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Pequi fruit (*Caryocar brasiliense* Camb.) pulp oil reduces exercise-induced inflammatory markers and blood pressure of male and female runners

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Abstract

The objective of this study was to investigate the anti-inflammatory properties of pequi (*Caryocar brasiliense*) fruit oil and its effects on the postprandial lipidemia and arterial blood pressure of male and female athletes. These athletes were evaluated after races in the same environment and under the same type, intensity, and length of weekly training conditions, both before and after ingestion of 400 mg pequi oil capsules for 14 days. Pequi fruit contains several antioxidants, and its oil has been associated with anti-inflammatory properties in other pequi species. Because the oil of pequi is mostly composed of oleic and palmitic fatty acids, the oil may alter the ratio of triglyceride to cholesterol in postprandial lipidemia. Epidemiologic studies suggest that an increased intake of monounsaturated fatty acids (such as oleic acid) is inversely related to blood pressure. Thus, we hypothesize that pequi oil could reduce exercise-induced inflammation and blood pressure, and modulate postprandial lipidemia in runners. To test this hypothesis, arterial blood pressures were checked before races; blood samples were taken after the races and submitted for analysis of leukocytes and platelets analysis, high-sensitivity C-reactive protein values, and postprandial lipids. Pequi oil resulted in anti-inflammatory effects and reduced the total cholesterol and low-density lipoprotein in the age group older than 45 years, mainly for men. The results showed a general trend for reduced arterial pressure, suggesting that pequi oil may have a hypotensive effect. However, this finding needs additional investigation. Thus, pequi oil, besides possessing many nutritional properties, may be a good candidate supplement for athletes.

Keywords: *Caryocar brasiliense* (pequi) fruit oil; Human athlete; Postprandial lipemia; Anti-inflammatory effects; High-sensitivity C-reactive protein; Arterial pressure

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; MDA, malondialdehyde; MPV, mean platelet volume; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ROS, reactive oxygen species; SFA, saturated fatty acids; TBARS, thiobarbituric acid reactive substances; TG, triglyceride; TRL, triglyceride-rich lipoprotein; VLDL, very low density lipoprotein.

1. Introduction

Reactive oxygen species (ROS) are constantly formed in the human body, mainly as a result of normal oxidative metabolism in the mitochondria, and neutralized by an elaborate antioxidant defense system [1]. Because exercise

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increases oxygen consumption, it also enhances ROS generation, which at relatively low cellular levels may influence metabolism in physiologic conditions [2]. A weak production of ROS is necessary for normal contractile activity of skeletal muscles [2], and physical training is known to induce antioxidant enzymes [3-5]. The generation of ROS by myocytes is potentially important because it seems to play a significant role in signaling molecules, modulating some regulatory systems involved in the skeletal muscle performance [2]. They also have effects on the immune system that are considered positive [4].

However, intensive or prolonged exercise, above habitual intensity of effort or training with very elevated frequency, overloads the endogenous antioxidant system's capacity, resulting in oxidative stress [3-6]. Thus, it results in increased levels of malondialdehyde (MDA) in the blood, which serve as indirect indicators of lipid peroxidation and can be measured by thiobarbituric acid reactive substances (TBARs) assay [5,7,8]. Moreover, it can initiate reactions that resemble the acute phase of the immune response to infection, inducing changes in the immune cell count and release of acute phase proteins, such as high-sensitivity C-reactive protein (hs-CRP) [6,9,10]. In this context, dietary antioxidant intake can help to prevent oxidative stress and injuries [1,4].

Many studies have investigated the impact of the antioxidant status in the exercise-induced damages [1,4,7,11]; and with this purpose, most dietary interventions have focused on nutritive factors such as vitamin antioxidants or drugs that mediate exercise-induced oxidative stress [12]. Nutritional supplements have been widely studied, among which vitamin E, vitamin C, creatine, and glutamine supplementation is included [4,7]. However, no study has examined individually the antioxidant roles of carotenoids or carotenoid-rich dietary food or supplements; only β-carotene (30 mg) has been tested in a mixture with vitamins C (1000 mg) and E (592 mg) [1,4,7]. Thus far, evaluation of the effectiveness of dietary antioxidant intervention in oxidative stress and exercise-induced damages remains incomplete because it can depend not only on the nature of the antioxidant in use, but also on the exercise type and the partial pressure of oxygen in the tissues.

Biological antioxidants, such as those found in plant-based foods, contain bioactive phytochemicals [12] and may play a vital role in protecting the cell from exercise-induced oxidative stress [11]. Pequi (*Caryocar brasiliense* Camb.) is a typical tree found in the Brazilian Cerrado. Pequi fruit pulp oil contains natural antioxidants, such as different carotenoids [13-16]. These substances are effective chain-breaking antioxidants at low partial pressure of oxygen [17,18] and can prevent oxidative injuries in those endurance athletes who overload their endogenous antioxidant system capacity through elevated training frequency or intense and prolonged exercise. In addition, the fatty acid composition of the oil is mainly composed of oleic acid (51.37% to 55.87%) and palmitic acid (35.17% to 46.79%) [14,19], which are involved in the modulation of the ratio of triglyceride (TG) to cholesterol in postprandial triglyceride-rich lipoprotein (TRL) [20].

In a previous study, pequi oil was efficient in reducing DNA damages and tissue injuries evaluated for aspartate aminotransferase and alanine aminotransferase [21]. In addition, according to ethnobotanical studies, the oils of the fruit pulp from *C coriaceum* (another species of pequi) possess significant anti-inflammatory effects [22,23]. Because these oils are similar to those of *C brasiliense*, where oleic and palmitic acids are also the major components [24], we hypothesized that pequi oil could efficiently reduce exercise-induced inflammation and modulate postprandial lipidemia of runners. Because some epidemiologic studies have suggested that an increased intake of monounsaturated fatty acids (MUFA, such as oleic acid) is inversely related to blood pressure [25], we also hypothesized that pequi oil intake could influence arterial pressure. Thus, the objective of this study was to investigate the anti-inflammatory properties of pequi (*C brasiliense*) fruit oil and its effects on the postprandial lipidemia and arterial blood pressure of runners after races run in the same environment and under the same conditions, intensity, and length of weekly training conditions (before and after taking the pequi oil supplement). This research is important to further understand the benefits of carotenoid supplements in the prevention of exercise-induced damage, mainly for those athletes who exercise strenuously and surpass their endogenous antioxidant defenses.

2. Methods and materials

2.1. Study design and participants

The trial was conducted from August 2007 to April 2008, after preclinical and toxicologic tests in mice [26]. Volunteers of both sexes (76 men and 49 women) and different age groups (15 to 67) were recruited in high schools, colleges, universities, clubs, and companies in Brasília (Federal District/Brazil). The selection criterion (inclusion/exclusion criterion) used for the runners was that they had at least a 4000-m run, which means that only trained athletes were included. They were to participate in 2 races, before (control group) and after (treatment group) ingestion of 400 mg of pequi oil in capsules supplied daily for 14 consecutive days. The decision for this daily ingestion took into account the data from pequi literature and the maximum daily dose of provitamin A carotenoids (25 mg) recommended by the National Agency for Sanitary Surveillance.

To avoid plasma volume changes in function of variability among individuals, each athlete participated as control group and treatment group, being compared in the statistical tests with him-or herself. There was no change in the daily routine, training, or lifestyle of all runners between the first race and second race, except for ingestion of pequi oil capsules. No volunteer was excluded by having chronic diseases such as slight hypertension or treated dyslipidemia,

and the effects of pequi oil supplementation on these volunteers were also analyzed.

The races before and after ingestion of pequi oil were conducted outdoors on flat tracks, under the same environmental conditions; and the athletes could choose the distance that they would cover (4-21 km), according to their type, intensity, and length of weekly training. Both races for each athlete were the same distance. The time needed by each athlete to finish the races was similar in the 2 races, guaranteeing the same intensity (time needed to finish the race) of races before and after pequi oil supplementation. The volunteers were informed about the purpose of the study; all of them received a random number generated by computer and were free to withdraw at any time during the study. After the first race, they received the capsules and were instructed to take them for 14 days during or immediately after lunch until the second race.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee for Health Sciences Faculty Research of the University of Brasília and by the National Commission for Ethics in Research (0.001668/2005-18). Written informed consent was obtained from all subjects.

2.2. Preparation of capsules

Pequi fruit was obtained *in natura* from the local markets of Brasília/DF (Brazil) and surrounding areas. The internal mesocarp was peeled or grated to obtain the pulp, which was packed in a covered pot and frozen at −86°C. Pequi pulp oil was extracted by cold maceration using chloroform as a solvent. The extract was subjected to evaporation under reduced pressure and dried at high vacuum for complete solvent removal. Pequi oil was incorporated in Aerosil (colloidal silicon dioxide) qsp so that the users ingested a daily dose of 400 mg of pequi oil. Its relative composition is shown in Table 1. The capsule production was patented as number PI0601631-6 (National Institute of Industrial Property).

2.3. Procedures and measurements

Waist circumference, hip circumference, waist-hip ratio, and body mass index (BMI) were verified before the first race, according to Wang and Hoy (2004) [28]. Waist circumferences were measured at the narrowest point below the ribs or halfway between the lowest ribs and the iliac crests. Hip circumferences were measured at the level of the anterior superior iliac spine, where this could be felt; otherwise, measurement was done at the broadest circumference below the waist (Table 2). The arterial pressure of volunteers was checked before the races, and blood samples were drawn with EDTA during the morning immediately after the races in 2 rounds: first in the race without pequi oil supplementation and second in the race after ingestion of 400 mg of pequi oil in capsules supplied daily for 14 consecutive days. Blood samples were submitted to immune cell and platelet counting; serum samples were submitted to determine postprandial lipid profiles and hs-CRP levels.

2.4. Biochemical analyses and cell count

Serum postprandial lipid profile analysis was run on the automated chemistry analyzer ADVIA 1650 (Bayer Diagnostics), using the appropriate Advia chemistry reagents. Leukocyte and platelet counts were carried out in the Cell-Dyn 3700 automated analyzer (Abbott Diagnostics), and hs-CRP was measured by an immunometric assay (Immulite 2000; DPC Medlab). The TBARS assay was carried out according to Wasowicz et al (1993) [8], with slight modifications. The TBA solution was prepared by dissolving TBA (Merck; final concentration of 29 mmol/L) in acetic acid (8.75 mol/L, Merck). The standard stock solution of MDA was prepared by dissolving 480 μL of 1,1,3,3-tetraethoxypropane (Sigma) in 100 mL of ethanol (Merck). Immediately before use, the solution was diluted in Milli-Q water to yield a working solution of 10 μmol/L. Working aqueous solutions of EDTA (67.3 mmol/L) and GSH (32.5 mmol/L) were freshly prepared immediately before use (to avoid GSH oxidation). After centrifugation (1500*g*, 10 minutes, 4°C), the plasma was carefully removed; and EDTA and GSH were added to a final concentration of 1.34 and 0.65 mmol/L, respectively. The samples were then quickly frozen in liquid nitrogen and stored at −80°C until analyzed.

For the TBARS test, 50 μL of plasma or an equal volume of MDA (working standard solution) was added to 10-mL glass tube containing 1 mL of Milli-Q water, followed by 1 mL of solution containing TBA (29 mmol/L) in acetic acid (pH of the reaction mixture, 2.4-2.6), mixed, and heated in a water bath for 1 hour at 95°C to 100°C. The samples were then cooled; and 25 μL of 5 mol/L HC1 was added (final pH, 1.6-1.7), followed by extraction with 3.0 mL of *n*-butanol

Table 1
Relative fatty acid and carotenoid composition of pequi (*C brasiliense* Camb.) pulp oil capsules

Fatty acids[a] [27] (% of pequi pulp fruit oil)						Carotenoids [13-16] (mg/100 g of pequi pulp fruit)		
Saturated		Monounsaturated		Polyunsaturated		Provitamin A	Lycopene	Total
Palmitic	41.78	Oleic (ω9)	54.28	Linoleic (ω6)	1.36	6.26-11.5	1.12-2.08	6.75-28.66
Stearic	1.28	Palmitoleic (ω7)	0.67	Linolenic (ω3)	0.51			
Araquidic	0.12							

[a] Present study. The omega nomenclature (ω), which is defined according to the carbon numeration associated with the first double bonds (3, 6, 7, or 9) from the methyl radical, was added (to the unsaturated fatty acids).

Table 2
Sample size and anthropometric variables of the athletes

	n (%)	Anthropometric variables			
		BMI	Waist circumference (cm)	Hip circumference (cm)	Waist-hip ratio
Total	125 (100)	23.65 ± 0.47	83.62 ± 1.48	95.84 ± 1.24	0.87 ± 0.01
Men	76 (60.8)	24.46 ± 0.66	86.44 ± 2.04	94.39 ± 1.79	0.92 ± 0.01
Women	49 (39.2)	22.43 ± 0.54	79.29 ± 1.65	98.06 ± 1.4	0.81 ± 0.02

Data are expressed as means ± SEM.

and vortex mixing for 30 seconds. The butanol phase was separated by centrifugation (1500*g*, 10 minutes), and its fluorescence was measured with a Jasco FP-777 spectrofluorometer (excitation, 525 nm; emission, 547 nm). A standard curve was prepared with MDA (0-0.15 μmol/L).

2.5. Statistical analyses

Statistical analyses were carried out using Statistical Package for the Social Sciences version 15.0 (SPSS, Chicago, IL), and the continuous variables were tested for normal distribution with Shapiro-Wilk. Data were expressed as means ± standard error of the mean (SEM). Differences were considered statistically significant when $P < .05$. The statistical factors analyzed were total (N = 125), sex (76 men and 49 women), age group (15-19, 20-24, 25-29, 30-34, 35-39, 40-44, and 45 years up), and covered distance (4-5, 6-7, 8-10, and 16-21 km). Afterward, preexisting cardiovascular disease (slight hypertension and treated dyslipidemia) was also analyzed. Differences between sexes were evaluated by the Student *t* test. For the other parameters, differences in the comparison of before and after values were assessed by the nonparametric Wilcoxon matched-pairs test because the data presented heterogeneous variability. The possible correlations between the parameters sex/age groups, sex/distance covered, and age groups/distance covered were also analyzed through χ^2 correlation test.

3. Results

3.1. Correlation test

There was a significant correlation between age groups and the distance covered ($P = .000$). No other correlation was found.

Table 3
Influence of pequi (*C brasiliense* Camb.) fruit pulp oil intake on the total and sex groups

	Total		Men		Women	
	Before	After	Before	After	Before	After
Leukocytes						
Total leukocytes (/mm^3)	7449.15 ± 207.22	7470.34 ± 178.91	7391.78 ± 237.25	7394.52 ± 226.14	7542.22 ± 386.90	7593.33 ± 294.76
Lymphocytes (/mm^3)	2671.08 ± 89.20	2624.26 ± 92.10	2737.96 ± 110.53	2668.45 ± 114.95	2562.58 ± 150.41	2552.58 ± 154.55
Segmented (/mm^3)	4017.89 ± 159.85	3976.42 ± 133.07	3871.44 ± 188.62	3855.45 ± 174.32	4255.47 ± 285.74	4172.67 ± 203.47
Rods (/mm^3)	26.47 ± 7.75	26.49 ± 7.81	29.79 ± 11.36	25.72 ± 9.87	21.22 ± 8.91	27.71 ± 12.89
Basophils (/mm^3)	85.46 ± 4.69	98.49 ± 4.32	81.99 ± 6.30	102.05 ± 5.55 *	91.09 ± 6.86	92.71 ± 6.85
Eosinophils (/mm^3)	146.40 ± 10.70	155.92 ± 11.62	160.93 ± 15.19	168.01 ± 16.57	122.82 ± 12.84	136.31 ± 14.08
Monocytes (/mm^3)	497.07 ± 20.41	549.18 ± 18.99 *	509.15 ± 27.47	555.82 ± 25.41	477.47 ± 29.81	538.40 ± 28.22 †
Platelets						
Platelet (1000/mm^3)	335.27 ± 6.34	312.16 ± 5.92 †	336.72 ± 7.99	309.65 ± 7.26 †	332.89 ± 10.50	316.29 ± 10.19 *
Plateletocrit (%)	0.36 ± 0.01	0.33 ± 0.01 †	0.35 ± 0.01	0.31 ± 0.01 †	0.37 ± 0.02	0.35 ± 0.01
MPV (fL)	10.54 ± 0.15	10.37 ± 0.16	10.34 ± 0.18	10.02 ± 0.18	10.88 ± 0.27	10.99 ± 0.26
PDW (%)	18.01 ± 0.11	18.11 ± 0.10	17.99 ± 0.14	17.98 ± 0.13	18.04 ± 0.18	18.32 ± 0.15
hs-CRP (mg/dL)	1.59 ± 0.21	1.55 ± 0.17	1.98 ± 0.33	1.61 ± 0.22	1.05 ± 0.16	1.48 ± 0.26
Postprandial lipid profile						
Total cholesterol (mg/dL)	187.78 ± 3.53	187.29 ± 3.22	187.23 ± 4.75	186.99 ± 4.29	188.65 ± 5.23	187.76 ± 4.84
TG (mg/dL)	113.09 ± 5.58	118.26 ± 4.83	116.88 ± 6.92	122.99 ± 6.56	107.17 ± 9.38	110.87 ± 6.87
HDL (mg/dL)	54.25 ± 1.21	54.48 ± 1.26	49.53 ± 1.19	49.70 ± 1.26	61.57 ± 2.09	61.89 ± 2.17
LDL (mg/dL)	110.82 ± 2.94	108.7 ± 2.64	114.08 ± 4.22	112.07 ± 3.67	105.8 ± 3.61	103.5 ± 3.53
VLDL (mg/dL)	22.62 ± 1.12	23.65 ± 0.97	23.38 ± 1.38	24.6 ± 1.31	21.44 ± 1.88	22.17 ± 1.37
Arterial pressure						
Systolic (mm Hg)	115.17 ± 0.70	112.2 ± 0.75 *	117.12 ± 0.86	114.38 ± 0.89 *	112,00 ± 1.04	108.67 ± 1.17 *
Diastolic (mm Hg)	72.63 ± 0.81	69.15 ± 0.75 *	74.66 ± 1.05	71.10 ± 0.95 *	69.33 ± 1.12	66.00 ± 1.07 *
TBARS (nmol/mL of MDA)	0.0267 ± 0.001	0.0264 ± 0.001	0.0265 ± 0.001	0.0259 ± 0.001	0.0271 ± 0.001	0.0271 ± 0.001
n (%)	125 (100)		76 (60.8)		49 (39.2)	

Data are expressed as means ± SEM. PDW indicates platelet deviation weight.

* Significant ($P < .05$) difference in the comparison of before and after values by the Wilcoxon matched-pairs test.

† Highly significant ($P < .01$) differences in the comparison of before and after values by the Wilcoxon matched-pairs test.

Table 4
Influence of pequi (*C brasiliense* Camb.) fruit pulp oil intake on the age groups (years)

	15-19		20-24		25-29	
	Before	After	Before	After	Before	After
Leukocytes						
Total leukocytes (/mm^3)	7180.00 ± 348.88	7420.00 ± 398.85	7826.09 ± 587.36	7873.91 ± 379.47	7978.26 ± 347.60	7739.13 ± 366.68
Lymphocytes (/mm^3)	2750.35 ± 207.54	2740.75 ± 292.04	2731.96 ± 208.32	2680.67 ± 202.63	2943.74 ± 223.27	2882.43 ± 180.61
Segmented (/mm^3)	3693.65 ± 222.97	3799.15 ± 258.84	4256.33 ± 454.93	4247.04 ± 298.69	4244.87 ± 260.01	4054.13 ± 288.36
Rods (/mm^3)	28.05 ± 25.51	33.47 ± 24.39	32.79 ± 15.43	23.21 ± 14.83	19.68 ± 13.48	19.41 ± 16.27
Basophils (/mm^3)	83.95 ± 12.4	81.50 ± 7.38	84.58 ± 10.86	104.21 ± 9.85	89.13 ± 9.59	107.96 ± 10.67
Eosinophils (/mm^3)	129.15 ± 21.55	173.75 ± 24.36 *	147.75 ± 19.83	174.88 ± 28.45	183.13 ± 27.85	157.87 ± 28.34
Monocytes (/mm^3)	490.75 ± 45.69	587.35 ± 45.92 *	570.29 ± 59.24	644.13 ± 33.40	500.26 ± 42.27	512.00 ± 39.14
Platelets						
Platelet (1000/mm^3)	322.80 ± 16.34	320.95 ± 16.42	316.04 ± 11.63	290.42 ± 10.81 *	350.35 ± 13.98	315.17 ± 10.30 †
Plateletocrit (%)	0.33 ± 0.02	0.33 ± 0.02	0.36 ± 0.02	0.30 ± 0.01 †	0.37 ± 0.02	0.33 ± 0.02 †
MPV (fL)	10.23 ± 0.29	10.33 ± 0.3	11.16 ± 0.43	10.11 ± 0.35 †	10.73 ± 0.41	10.49 ± 0.40
PDW (%)	17.76 ± 0.24	18.02 ± 0.22	18.58 ± 0.29	18.07 ± 0.21	17.96 ± 0.23	18.30 ± 0.26
hs-CRP (mg/dL)	1.35 ± 0.57	1.66 ± 0.59	1.97 ± 0.70	2.39 ± 0.51	1.83 ± 0.42	1.76 ± 0.36
Postprandial lipid profile						
Total cholesterol (mg/dL)	150.95 ± 6.27	158.00 ± 6.34	173.42 ± 7.00	174.75 ± 7.14	193.29 ± 6.25	195.33 ± 6.27
TG (mg/dL)	88.10 ± 12.78	98.75 ± 11.29	97.79 ± 8.53	116.67 ± 11.54 *	117.43 ± 11.63	118.39 ± 9.17
HDL (mg/dL)	45.60 ± 2.31	48.55 ± 2.30	50.00 ± 2.09	50.37 ± 2.13	56.08 ± 3.66	56.38 ± 3.69
LDL (mg/dL)	87.26 ± 4.50	88.09 ± 4.95	103.86 ± 6.36	101.04 ± 6.14	112.17 ± 7.29	113.76 ± 6.23
VLDL (mg/dL)	17.62 ± 2.56	19.75 ± 2.26	19.56 ± 1.70	23.33 ± 2.31 *	23.49 ± 2.33	23.68 ± 1.83
Arterial pressure						
Systolic (mm Hg)	114.50 ± 1.35	111.00 ± 1.43	115.83 ± 1.69	113.75 ± 2.16	116.36 ± 1.40	113.64 ± 1.55
Diastolic (mm Hg)	71.50 ± 1.50	69.00 ± 1.61	72.08 ± 1.90	69.58 ± 1.75	75.00 ± 2.05	71.82 ± 1.70
TBARS (nmol/mL of MDA)	0.0219 ± 0.001	0.0238 ± 0.002	0.0258 ± 0.001	0.0269 ± 0.001	0.0288 ± 0.001	0.0276 ± 0.001
(%)	20 (16.0)		25 (20.0)		25 (20.0)	

Data are expressed as means ± SEM.
* Significant (P <.05) difference in the comparison of before and after values by the Wilcoxon matched-pairs test.
† Highly significant (P <.01) difference in the comparison of before and after values by the Wilcoxon matched-pairs test.

3.2. Leukocytes and platelets

There was a general downward trend for lymphocytes, mature neutrophils (segmented), and platelet parameters, whereas monocyte numbers were enhanced after pequi oil supplementation; mean values of immature neutrophils (rods) were not changed (Table 3). Similar trends were observed during analyses of age groups (Table 4) and distance covered (data not shown), and there was a general tendency for monocytes to increase after pequi oil supplementation but decrease with age. For the total group, pequi oil intake resulted in a significant reduction of platelets (P = .000) and plateletocrit (P = .000) and in increased monocytes (P = .020). For men, the results were significant for increased basophils (P = .017) and decreased platelets (P = .000) and plateletocrit (P = .000). For women, they were significant for an increase in platelets (P = .025). Significant values were also observed for increased eosinophils and monocytes in the age group of 15 to 19 years (P = .048 for both) and for monocytes in the age group of 30 to 34 years (P = .015) and distance of 4 to 5 km (P = .011); for decreased platelets, they were significant for the age group of 20 to 34 years (Table 4) and distances of 6 to 7 (P = .01) and 8 to 10 km (P = .04) (data not shown). Significant decreases in the plateletocrit values were observed in the age groups of 20 to 24 (P = .003) and 25 to 29 years (P = .009). Men included in the age group older than 45 years also presented a significant decrease in the values of platelets (P = .018), and a significant increase in the monocyte number observed for the distance of 4 to 5 km was found in women from the age group of 20 to 24 years (P = .049), which also presented a significant fall in the number of platelets (P = .025). The significant platelet reduction observed for the distance of 6 to 7 km was mainly related to men from the age groups of 20 to 24 (P = .046) and 25 to 34 (P = .043) years. The t test showed significant differences between sexes for eosinophils before (.029) and after (.036) pequi oil supplementation.

3.3. Postprandial lipid profile

There was a general downward trend in the values of total cholesterol and low-density lipoprotein (LDL), whereas

30-34		35-39		40-44		≥45	
After	Before	After	Before	After	Before	After	Before
6391.67 ± 485.93	7608.33 ± 820.33	7700.00 ± 828.61	7385.71 ± 549.61	7555.56 ± 413.36	6988.89 ± 244.63	6968.75 ± 686.22	6693.75 ± 546.08
2416.58 ± 286.93	2523.58 ± 339.29	2667.64 ± 216.26	2426.29 ± 207.91	2621.00 ± 211.34	2820.44 ± 288.75	2310.75 ± 265.48	2161.31 ± 205.57
3317.00 ± 357.48	4240.83 ± 605.04	4287.36 ± 745.67	4001.29 ± 503.19	4197.33 ± 340.97	3429.56 ± 138.37	3928.19 ± 442.55	3767.94 ± 323.82
22.50 ± 10.46	29.25 ± 22.30	62.29 ± 40.89	47.14 ± 32.50	9.67 ± 9.67	0.00 ± 0.00	5.50 ± 5.50	27.63 ± 19.73
65.00 ± 15.09	101.83 ± 20.25	82.14 ± 14.61	92.29 ± 8.58	117.33 ± 15.18	126.11 ± 12.76	83.69 ± 11.82	84.94 ± 10.63
172.67 ± 40.14	146.50 ± 30.57	94.71 ± 18.69	107.36 ± 18.29	138.00 ± 24.96	151.78 ± 21.02	143.38 ± 40.28	154.31 ± 46.53
395.75 ± 62.64	572.67 ± 79.61 *	477.29 ± 51	489.93 ± 32.42	473.22 ± 53.62	458.67 ± 39.5	497.25 ± 52.32	497.63 ± 70.63
332.33 ± 15.16	304.00 ± 17.69 *	376.33 ± 22.48	337.93 ± 23.04 *	336.44 ± 22.20	309.89 ± 21.31	321.06 ± 17.21	312.69 ± 15.99
0.36 ± 0.02	0.33 ± 0.03	0.37 ± 0.03	0.34 ± 0.03	0.37 ± 0.02	0.34 ± 0.03	0.33 ± 0.02	0.33 ± 0.02
10.75 ± 0.44	10.82 ± 0.68	9.74 ± 0.34	10.01 ± 0.40	10.30 ± 0.18	10.44 ± 0.37	10.42 ± 0.34	10.61 ± 0.39
17.96 ± 0.30	18.23 ± 0.43	17.93 ± 0.34	17.93 ± 0.16	17.93 ± 0.17	18.11 ± 0.36	17.67 ± 0.29	18.08 ± 0.26
1.64 ± 0.48	0.70 ± 0.12 †	1.92 ± 0.75	1.46 ± 0.45	0.74 ± 0.26	0.79 ± 0.25	1.28 ± 0.29	1.30 ± 0.25
188.73 ± 8.48	189.00 ± 7.30	190.43 ± 6.97	185.07 ± 6.91	208.44 ± 9.75	208.44 ± 10.30	229.88 ± 8.87	217.59 ± 7.62 †
119.18 ± 24.56	125.91 ± 19.36	137.86 ± 17.59	137.71 ± 9.15	121.89 ± 15.26	112.67 ± 14.37	229.88 ± 8.87	125.29 ± 16.16
59.27 ± 2.68	59.00 ± 3.12	53.40 ± 2.19	50.33 ± 2.70	56.00 ± 2.9	56.22 ± 3.41	64.41 ± 3.15	64.35 ± 3.81
105.62 ± 8.40	104.82 ± 6.18	110.57 ± 6.63	107.39 ± 6.64	128.07 ± 8.88	129.69 ± 9.08	139.62 ± 6.28	128.18 ± 5.09 †
23.84 ± 4.91	25.18 ± 3.87	27.57 ± 3.52	27.54 ± 1.83	24.38 ± 3.05	22.53 ± 2.87	25.85 ± 3.57	25.06 ± 3.23
112.73 ± 2.37	114.55 ± 2.47	113.33 ± 2.11	109.33 ± 2.28	116.00 ± 3.40	112.00 ± 2.49	116.25 ± 1.80	110.63 ± 1.70 *
70.00 ± 3.30	67.27 ± 2.73	74.67 ± 2.36	68.00 ± 2.23	72.00 ± 2.91	70.00 ± 2.11	71.88 ± 1.88	66.88 ± 2.18
0.0284 ± 0.003	0.0261 ± 0.002	0.0273 ± 0.002	0.0254 ± 0.001	0.0263 ± 0.002	0.0263 ± 0.002	0.0292 ± 0.002	0.028 ± 0.002
		16 (12.8)		10 (8.0)		17 (13.6)	

high-density lipoprotein (HDL) increased after pequi oil treatment for the total and sex groups (Table 3). Moreover, there was a general trend for total cholesterol and LDL to go down with aging, particularly from 30 to 34 years. Apart from the age group of 40 to 44 years, a similar trend occurred for LDL where the values fell from 30 to 34 years (Table 4). Total cholesterol and LDL also presented a downward trend as the distance covered increased (data not shown). Significant results were observed for TGs and very low density lipoprotein (VLDL), which increased in the age group of 20 to 24 years ($P = .030$ and $P = .029$, respectively), and for a fall in total cholesterol and LDL in the age group older than 45 years ($P = .003$ and $P = .006$, respectively). For the latter, results were particularly significant for men ($P = .012$ for total cholesterol, $P = .036$ for LDL). The t test showed significant differences between sexes for LDL before ($P = .021$) and HDL after ($P = .045$) pequi oil supplementation.

3.4. Arterial pressure and hs-CRP

After pequi oil treatment, there was a significant decrease in the values for systolic and diastolic blood pressures for the total ($P = .001$ for both pressures), men ($P = .0012$ for both pressures), and women ($P = .036$ and $P = .043$, respectively) groups. No difference in the hs-CRP values was observed for the total group. Men presented a decrease of 18.6% in mean values of hs-CRP as well as 33.3% in SE values (Table 3). Significant values were found for systolic ($P = .012$) and diastolic pressure ($P = .011$) decreases in the age group older than 45 years, and this fall was particularly related to men ($P = .018$ for systolic and $P = .005$ for diastolic pressures). For hs-CRP, significant differences appeared in the age group of 30 to 34 years ($P = .005$), whose values decreased; for men, it was also observed in the age group of 25 to 34 years ($P = .004$) (Table 4). No significant difference was observed for the analysis of the distance covered (data not shown). The t test showed significant differences between sexes for hs-CRP (.002) and diastolic pressure ($P = .024$) before pequi oil treatment.

3.5. TBARs assay

After pequi oil supplementation, no significant changes in MDA values were observed for the total group (men). No effect was observed in women (Table 3). There was a

decrease in MDA values in almost all age groups and distances, except for the age groups of 15 to 19 and 20 to 24 years (Table 4) and 4 to 5 km (data not shown). The TBARs assay revealed no significant differences.

3.6. Preexisting cardiovascular diseases (slight hypertension and treated dyslipidemia)

In all analyses of this group (n = 9), there were no significant quantitative changes after pequi oil intake (data not showed).

3.7. Adverse effects

No subjects withdrew from the study because of discomfort or adverse effects associated with the treatment. Eleven subjects experienced heavy drowsiness, whereas 4 reported insomnia; 6 volunteers had a mild intestinal disruption, 2 reported intestinal constipation, 3 subjects reported increased flatulence, 1 man complained of heartburn, 2 women reported increased acne, and 1 woman described the appearance of painful subcutaneous nodules in the arms. All symptoms were noticed within the first 3 to 4 days of treatment, disappearing soon afterward.

4. Discussion

Regular aerobic exercise expands the baseline plasma volume, and there is a high variability of plasma volume changes between individuals and within an individual due to exercise performance [29]. In this study, to avoid plasma volume changes as a function of variability among individuals, each athlete participated as control group and treatment group, being compared in the statistical tests with him- or herself. The athletes ran the same distance in both races in the same time and under the same environmental conditions. They also chose the distance they would cover, according to the type, intensity, and length of their weekly training; there was no change in the daily routine, training, or lifestyle of all runners between the first race and second race, except for ingestion of pequi oil capsules. Most physiologic and biochemical effects of exercise have already been well studied [1-10,20,29-34], and we did not aim to evaluate such effects. We investigated the anti-inflammatory effects of *C brasiliense* fruit pulp oil and the effects on postprandial lipemia and arterial pressure of runners after races run under the above conditions (before and after taking the pequi oil supplements). Thus, the results obtained in this study were caused by the pequi oil intervention instead of other factors.

Acute response to exercise provokes temporary changes in the immune cell count as a response to adrenalin and cortisol secretion. In the immediate postexercise period, an increment of 50% to 100% of total leukocytes occurs, mainly because of neutrophilia and lymphopenia, as well as monocytosis, which also occurs at a lower rate. After a period of 30 minutes of recovery, an accentuated fall in the lymphocyte number (30%-50%) and persistence of the neutrophilia is observed [34]. In view of the fact that evaluations of the present study were accomplished immediately after exercise and there was a fall in the number of mature (segmented) neutrophils and lymphocytes after supplementation, we can suggest that pequi oil was biologically efficient in reducing exercise-induced inflammation. This suggestion is supported by results of platelet parameters and hs-CRP. Although significant results for monocytes increased after supplementation, this was mainly related to the age group of 30 to 34 years, which also presented a higher reduction in the values of hs-CRP, an acute-phase reactant and a sign of inflammation [35], implying that inflammation fell. This age group also presented a higher decrease in the values of TBARS assay, a technique used to evaluate lipid peroxidation in blood or serum [7,8], suggesting that these protective results were probably due to the antioxidant activity of pequi oil.

Evidence has emerged indicating interactions between neutrophils and platelets and suggesting an ability of platelets to enhance neutrophil-induced endothelial dysfunction [36]. Because after pequi oil treatment there was a significant fall in the platelet numbers and mature neutrophils decrease, these can also indicate an improvement in exercise-induced endothelial dysfunction. Moreover, increased mean platelet volume (MPV) is an indicator of platelet activation [37,38]; and after supplementation, there was a significant reduction in this parameter for men, indicating that the anti-inflammatory activity of the pequi oil was higher for men. In addition, although the general tendency was for monocytes to increase after pequi oil supplementation, this increase decreased with age. At the same time, there was a general tendency for platelet numbers to fall in all age groups, with the latter reduction especially associated with the age group older than 45 years.

Although this study was limited by the lack of a placebo group, the anti-inflammatory effects of *C coriaceum* have been demonstrated in the literature; and its effects are related to the fixed oils of the fruit pulp, whose components are similar to that of *C brasiliense* [22-24]. Because pequi (*C brasiliense*) oil reduced exercise-induced DNA and tissue damages in a previous study [21], our results are also in accordance with anti-inflammatory effects obtained for *C coriaceum*. Both studies suggest that these effects can be related to both fixed oils and carotenoid compositions of pequi fruit pulp oil, which cause us to accept the hypothesis that pequi oil can efficiently reduce exercise-induced inflammation.

Generally, fats rich in MUFA such as oleic acid have been found to cause pronounced postprandial lipemia, with a tendency to have large TRL particles [39]; fats rich in long-chain saturated fatty acids (SFA) such as palmitic acid have been shown to exacerbate postprandial lipemic responses [20]. In the postprandial state, high concentrations of TRL represent a potentially rich source of fatty acids for contracting muscles and may be important in replenishing muscle TG after exercise [40]. Given that oleic and palmitic acids are the major components of pequi fruit pulp oil, an enhanced postprandial lipemia could be expected after treatment as well as a higher total cholesterol and LDL

reduction as distance increases. However, because of its fatty acid composition, there is evidence to suggest that consumption of pequi oil can influence lipid metabolism [20] and that this influence depends on the age and the sex of the consumer. In the present study, there was a general tendency for total cholesterol and LDL to decrease with age, mainly for men, after consumption of pequi oil, supporting our hypothesis that pequi oil can modulate postprandial lipemia.

The effects of diet on blood lipids are known best in men. Several studies, but not all, noted that blood lipids in women may be less responsive to diet than are blood lipids in men [41]. However, most studies included only small numbers of women; and statistical comparisons of lipid responses between men and women were not always made [41]. Our study did not corroborate those results because in the comparisons between sexes (t test), women present a higher HDL increase (0.52%) than men (0.34%) after pequi oil supplementation. In addition, there was a significant difference between sexes for decreased LDL only before pequi oil supplementation; and this difference disappeared after the treatment, although the significant fall in the values of LDL for the total group and age group older than 45 years was also particularly related to the men. These results suggest that pequi oil can diminish the differences in the LDL responses between men and women, besides presenting a good antioxidant activity. Thus, it can affect the atherogenicity of these particles, at least for men, considered an independent risk factor for cardiovascular disease [42].

Epidemiologic studies of dietary fats showed no clear relation between blood pressure and the total amount of fat consumed [25,43]. A positive association between the estimated amount of dietary saturated fat and blood pressure was reported in some epidemiologic studies, but not in others [25]. However, an association between dietary saturated fat reduction and lower blood pressure has already been demonstrated in the literature [25,42]. Although a few epidemiologic studies with small sample populations suggested that an increased intake of MUFA is inversely related to blood pressure, other studies with larger sample populations were unable to find this correlation [25]. However, there was some evidence that supports the suggestion that a diet with a high polyunsaturated fatty acids (PUFA)/SFA ratio exerts a hypotensive effect. Although the presumed influence of dietary fat on blood pressure is small, it is of an order to have a considerable impact on public health [43]. In this study, the general tendency of arterial pressure (systolic and diastolic) to decrease after pequi oil supplementation supports the previous suggestion that a higher intake of MUFA is inversely related to blood pressure and that not only a diet with a high PUFA/SFA ratio, but also a diet with high PUFA-MUFA/SFA ratio, can exert a hypotensive effect. Although these results support our hypothesis that pequi oil can reduce blood pressure, a future study involving systematic measurement of arterial pressure can help to elucidate this question further.

In conclusion, we accept our initial hypothesis that *C brasiliense* fruit pulp oil presents anti-inflammatory effects similar to those demonstrated for *C coriaceum*, besides reducing significantly the total cholesterol and LDL in the age group older than 45 years, mainly for men. The present data also suggest that pequi oil can have a possible hypotensive effect. However, this effect needs further investigations. Thus, pequi oil, as well as possessing other nutritional properties, is a good candidate as a supplement for athletes.

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