, and moisture contents) were carried out following the methods of the Association of Official Analytical Chemists (2000) to determine the proximal composition of the AB.

Antioxidant activity, polyphenol, and anthocyanins determination

Total polyphenols in the AB were quantified by a spectrophotometric method using the Folin–Ciocalteu reagent according to Singleton and Rossi (1965). Total anthocyanin concentration was determined using the differential pH method (Lee et al. 2005) and was expressed as cyanidin 3-glucoside. In addition, the total anthocyanin content was calculated by the high-performance liquid chromatography (HPLC) method and defined as mg/100 g of cyanidins in the mixture (Zanatta et al. 2005). The antioxidant activity was determined by the ORAC method (Dávalos et al. 2004) and the reaction was conducted in phosphate 75 mmol·L^{-1} buffer (pH 7.4) at 37 °C using Trolox (100 nmol·L^{-1}) as standard. Fluorescence readings (λexcitation: 485 nm and λemission: 520 nm) were taken every minute for 80 min with a spectrofluorimeter (FLUOstar Omega by BMG Labtech).

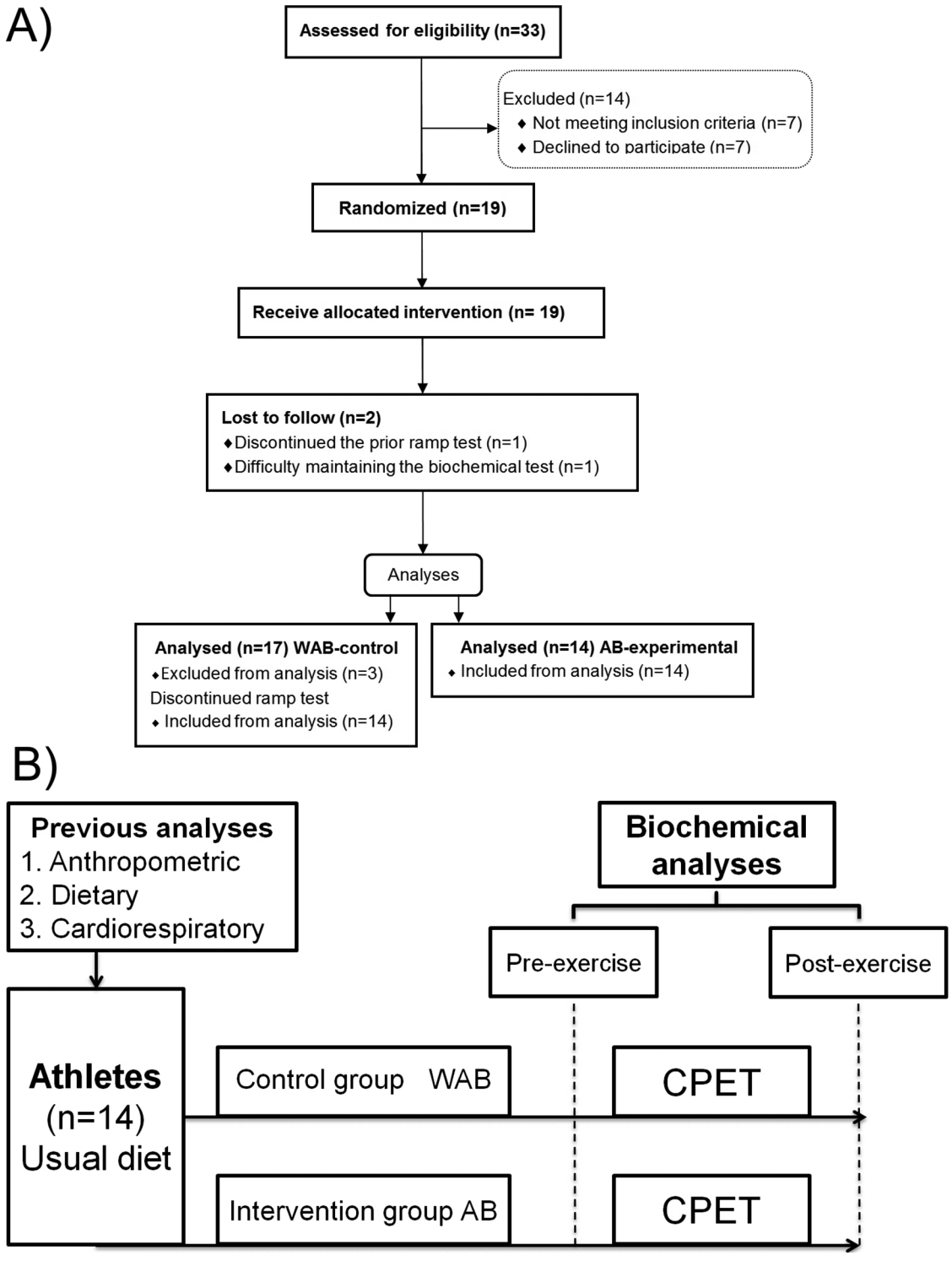
Participants and anthropometric measurements

A group of 14 male athletes (aeronautical pentathlons, runners, and sprinters) that were in their competitive training phase volunteered for the intervention study and had the following characteristics (mean (±SD)): age, 26 (6) years; height, 177.9 (7.2) cm; body mass, 72.3 (7.5) kg; body mass index, 22.8 (1.5) kg·m^{-2}; body fat, 5.9 (2.8) %; resting heart rate, 60 (10) beats·min^{-1}; systolic blood pressure, 115 (6); and diastolic blood pressure, 71 (9). Body mass and height were assessed respectively by using digital balance scales (Welmy, São Paulo, SP, Brazil) and a stadiometer graded in millimetres (American Medical do Brasil, São Paulo, SP, Brazil). Skinfold thicknesses were obtained at 3 sites (chest, abdomen, and thigh) using a Lange compass (Beta Technology Inc., Cambridge, Mass., USA), and body density and body fat percentage were estimated using the equations of Siri (Guerra et al. 2010) and Jackson and Pollock (1978). Fat mass and fat-free mass were derived from body mass and body fat percentage values. The same experienced investigator obtained all skinfold measurements. Exclusion criteria were (*i*) the use of cardiovascular or metabolic medication; (*ii*) smoking or use of alcohol or ergogenic substances that could affect exercise performance; and (*iii*) cardiovascular, respiratory, bone, muscle, or joint problems that could limit physical function. The protocol was approved by the Ethics Committee for Human Research of the Federal University of Rio de Janeiro (CEP/UFRJ-Protocol 112/10). All volunteers signed a participation agreement.

Study design

Each athlete (n = 14) visited the laboratory 4 times on 4 separate days. On the first day, a dietary record was obtained for information on the athletes' eating habits and anthropometric measurements were taken. On the second day, the participants performed a ramp-incremented maximal cardiopulmonary exercise test (CPET) to assess their cardiorespiratory fitness. On the third and fourth visits, 72 h after the CPET, the participants performed 2 continuous exercise bouts to exhaustion at 90% $\dot{V}O_{2max}$ in 2 conditions, with AB and without AB (WAB; control), to verify the cardiorespiratory responses, time to exhaustion, and the control of muscle and oxidative stress biomarkers before and after the maximal treadmill running. Each exercise bout was separated by 48 h (Fig. 1). All participants had previous experience with a treadmill and none presented difficulty or movement limitation. The tests were performed on the same motorized treadmill (Inbramed Super ATL, Porto Alegre, RS, Brazil). The ambient temperature ranged from 19 °C to 22 °C and the relative humidity ranged from 50% to 70%.

Fig. 1. (A) Overview of all phases of the study. (B) Experimental design. AB, açai functional beverage; CPET, cardiopulmonary exercise test; WAB, without AB (control condition).



Dietary intervention and acute supplementation

The participants ($n = 14$) were instructed to maintain their usual diet and not to use any supplements and/or sports beverages containing antioxidants or caffeine for at least 48 h before the tests, and were advised to consume a more balanced diet (amount and proportion of macro and micronutrients as recommended by the DRIs (Zello 2006)). The athletes were instructed before the tests to avoid anthocyanin-rich food (e.g., red fruits, juices, and red tea). A trained nutritionist gave written and verbal instructions to "adequate" the athletes' diets. The nutrient intakes were assessed by nutrition software based on dietary reference intake (Rodriguez et al. 2009). In addition, the athletes were asked to consume a balanced breakfast before the test to avoid a decrease in treadmill exercise performance. The mean (±SD) total energy intake at breakfast (bread, fruits, and dairy products) given 1 h before the tests was 595 (49) kcal. In the WAB (control condition), besides the usual break-

Table 1. Mean (±SD) macronutrients, phytochemical content, and antioxidant activity for the açai (*Euterpea oleracea* Mart.) functional beverage (AB).

Variables	Mean (±SD)
Energy, kcal	402 (3.10)
Protein, g	10.65 (0.60)
Carbohydrates, g	75.35 (1.20)
Fat, g	6.51 (0.87)
Total anthocyanins, mg cyd-glu	27.58 (0.81)
Total phenolics, mg·mL^{-1} GAE	28.99 (3.76)
ORAC-FL, μg·mL^{-1} trolox	74.35 (3.72)
ORAC-FL value	28.14 (0.54)

Note: cyd-glu, cyanidin-glucoside equivalents; GAE, gallic acid equivalents; ORAC-FL, oxygen radical absorbance capacity-fluorescein.

fast, the consumption of 300 mL of yellow fruit juice (peach) was recommended for the test. After the first exercise bout to exhaustion in the control condition, the athletes immediately drank 300 mL of the AB, which comprised freeze-dried açai (4%), 27.6 mg of total anthocyanins, glutamine (2.5%), and carbohydrates (20%) as the predominant ingredients, and were instructed to consume the AB for 3 consecutive days (acute supplementation) and 1 h before the exercise bout to exhaustion.

Sample collection and biochemical analyses

The athletes were submitted to previous haematological analyses to verify their health status for inclusion in the intervention study. The blood was collected after arm venipuncture at baseline and postmaximal treadmill running exercise. Blood analyses were performed in triplicate using clinical kits. Clinical analyses were performed to verify the immune response (blood white cell, segmented, and lymphocyte cell counts); determination of muscle injury markers (ammonia, creatinine, urate, urea); hepatic enzyme activity (alanine aminotransferase (ALT; EC 2.6.1.2), aspartate aminotransferase (AST; EC 2.6.1.1), lactate dehydrogenase (LDH; EC 1.1.1.27), creatine kinase (CK; EC 2.7.3.2)); as well as oxidative stress markers such as malondialdehyde (MDA) and glutathione peroxidase (GPx; EC 1.11.1.9), after continuous exercise bouts to exhaustion in the WAB and AB conditions.

The automated method for electrical impedance (Cell-Dyn 3700 automated analyzer) was used for blood cell analyses. Ammonia was measured by an enzymatic system using the Trinder method (Kageyama 1971) by glutamate dehydrogenase reaction. Urate, urea, and creatinine were analyzed respectively by Labtest method. Hepatic enzymes were analyzed using the kinetic method ultraviolet of International Federation of Clinical Chemistry (Roscoe 1953). MDA was analyzed from separation by HPLC with ultraviolet detection at MDA and thiobarbituric acid (Therond et al. 2000). GPx was analyzed in blood hemolysate using the kinetic enzymatic method (Flohé and Günzler 1984). The blood samples were collected in tubes with or without coagulation enhancer and splitting gel using a vacuum system (Vacuette, Greiner Bio-one, Bad Haller Strabe, Kremsmünster Austria and BD Vacutainer, Franklin Lakes, N.J. USA). The collected blood was immediately centrifuged (3000*g*, 10 min) and the plasma and serum were aliquoted and stored (–20 °C) until the time of analysis.

Exercise and control conditions

The treadmill ramp protocol was individualized to elicit each subject's limit of tolerance within 10–12 min and to determine $\dot{V}O_{2max}$ (Buchfuhrer et al. 1983). The running treadmill test was characterized by simultaneous changes in speed and slope (Porszasz et al. 2003). Based on the $\dot{V}O_{2max}$ values obtained in the ramp protocol, the values of 60% and 90% $\dot{V}O_{2max}$ were calculated to determine the running speeds and slopes for the 2 exercise bouts performed to exhaustion (Table 1), following strict recommendations published elsewhere (Howley et al. 1995; Porszasz et al. 2003).

Central (C-RPE) and local ratings of perceived exertion (L-RPE) were assessed by the Borg CR10 scale at the end of each minute of exercise (Borg 1982). Subjects provided an L-RPE for feelings pertaining to exercising muscles and joints and a C-RPE for the respiratory and cardiovascular systems. Prior to the exercise bout, detailed and standardized instructions were given about the characteristics and correct classification of both central and local fatigue. Breath-by-breath pulmonary gas exchanges and minute ventilation were determined using a VO2000 analyzer (Medical Graphics, Saint Louis, Mo., USA) and silicone face mask (Hans Rudolph, Kansas, Mo., USA). Data were then retrospectively time-averaged into 30-s bins. Prior to testing, the gas analyzers were calibrated according to the manufacturer's instructions using a certified standard mixture of oxygen (17.01%) and carbon dioxide (5.00%), balanced with nitrogen (AGA, Rio de Janeiro, RJ, Brazil). Flows and volumes of the pneumotacograph were calibrated with a syringe graduated for a 3-L capacity (Hans Rudolph). The room temperature during all the tests ranged from 21 °C to 23 °C and relative humidity ranged from 55% to 70%.

Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics version 19.0 (SPSS Inc., Chicago, Ill., USA). To compare physical performance with and without the AB across time, C-RPE, L-RPE, oxygen uptake ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$), and pulmonary ventilation ($\dot{V}_E$) assessed during the 2 exercise bouts to exhaustion were split into quartiles. Two-way (condition × time) general linear mixed models for repeated measures were used to investigate whether physiological and perceptual effort variables increased over time, and whether the time effect was influenced by the supplemented condition (i.e., AB vs. WAB). The normality of difference scores was checked using quantile–quantile plots and was confirmed in each instance. The time to exhaustion and oxidative stress biomarkers observed pre- and postexercise in the AB and WAB conditions were compared using the Student's *t* test for paired samples, and differences in nutrient intake for habitual diet and habitual diet with AB were compared using the Student's *t* test. The distribution of these differences was graphically displayed using Bland–Altman plots, which include the associated 95% limits of agreement (mean difference ± 1.96 times standard deviation of differences). All results are presented as means ± SD. Two-tailed statistical significance for all analyses was accepted as $p \leq 0.05$.

Results

The experimental design was drawn from the minimum and maximum concentration of its main ingredients. The açaí beverage was selected for this intervention because of its outstanding antioxidant activity, which was determined by the ORAC-FL assay and the content of anthocyanins per dose ingested (300 mL). The beverage consumed by athletes showed 28.1 ± 0.54 in ORAC-FL relative values, corresponding to 74.1 ± 2.6. μg·mL^{-1} in Trolox equivalents. Table 1 presents the AB nutritional content, antioxidant activity by the ORAC-FL assay, total polyphenols, and anthocyanins, respectively. Table 2 showed the athletes' dietary intake in the AB and WAB conditions. The data showed an improvement of approximately 10% with the addition of AB to the usual diet when compared with control (WAB), but this dietary increment did not significantly alter the total nutrient intake of the group ($p > 0.05$).

In the simple-blinded randomized intervention study (Fig. 1), 33 athletes were eligible for inclusion in the clinical trial. Of these, 19 (57.6%) discontinued the clinical intervention study, of which 14 (42.4%) did not complete the cardiorespiratory fitness test in the preliminary phase, and 5 (15.2%) discontinued the study in the AB supplementation phase. Therefore, only 14 athletes (42.4%) com-

Table 2. Dietary intake of mean (±SD) daily total energy, macronutrients, and antioxidants before each trial (n = 14).

	Mean ± SD	
Variables	WAB	AB
Energy, kcal	3107.21 (763.45)	3509.71 (763.45)
Protein, g	136.21 (39.49)	146.86 (50.14)
Carbohydrates, g	434.38 (156.6)	509.73 (156.60)
Fat, g	91.65 (33.63)	98.16 (40.14)
Vitamin C, mg	127.43 (92.62)	129.52 (90.03)
Vitamin E, mg	17.67 (8.95)	19.70 (8.72)
Vitamin A, mg	1232.51 (775.60)	1301.40 (731.20)

Note: There were no significant differences (the effects of dietary and antioxidants vitamin intake between WAB and AB conditions were not given). AB, açai beverage; WAB, without açai beverage.

pleted all phases of the study for WAB (control) and AB (experimental) conditions (Fig. 1).

Table 3 shows the results for the outcome variables from CPET and exercise bouts to exhaustion in the WAB and AB conditions (running speeds, slopes, and time to exhaustion). All subjects satisfied at least 3 of the 4 criteria stipulated in the Exercise and control conditions section during the incremental exercise test.

Table 4 exhibits data for immune system markers (total leucocytes, lymphocytes, and segmented count) and muscle, hepatic, and oxidative stress injury markers (ammonia, blood urate, urea, creatinine, LDH, CK, ALT, AST, GPx, and MDA) at baseline and postexercise bouts to exhaustion in the WAB and AB conditions. First, the WAB condition caused a 197% increase in ammonaemia after exercise (30.6 ± 18.2 μmol·L^{-1} to 91.0 ± 31.6 μmol·L^{-1}), while the exercise performed in the AB condition increased blood ammonia by 103% (36.14 ± 23.14 μmol·L^{-1} to 72.93 ± 29.66 μmol·L^{-1}), showing that the exercise protocol was able to induce muscle stress in the volunteers ($p < 0.001$). Furthermore, the WAB condition caused a significant increase in injury markers ($p < 0.001$) (i.e., blood creatinine = 16%, LDH = 19%, ALT = 16%, AST = 22%, and CK = 15%) in response to exercise. The AB consumption controlled the increase of blood creatinine at baseline by about 16% (p = 0.02) (WAB: 71.6 ± 16.8 μmol·L^{-1}; AB: 60.1 ± 11.5 μmol·L^{-1}) and 22% postexercise when compared with the WAB (p = 0.041). The AB condition also increased the levels of liver and muscle injury markers, but was significantly less than the WAB (i.e., LDH = 7%, ALT = 12%, AST = 16%, and CK = 17%). Nevertheless, the mean observed values for CK were not significantly different between conditions ($p > 0.05$).

The LDH at baseline was higher in the AB than in the WAB condition (p = 0.005), but after exercise WAB increased by 19% compared with a 7% increase in AB (WAB: 326.64 ± 71.74 U·L^{-1} to 388.35 ± 75.6 U·L^{-1}, $p < 0.001$; AB: 374.93 ± 86.56 U·L^{-1} to 402.78 ± 65.6 U·L^{-1}, p = 0.100). The AST increased significantly in both conditions ($p < 0.001$), but the increase was higher in the WAB (22%) compared with the AB (16%) (WAB: 32.9 ± 18.5 to 40.1 ± 21.5 U·L^{-1}, $p < 0.001$; AB: 33.9 ± 9.7 U·L^{-1} to 39.5 ± 10.6 U·L^{-1}, p = 0.009). There were no significant differences in ALT and blood urate at baseline and postexercise in either condition. The AB caused a greater increase in blood urea after the exercise compared with the WAB (p = 0.025). With regard to blood oxidative stress parameters, the WAB caused an increase of 7% and 2% in MDA and GPx, respectively ($p > 0.05$). The AB reduced the blood MDA increase (p = 0.048) by 3% (WAB: 3.9 ± 0.73 mmol·L^{-1}; AB: 3.2 ± 0.69 mmol·L^{-1}). The GPx concentration remained stable at baseline and postexercise in both conditions (p = 0.896), but the WAB caused a slight increase (2%) in this marker when compared with the slight decrease (2.5%) observed in the AB condition (p = 0.435).

Blood cell analyses were performed and nonsignificant differences were detected for platelets, red blood cells, or hemoglobin content in both conditions (data not shown). Changes in white blood cells were analyzed at baseline and postexercise. Total leukocytes count increased 73.9% and 68.5% in the WAB and the AB conditions, respectively, with a nonsignificant difference between conditions (p = 0.513). Mature neutrophils (segmented cells) showed no difference before or after the exercise bouts (p = 0.919). The AB significantly reduced lymphocytes at baseline (p = 0.017) and postexercise (p = 0.051).

Table 3. Mean (±SD) outcome variables for the incremental exercise test as well as treadmill velocity, slopes, and time of exhaustion during exercise bouts to exhaustion with continuous work rates at 60% and 90% $\dot{V}O_{2max}$ in the WAB and AB conditions (n = 14).

Variables	Mean (±SD)
Ramp-incremented maximal exercise CPET	
Maximal heart rate, beats·min^{-1}	184 (10.0)
$\dot{V}O_{2max}$, mL·kg^{-1}·min^{-1}	51.9 (7.9)
$\dot{V}O_{2max}$, L·min^{-1}	3.8 (0.5)
Minute ventilation, L·min^{-1}	98.5 (12.6)
Respiratory exchange ratio	1.20 (0.16)
Peak treadmill velocity, km·h^{-1}	15.6 (1.5)
Peak treadmill slope, %	5.2 (0.9)
Time to exhaustion, s	690 (105)
Continuous work rate at 60% $\dot{V}O_{2max}$	
Treadmill velocity, km·h^{-1}	9.4 (0.9)
Treadmill slope, %	3.0 (0.5)
Time, s	300
Continuous work rate at 90% $\dot{V}O_{2max}$	
Treadmill velocity, km·h^{-1}	14.1 (1.3)
Treadmill slope, %	4.5 (0.8)
Time to exhaustion during WAB condition, s	409 (175)
Time to exhaustion during AB condition, s	477 (2)

Note: AB, açai functional beverage; CPET, cardiopulmonary exercise testing; $\dot{V}O_{2max}$, maximal oxygen uptake; WAB, without AB (control).

Figure 2A shows the distribution of the differences between the test in the WAB condition and AB time to exhaustion for each of the 14 subjects. The mean (±SD) time to exhaustion observed during the energy drink condition was significantly higher than the time to exhaustion observed in the control condition (477 ± 237 vs. 409 ± 175 s) (mean difference = 69 s, p = 0.045). Figure 2B shows data for heart rate (HR), C-RPE, L-RPE, $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}_E$, observed within quartile time intervals during continuous exercise bouts to exhaustion in the WAB and AB conditions. HR exhibited a significant increase over time (C-RPE: F = 206.6, $p < 0.001$), the slope of which was influenced by the exercise condition (F = 15.9, $p < 0.001$). Mean HR in each quartile time interval of exercise was approximately 4 beats·min^{-1} higher during the WAB condition than in AB. Similar to HR, both C-RPE and L-RPE exhibited an increase over time (C-RPE: F = 295.5, $p < 0.001$; L-RPE: F = 191.4, $p < 0.001$), in which the RPE responses were also significantly attenuated in the AB condition (C-RPE: F = 15.4, $p < 0.001$; L-RPE: F = 48.5, $p < 0.001$). Each quartile time interval of exercise was associated with a mean difference between the WAB and AB conditions of approximately 0.8 and 1.5 for the central and local RPE, respectively. Finally, the $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}_E$ also increased over the duration of each exercise bout ($\dot{V}O_2$: F = 35.3, $p < 0.001$; $\dot{V}CO_2$: F = 204.1, $p < 0.001$; $\dot{V}_E$: F = 227.6, $p < 0.001$), but the AB did not significantly affect these variables ($\dot{V}O_2$: F = 0.2, p = 0.638; $\dot{V}CO_2$: F = 0.2, p = 0.686; $\dot{V}_E$: F = 2.9, p = 0.093). Nevertheless, the mean observed values for $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}_E$ in each quartile time interval of exercise were, on average, 0.17, 0.20, and 2.7 L·min^{-1} lower during the AB condition than in the WAB condition, respectively.

Discussion

The selected beverage (AB) consumed by athletes contained 12.0 g of freeze-dried açai per dose, with a good total phenolic and anthocyanins content, vitamin E, and ORAC-FL values per dose (Table 1), suggesting a positive correlation with its antioxidant

Table 4. Biomarkers analyses (mean (±SD)) during baseline and postexercise bouts to exhaustion for the WAB and AB conditions (n = 14 athletes).

	Baseline			Postexercise to exhaustion		
Variables	WAB	AB	p	WAB	AB	p
Total leukocytes, ×10^9/L	5636 (1695)	5716 (1376)	0.728	9868 (27.70)	9633 (29.5)	0.513
Lymphocytes[a]	42.14 (10.90)	23.0 (23.4)	0.017*	57.69 (7.25)	53.08 (9.8)	0.051*
Segmented cells[a]	47.57 (11.03)	42.92 (11.79)	0.194	33.14 (10.04)	33.43 (9.09)	0.919
Ammonia, μmol·L^{-1}	30.64 (18.24)	36.14 (23.14)	0.545	91.00 (31.57)	72.93 (29.66)	0.024*
Urate, μmol·L^{-1}	0.24 (0.04)	0.24 (0.039)	0.740	0.25 (0.039)	0.24 (0.04)	0.214
Urea, mmol·L^{-1}	4.59 (1.06)	5.27 (1.30)	0.151	4.72 (1.07)	5.44 (1.30)	0.025*
Creatinine, μmol·L^{-1}	71.60 (16.77)	60.11 (11.49)	0.02*	83.09 (79.56)	64.53 (61.88)	0.041*
LDH, U·L^{-1}	326.64 (71.74)	374.93 (86.56)	0.005*	388.35 (75.6)	402.78 (65.6)	0.153
CK, U·L^{-1}	254.21 (216.70)	337.43 (185.64)	0.287	293.64 (230.4)	395.42 (218.0)	0.104
ALT, U·L^{-1}	25.29 (10.21)	26.29 (12.26)	0.350	29.36 (11.8)	28.71 (9.99)	0.539
AST, U·L^{-1}	32.86 (18.48)	33.93 (9.70)	0.358	40.14 (21.47)	39.5 (10.6)	0.455
MDA, mmol·L^{-1}	3.64 (0.72)	3.26 (8.78)	0.186	3.89 (0.73)	3.20 (0.69)	0.048*
GPx, U·L^{-1}	6948.36 (1403.90)	7008.07 (1341.63)	0.896	7081.57 (1760.01)	6829.5 (1171.83)	0.435

Note: AB, usual diet + açai energy drink; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatinine kinase; GP_X, glutathione peroxidase; LDH, lactate dehydrogenase; MDA, malondialdehyde; WAB, usual diet without açai energy drink.

[a]Cells count (%).

*Significantly different ($p < 0.05$), both (WAB and AB conditions) at baseline versus postexercise to exhaustion.

activity. In previous work, samples of red grape juice showed ORAC-FL values of around 14.6 to 25 of Trolox equivalents, close to that observed in AB (Dávalos et al. 2004). Data from the present study suggest that the functional beverage ingested by the athletes before the exercise demonstrated good antioxidant activity, since the açai fruit is rich in anthocyanins (111 mg·L^{-1}), and also other flavonoids (91.3 mg per 100 g) (Heinrich et al. 2011).

The present study aimed to investigate the effects of AB consumption on the muscle and oxidative stress, cardiorespiratory responses, RPE, and effort tolerance during continuous exercise bouts close to maximal intensity work rates (i.e., 90% $\dot{V}O_{2max}$). The main finding was that AB consumption enhanced the time to exhaustion of athletes during a short-term high-intensity continuous bout. This improvement was followed by an attenuation of muscle and oxidative stress biomarkers, mainly by decreasing ammonaemia and MDA. Furthermore, the AB attenuated the cardiorespiratory responses, showing potential to minimize the hyperventilation induced by exercise. From a practical perspective, the AB may be a natural, useful, and practical tool to enhance athletic performance during high-intensity training protocols.

To the best of our knowledge, the present study is the first to investigate the extent to which AB consumption attenuates physiological responses and perception of effort during vigorous-intensity bouts to exhaustion. Our results showed that HR, $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}_E$ in each quartile time interval of exercise were, on average, 2%, 5%, 6%, and 3% lower during the AB condition than that of the WAB condition, respectively. Although the precise mechanisms by which exercise tolerance increased with the AB has not been elucidated, and can be partially explained by several physiological adjustments that occur in response to increasing CO_2 levels during the exercise bout performed to exhaustion. Nybo and Rasmussen (2007) showed that hyperventilation during strenuous exercise may decrease the arterial CO_2 tension and blunt the increase in cerebral blood flow, which could induce an inadequate oxygen delivery to the brain contributing to early fatigue. In fact, increased ventilation can also increase the total cost of the activity and create competition for blood flow between the respiratory and locomotor muscles, which may compromise exercise performance via a vasoconstriction response (Wells and Norris 2009).

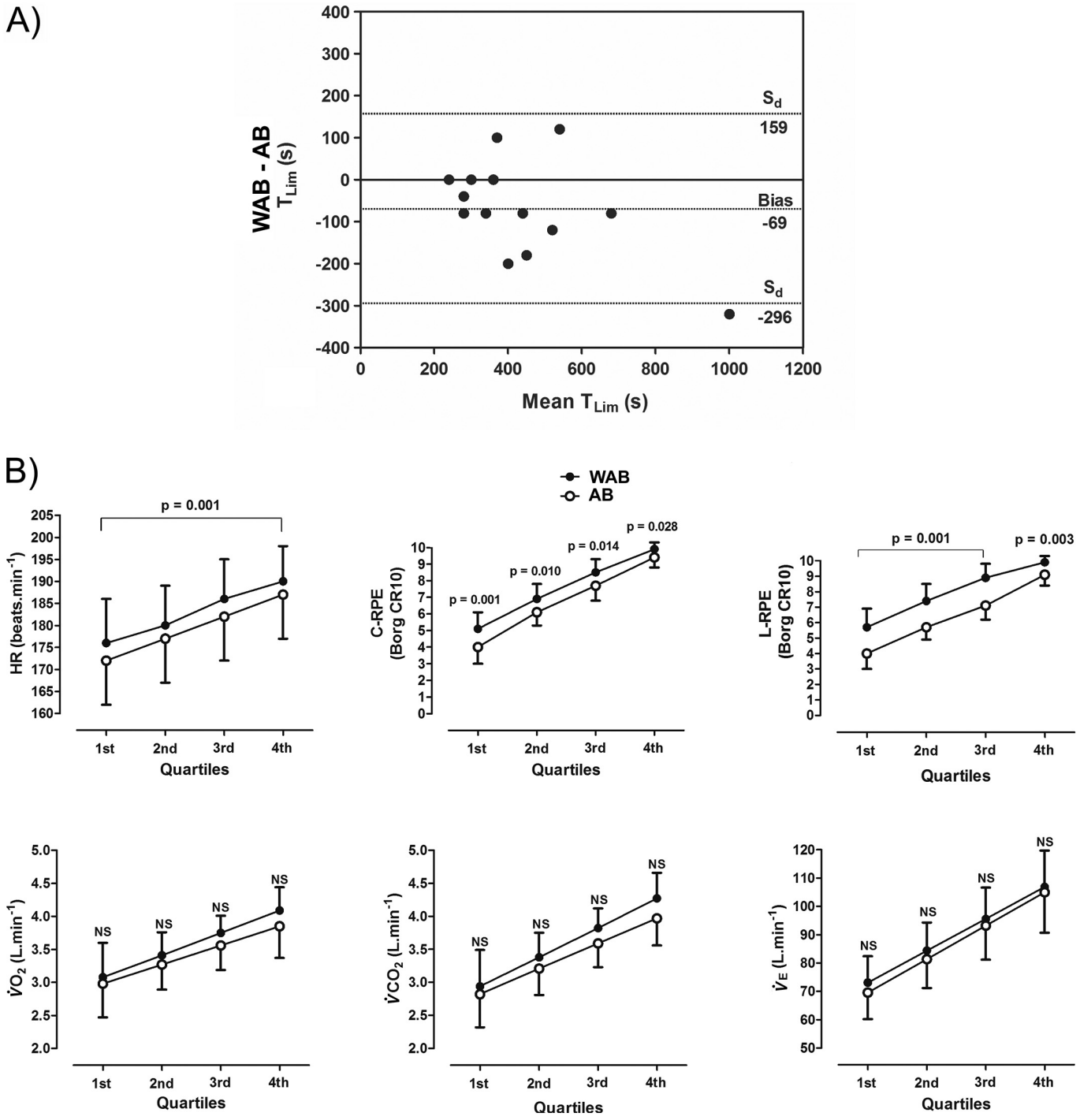
It has been suggested that the RPE is influenced by feedback mechanisms from either cardiopulmonary (central rated perceived exertion) or peripheral (L-RPE) factors. Cardiopulmonary factors include, among others, HR, $\dot{V}O_2$, and $\dot{V}_E$, while peripheral/metabolic factors include blood lactate, CO_2 production, mechanical strain, and skin–core–muscle temperatures (Chen et al. 2002). In this regard, in the present study it was observed that on average, C-RPE and L-RPE were 11% and 23% lower during the AB condition compared with the WAB condition, respectively (Fig. 2). The significant decrease in RPE observed during the AB condition, especially in L-RPE, may be explained by the attenuation of cardiorespiratory responses, improving the exercise tolerance and, therefore, the longer time to exhaustion (6.7 ± 2.9 min vs. 7.7 ± 3.9 min) (Fig. 1).

In this study, the exercise protocol was an inductor of muscle and oxidative stress that significantly influenced enzyme activity associated with muscle and liver injury, provoking an increase in CK, LDH, ALT, and AST, as well as in plasma ammonia. Several previous studies observed an increase in ammonaemia and muscle/liver injury markers as a consequence of intense efforts (Bassini-Cameron et al. 2008). An increase in muscle and liver stress markers has also been reported because of energy depletion from submaximal tests (60%–70% $\dot{V}O_{2max}$) performed until exhaustion (Langfort et al. 2004). Our data concur with these results, showing an increase in injury markers after exhaustive maximal exercise. As expected, according to a previous pilot study (Carvalho-Peixoto et al. 2010), the ammonaemia was higher after exercise in both conditions, but this increase was shown to be offset by the AB (Carvalho-Peixoto et al. 2007).

The increase in ammonia levels in high-intensity continuous exercise impairs ATP regeneration by the activation of NMDA receptors, which compromises mitochondrial function and decreases ATP synthesis while increasing intramitochondrial Ca^{2+}. This may affect the function of different enzymes of the respiratory chain, leading to an increased release of free radicals and early fatigue (Wilkinson et al. 2010). Our findings suggested that the high-intensity treadmill running exercise induced muscle damage, as reflected by the increase in ammonaemia and muscle injury markers, but also by the fact that such a response was reduced in the AB condition.

Previous studies reported that strenuous exercise induces oxidative stress by generating biomarkers that can be measured through the oxidative damage of lipids, genetic material, and proteins (Bassini-Cameron et al. 2007). Skeletal muscle cells generate ROS at a number of cellular sites and this production can be increased by contractile activity (Gonzalez et al. 2002). An early study suggested that the production of superoxide as a by-product of mitochondrial oxygen consumption would be a great source of muscle ROS generation (Jackson 2008). However, regular intense or prolonged exercise can overload the endogenous antioxidant

Fig. 2. (A) Bland–Altman plots showing individual differences between the WAB and AB conditions for the T_{Lim}. The first and third horizontal dashed lines in each graph represent the 95% limits of agreement. (B) Mean ± SD; HR, $\dot{V}O_2$, $\dot{V}_E$, $\dot{V}CO_2$, C-RPE, and L-RPE at quartile time intervals during continuous exercise bouts to exhaustion in the WAB (mean (±SD) 409 (175) s·quartile^{-1}) and AB conditions (mean (±SD) 477 (237) s·quartile^{-1}). C-RPE and L-RPE were assessed by the Borg CR10 scale (Borg 1982). *p* values indicate significant difference between the WAB and AB conditions ($p < 0.001$ to $p = 0.028$). AB, açai functional beverage; C-RPE, central rating of perceived exertion; HR, heart rate; L-RPE, local rating of perceived exertion; NS, nonsignificant; S_d, standard deviation of the differences; T_{Lim}, time to exhaustion; $\dot{V}CO_2$, carbon dioxide output; $\dot{V}_E$, pulmonary ventilation; $\dot{V}O_2$, oxygen uptake; WAB, control condition.



system's capability, impacting on the redox status; thus a dietary intervention with antioxidants, mainly for athletes with low dietary antioxidant intake, may be required since it can attenuate cytokine production by directly neutralizing ROS and/or inhibiting the activity of redox-sensitive signal transduction pathways (Peake et al. 2007). The antioxidant ingredient used in the AB was freeze-dried açai, with high antioxidant activity. The AB intake as an antioxidant beverage benefited the athletes in controlling lipid peroxidation, showing significant MDA control and also the potential to modulate the GPx activity, as reported in our pilot study (Carvalho-Peixoto et al. 2010). Some authors have demonstrated the role of antioxidants in oxidative stress control in athletes and

nonathletes. Morillas-Ruiz et al. (2006) verified the effects of polyphenolic antioxidants on exercise-induced oxidative stress and concluded that the antioxidant supplement offered protection against the oxidative stress caused by intense effort. In addition, Panza et al. (2008) showed that the consumption of green tea favourably affected oxidative stress biomarkers in trained men. Similarly Jensen et al. (2008) verified that the use of an açai juice blend improved the antioxidant status of the volunteers and caused a decrease in lipid peroxidation.

Our findings also demonstrated an increase in white blood cell count after the maximal treadmill running exercise at 90% $\dot{V}O_{2max}$ in both the AB and WAB conditions. As previously described, leukocytosis can occur because of muscle damage induced by intense effort (Gleeson 2007). Our data are in accordance with other researchers that have reported immune function impairment following acute bouts of intense exercise (Bassini-Cameron et al. 2008). The AB seemed to attenuate lymphocytosis in response to maximal exercise, probably because of its energetic ingredients since it is well established that carbohydrate and glutamine supplementation can decrease both lymphocyte and leukocyte count after high-intensity protocols (Bassini-Cameron et al. 2007; Gleeson 2007). Nantz et al. (2006) showed that the consumption of a dried, encapsulated fruit and vegetable juice concentrate resulted in increased immunity and plasma antioxidant capacity of healthy adult volunteers. In our case, the improvement in the immune response could be possibly attributed to the addition of freeze-dried açai to the energetic supplement, since studies have reported several anti-inflammatory and antioxidant properties of flavonoids present in the açai fruit (Heinrich et al. 2011; Xie et al. 2012).

Conclusion

The AB consumption attenuated ammonaemia, cardiorespiratory responses, and RPE, increasing the time to exhaustion within near-to-maximal treadmill exercise in aerobically trained athletes. Additionally, the AB presented good sensorial attributes for this group of athletes and good antioxidant activity. The AB acute supplementation attenuated the oxidative stress and muscle damage markers and also improved the lymphocyte response induced by maximal treadmill exercise. These effects improved exercise tolerance by attenuating physiological and psychological responses related to fatigue. From a practical perspective, the AB may be a useful and practical functional drink to enhance athletic performance and recovery postexercise during high-intensity training or competitions. Further research in vivo or with athletes are necessary to verify the beneficial effects of the AB for sports nutrition and health.

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