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Applied nutritional investigation

Brazilian nut consumption by healthy volunteers improves inflammatory parameters

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ABSTRACT

Objective: The aim of this study was to investigate the effect of a single dose of Brazil nuts on the inflammatory markers of healthy individuals.
Method: A randomized crossover study was conducted with 10 healthy individuals (mean age 24.7 ± 3.4 y). Each individual was tested four times regarding intake of different portions of Brazil nuts: 0, 5, 20 and 50 g. At each testing period, peripheral blood was collected before and at 1, 3, 6, 9, 24, and 48 h after intake of nuts, as well as at 5 and 30 d after intake of various Brazil nut portions. Blood samples were tested for high-sensitivity to C-reactive protein, interleukin (IL)-1, IL-6, IL-10, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ, aspartate and alanine aminotransferases, albumin, total protein, alkaline phosphatase, gamma-glutamyltransferase, urea, and creatinine.
Results: Consumption of nuts did not affect biochemical parameters for liver and kidney function, indicating absence of hepatic and renal toxicity. A single intake of Brazil nuts (20 or 50 g) caused a significant decrease in serum IL-1, IL-6, TNF-α, and IFN-γ levels ($P < 0.05$), whereas serum levels of IL-10 were significantly increased ($P < 0.05$).
Conclusion: The results indicate a long-term decrease in inflammatory markers after a single intake of large portions of Brazil nuts in healthy volunteers. Therefore, the long-term effect of regular Brazil nut consumption on inflammatory markers should be better investigated.

Introduction

Nuts are recommended as an important constituent of a healthy diet [1]. They are energy-dense and provide 23.4 to 26.8 kJ/g of food with a high-fat content (45%–75% of weight), but mostly unsaturated fat [2]. For instance, Brazil nuts are a good source of unsaturated fatty acids and selenium [3,4]. The concentration of selenium in Brazil nuts varies from 8 to 83 μg/g and is among the highest found within foods consumed by humans [5,6].

Selenium is an essential nutrient for human health [7] and its biological functions are mediated by the expression of about 20 selenoproteins, which have selenocysteine at their active sites [8,9]. Some selenoproteins, e.g., the glutathione peroxidase (GPx) and thioredoxin reductase isoforms, are important enzymes involved in the metabolism of reactive oxygen species [10]. However, the physiological role played by various other selenoproteins remains poorly understood.

In humans, selenium is implicated as a modulator of the immune function [11]. Accordingly, selenium supplementation has been reported to increase lymphocyte proliferation in response to mitogens [11,12], and the expression of high-affinity interleukin (IL) -2 receptor [11,13]. Selenium can also improve

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Table 1
Anthropometric measurements and biochemical characteristics of volunteers

Parameters	Before start of experiment		After end of experiment	
	Males (n = 6)	Females (n = 4)	Males (n = 6)	Females (n = 4)
Age (y)	27.6 ± 3.1	29.0 ± 4.4	28.0 ± 2.9	29.2 ± 4.8
Body weight (kg)	86.6 ± 12.5	59.5 ± 6.5	86.9 ± 12.5	59.2 ± 6.4
BMI (kg/m^2)	26.7 ± 3.5	23.4 ± 1.6	26.8 ± 3.7	23.1 ± 1.4
Leukocytes (10^3/mm^3)	7.3 ± 0.9	7.3 ± 2.2	7.4 ± 1.9	7.6 ± 1.5
Erythrocytes (10^3/mm^3)	5.4 ± 0.3	4.6 ± 0.2	5.4 ± 0.3	4.6 ± 0.4
Hemoglobin (g/dL)	14.5 ± 0.6	12.9 ± 1	15.3 ± 0.5	13.2 ± 1.1
Hematocrit (%)	44.6 ± 1.7	39.6 ± 3.3	43.7 ± 1.4	38.4 ± 3.6
Platelet (10^3/mm^3)	250.5 ± 88	279.5 ± 50	260.5 ± 65.5	266.2 ± 10.2
Glucose (mg/dL)	82.4 ± 7.8	82.9 ± 7.4	89.0 ± 9.3	86.9 ± 6.7
AST (UL)	18.6 ± 6.1	19.7 ± 6.6	19.4 ± 5.5	19.2 ± 6.4
ALT (UL)	20.5 ± 8.6	23.2 ± 8.6	21.3 ± 8.7	23.3 ± 9.4
Gamma GT (UL)	9.40 ± 5	10.50 ± 4.6	10.30 ± 4.7	10.40 ± 5
Phosphatase alkaline (UL)	38.2 ± 12.4	43.9 ± 10.2	38.2 ± 11.5	43.7 ± 7.8
Total protein (g/dL)	6.5 ± 0.6	6.8 ± 0.3	6.6 ± 0.6	6.8 ± 0.32
Albumin (g/dL)	4.7 ± 0.4	4.8 ± 0.4	4.7 ±0.4	4.7 ± 0.32
Urea (mg/dL)	20.0 ± 7.5	20.2 ± 7.3	19.7 ± 6.3	21.4 ± 7.2
Creatinine (mg/dL)	0.75 ± 0.1	0.80 ± 0.1	0.78 ± 0.13	0.77 ± 0.13
C-reactive protein (mg/dL)	0.50 ± 0.02	0.52 ± 0.04	0.48 ± 0.08	0.52 ± 0.04

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; gamma GT, gamma-glutamyltransferase
Data are expressed as means ± SD. Test paired (Wilcoxon) in relation to gender

lymphocyte-mediated tumor cytotoxicity and killer cells activity [14], and may affect the immune system by regulating peroxide levels in the immune cell microenvironment.

Compared with other nuts, Brazil nuts are highly rich in unsaturated fatty acids and they have been associated with improved lipid profile and reduction in blood pressure, insulin resistance, and systemic levels of inflammatory markers [1,15]. Fatty acids from nuts are important contributors to the beneficial health effects, which protect from the development of coronary heart disease [2]. It was previously reported [16] that high monounsaturated fatty acid (MUFA) diets are associated with reduced cardiovascular disease–associated mortality. Recently, we observed that acute ingestion of Brazil nuts was non-toxic and modulated favorably the lipid profile of healthy volunteers, increasing their serum levels of high-density lipoprotein cholesterol and lowering low-density lipoprotein cholesterol [6].

However, blood levels of inflammatory mediators in response to acute consumption of Brazil nuts in humans, has yet to be investigated. Here, we hypothesized that consumption of Brazil nuts would be associated with lower levels of inflammatory markers. Thus, the objective of the present study was to investigate the effect of a single dose of Brazil nuts on inflammatory markers in healthy volunteers.

Table 2
Fatty acids composition of the Brazil nut

Fatty acid	g/100 g
C14:0	0.07 ± 0.03
C16:0	16.74 ± 1.06
C16:1	0.43 ± 0.09
C17:0	0.14 ± 0.15
C18:0	9.97 ± 1.42
C18:1n9	28.52 ± 1.99
C18:2n6	36.04 ± 2.26
C20:0	0.17 ± 0.05
C20:1n9	0.09 ± 0.02
C18:3n3	0.11 ± 0.03

Results are expressed as mean ± SD of three determinations

Table 3
Phenolics and flavonoids composition of Brazil nuts (*Bertholletia excels*) aqueous extracts

Compounds	Aqueous extract (mg/g)
Gallic acid	0.35 ± 0.1
Catechin	0.92 ± 0.2
Resveratrol	1.37 ± 0.1
Ellagic acid	1.74 ± 0.2
Epicatechin	0.84 ± 0.5
Rutin	0.60 ± 0.1
Quercitrin	0.64 ± 0.4
Quercetin	1.31 ± 0.1
Kaempferol	0.67 ± 0.3

Results are expressed as mean ± S.D. of three determinations

Materials and methods

Study participants

The study was a randomized crossover trial with 10 adult volunteers, ages 23 to 34 y. The mean age of the participants was 24.7 ± 3.4 y and 60% were men. Each participant was tested four times after the administration of the different amounts of Brazil nuts: 0, 5, 20, and 50 g. Two Latin squares of 4 × 4 for the four tests were used to randomize participants into four orders of treatment. Before each treatment, the volunteers underwent a 30-d washout period, to ensure that the active compounds or ingredients in the nuts were completely eliminated [6]. We recommended a balanced diet with daily energy containing about 25 kcal/kg. Furthermore, we requested volunteers avoid the ingestion of foods containing high quantities of selenium (eggs, egg yolks, garlic, Brazil nuts, whole wheat cereal, viscera, shellfish, and fish). We applied 24-h dietary recall and food frequency questionnaires after the last blood sampling to verify the types of foods consumed during the study period [6]. The volunteers always reported not ingesting the restricted foods.

This study has been reviewed and approved by the Universidade Federal de Santa Maria's Internal Review Board (No. 0240.0.243.000-11) and informed consent was obtained from all participants.

Body weight was measured to the nearest 0.01 kg using a digital scale, and height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. The body mass index was calculated and the participants were classified according to World Health Organization guidelines [17].

According to the U.S. Department of Agriculture, Brazil nuts contain (per 100 g) 14.5 g of proteins, 15.1 g of carbohydrates, 63.7 g of total fats (15.3 g saturated fatty acids, 27.4 g MUFAs, 21 g polyunsaturated fatty acids [PUFAs]), as well as 7.9 g of dietary fibers, for a total of 2.690 kJ [18]. Corroborating earlier studies, Brazil nuts given to the participants contained 31.25 ± 18.7 μg/g of selenium [6].

Fatty acid determination Brazil nuts

The extraction of Brazil nut lipids was performed according to a previously described method [19], grinding a known amount of Brazil nuts in the presence

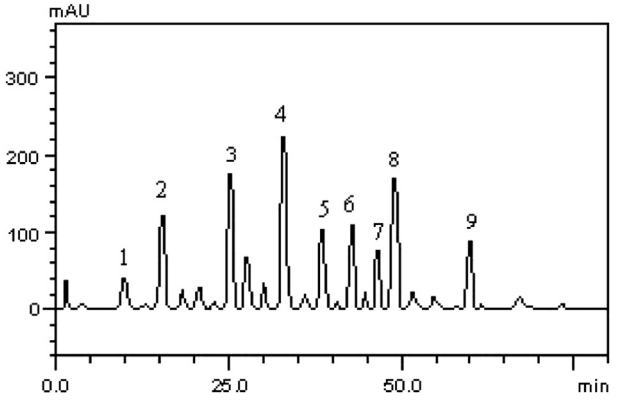

Fig. 1. Representative high performance liquid chromatography profile of *Bertholletia excelsa* aqueous extract. Gallic acid (peak 1), catechin (peak 2), resveratrol (peak 3), ellagic acid (peak 4), epicatechin (peak 5), rutin (peak 6), quercitrin (peak 7), quercetin (peak 8), and kaempferol (peak 9).