

# Brazil nut ingestion increased plasma selenium but had minimal effects on lipids, apolipoproteins, and high-density lipoprotein function in human subjects

Célia C. Strunz<sup>a</sup>, Tatiane V. Oliveira<sup>a</sup>, Juliana C.M. Vinagre<sup>a</sup>, Adriana Lima<sup>b</sup>,  
Sílvia Cozzolino<sup>b</sup>, Raul C. Maranhão<sup>a,b,\*</sup>

<sup>a</sup>Lipid Metabolism Laboratory, the Heart Institute (InCor) of the Medical School Hospital, São Paulo, Brazil

<sup>b</sup>Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil

Received 1 August 2007; revised 21 December 2007; accepted 16 January 2008

## Abstract

The Brazil nut (*Bertholletia excelsa*) of the Amazon region is consumed worldwide. It is rich in both monounsaturated fatty acids and polyunsaturated fatty acids and is known for its high selenium content. This study tested the hypothesis whether the consumption of this nut could affect the plasma lipids and apolipoproteins and some functional properties of the antiatherogenic high-density lipoprotein (HDL). Fifteen normolipidemic subjects aged  $27.3 \pm 3.9$  years and with body mass index of  $23.8 \pm 2.8$  kg/m<sup>2</sup> consumed 45 g of Brazil nuts per day during a 15-day period. On days 0 and 15, blood was collected for biochemical analysis, determination of HDL particle size, paraoxonase 1 activity, and lipid transfer from a lipoprotein-like nanoparticle to the HDL fraction. Brazil nut ingestion did not alter HDL, low-density lipoprotein cholesterol, triacylglycerols, apolipoprotein A-I, or apolipoprotein B concentrations. HDL particle diameter and the activity of antioxidative paraoxonase 1, mostly found in the HDL fraction, were also unaffected. Supplementation increased the reception of cholesteryl esters ( $P < .05$ ) by the HDL yet did not alter the reception of phospholipids, free cholesterol, or triacylglycerols. As expected, plasma selenium was significantly increased. However, the consumption of Brazil nuts for short duration by normolipidemic subjects in comparable amounts to those tested for other nuts did not alter serum lipid profile. The only alteration in HDL function was the increase in cholesteryl ester transfer. This latter finding may be beneficial because it would improve the nonatherogenic reverse cholesterol transport pathway.

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## Keywords:

Humans; Cholesterol; Dietary selenium; Lipoproteins; Nanoparticles; Emulsions

## Abbreviations:

Apo A-I, apolipoprotein A-I; Apo B, apolipoprotein B; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MUFA, monounsaturated fatty acids; PON1, paraoxonase 1; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

## 1. Introduction

Epidemiological studies indicate that frequent consumption of nuts may decrease the risk of coronary artery disease [1]. A lipid-lowering effect has been documented in studies with almonds [2], walnuts [3], pecans [4], pistachios [5], and peanuts [6] consumed in daily amounts ranging from 45 to 100 g/d.

\* Corresponding author. Laboratório de Metabolismo de Lípidos Instituto do Coração do HC-FMUSP, São Paulo, Brazil, Tel.: +55 11 30695951; fax: +55 11 3095574.

E-mail address: [ramarans@usp.br](mailto:ramarans@usp.br) (R.C. Maranhão).

Brazil nut (*Bertholletia excelsa*) found in the Amazon region in South America is consumed worldwide. As a distinct characteristic, this fruit has a high-selenium content [7]. Selenium is found in the active site of many enzymes such as thioredoxin reductase, which catalyzes oxidation/reduction reactions and is essential for the synthesis of glutathione peroxidase that prevents oxidative processes [8]. Diminished plasma selenium levels have been associated with increased risk of coronary artery disease development [9].

With regard to fat composition, the Brazil nut is rich in monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) that have the potential of reducing proatherogenic low-density lipoprotein (LDL) cholesterol [10]. However, when compared with other nuts such as almonds, walnuts, pecans, pistachios, and peanuts, the Brazil nut has a relatively higher content of LDL cholesterol-raising saturated fatty acids (SFA) [11]. Brazil nuts are also rich in magnesium and sulfur amino acids.

To the best of our knowledge, the effects of Brazil nut consumption on plasma lipids have not yet been explored. Because of the nutritional composition of this nut, the health benefits of this food are of special interest. Few investigations have evaluated the effects of selenium on plasma lipids with the interaction of PUFA, MUFA, and SFA in the Brazil nut. Therefore, this study was designed to evaluate the effects of consumption of this nut at a level similar to other edible nuts reported [11] so to make an important comparison. Some functional properties of high-density lipoprotein (HDL), HDL particle size, and the antioxidant enzyme paraoxonase 1 (PON1) that is associated with the HDL fraction were also tested. These measurements are valuable in understanding the HDL antiatherogenic properties that cannot be evaluated by simply measuring HDL cholesterol concentration.

## 2. Methods and materials

### 2.1. Subjects

Fifteen healthy volunteers (5 males and 10 females) participated in the study. All were personnel of our laboratory, aged  $27.3 \pm 3.9$  (23–34) years, and with body mass index  $23.8 \pm 2.8$  (19.8–28.4) kg/m<sup>2</sup>. Inclusion criteria were plasma total cholesterol concentration of less than 200 mg/dL, LDL cholesterol of less than 100 mg/dL, and triacylglycerols of less than 200 mg/dL. None were taking vitamin supplements, hypolipidemic agents, or any other type of medication. The study was approved by the ethics committee of the Heart Institute of the Medical School Hospital (São Paulo, Brazil), and an informed written consent was obtained from each participant.

### 2.2. Experimental design

Before the commencement of the study, the usual food intake of each participant was assessed by a dietary inquiry.

Participants had not ingested Brazil nuts for at least the previous 2 months.

All participants were simultaneously included in the study protocol that consisted in the consumption of 45 g/d of Brazil nuts, roughly 11 nuts, for a 15-day period. The nuts were consumed as snacks or with meals in salads. On the last day of ingestion, the dietary inquiry was once again applied to verify whether or not nut consumption had altered the composition of the diet. Blood samples were drawn after a 12-hour fast on the first and last day of the study for the biochemical determinations described below.

As the nut consumption period was relatively short, it was not necessary to design a control group without supplementation of Brazil nuts. The specified supplement of 45g Brazil nuts used for the study contains 6.44 g of protein, 5.52 g of carbohydrates, and 29.89 g of total fat (6.81 g of SFA, 11.06 g of MUFA, 9.26 g of PUFA), as well as 3.38 g of dietary fiber and 862.65  $\mu$ g of selenium, for a total of 1235 kJ [12].

### 2.3. Plasma lipid and apolipoprotein determinations

Plasma total cholesterol (CHOD-PAP; Roche, Basel, CHE) and triacylglycerols (Triacylglycerol Rapid, Roche) were determined by enzymatic methods using a Cobas Mira analyzer (Roche). High-density lipoprotein cholesterol was measured after precipitation of the very low-density lipoproteins and LDL with HDL reagent (Roche; phosphotungsten acid/MgCl<sub>2</sub> method) with automatic equipment. Low-density lipoprotein C was estimated by the Friedewald formula [13]. Apolipoproteins A-I and B were determined by immunoturbidimetric assay (Roche) in a Cobas MIRA analyzer.

### 2.4. High-density lipoprotein particle size

The mean particle diameter of the HDL fraction was measured by laser-light scattering analysis with a ZetaPALS Zeta Potential Analyzer (Brookhaven Instruments, Holtsville, NY) after the separation of HDL from the plasma by chemical precipitation of the apo B-containing lipoprotein as described previously [14].

### 2.5. Preparation of the labeled nanoemulsion for the lipid transfer assay

The lipid nanoemulsion was prepared from a lipid mixture of 40 mg cholesteryl oleate, 20 mg egg phosphatidylcholine, 1 mg triolein, and 0.5 mg cholesterol purchased from the Sigma Chemical Company (St Louis, Mo). Lipid emulsification by prolonged ultrasonic irradiation in aqueous media and a 2-step ultracentrifugation of the crude emulsion with density adjustment by addition of KBr to obtain the nanoemulsion was carried out by the method previously described [15] and modified by Maranhão et al [16]. The nanoemulsion was dialyzed against a 0.9% NaCl solution. Trace amounts of cholesteryl [<sup>1-14</sup>C] oleate and glycerol tri

Table 1

Estimate by dietary inquiry approach of the daily composition of the diet immediately before and on the last day of Brazil nut consumption (45 g/d)

Nutrients	Before Brazil nut consumption	Last day of Brazil nut consumption	% Variation
Total lipid (fat) (g)	61.1 ± 22.4	89.2 ± 19.8 *	+46.0
SFA	18.9 ± 8.1	24.7 ± 7.7 *	+30.8
MUFA	22.0 ± 10.8	31.8 ± 9.7 **	+44.9
PUFA	6.6 ± 4.2	16.0 ± 4.3 **	+146.6
Cholesterol (mg)	210.6 ± 93.7	218.3 ± 97.6	+3.7
Protein (g)	79.9 ± 28.0	84.1 ± 28.0	+5.1
Carbohydrates (g)	159.2 ± 77.7	154.4 ± 57.1	–
Dietary fiber (g)	9.9 ± 7.3	12.2 ± 6.0	+23.6
Selenium (μg)	94.4 ± 23.1	955.1 ± 21.2 **	+912.1
Energy intake (kJ)	5997.3 ± 1438.0	7076.0 ± 1171.0 *	+18.0

Results are expressed as means ± SD (n = 15; 10 women and 5 men).

\*  $P < .05$  compared to baseline diet (paired  $t$  test).

\*\*  $P < .01$  compared to baseline diet (paired  $t$  test).

[9,10( $n$ )- $^3$ H] oleate or [7( $n$ )- $^3$ H] cholesterol and L-3-phosphatidylcholine, 1-stearoyl-2-[1- $^{14}$ C]arachidonoyl (Amersham, Little Chalfont, Buckinghamshire, UK) were added to the initial solution.

## 2.6. Assay for the lipid transfer from the donor nanoemulsion to HDL

Plasma with EDTA in a volume of 200 μL was incubated with 50 μL of the nanoemulsion labeled with  $^{14}$ C-cholesterol and  $^3$ H-triacylglycerols or with  $^3$ H-cholesteryl esters and  $^{14}$ C-phospholipids. After a 1-hour shaking bath at 37°C, 250 μL dextran sulfate/MgCl<sub>2</sub> was added as a precipitation reagent. The mixture was then mixed for 30 seconds and centrifuged for 10 minutes (3000g). Finally, 250 μL of the supernatant was added to counting vials containing 5 mL scintillation solution (Packard BioScience, Groningen, The Netherlands) [17]. Radioactivity was measured with a liquid scintillation analyzer (1600 TR model, Packard, Palo Alto, CA). The blank samples consisted of 200 μL Tris solution with added labeled nanoemulsion and the precipitation reagent after incubation, as described above. The results of the radioactive transfer from the lipid nanoemulsion to the HDL fraction were expressed as a percentage of the total incubated radioactivity, determined in a plasma sample containing no precipitation reagents.

## 2.7. Paraoxonase 1 activity

Paraoxonase 1 activity was measured by adding serum to 1 mL Tris-HCl buffer (100 mmol/L, pH 8.0) containing 2 mmol/L CaCl<sub>2</sub> and 5.5 mmol/L paraoxon (Sigma Chemical Company, London, UK). The generation of  $p$ -nitrophenol was measured at 405 nm, at 37°C in a Bio-Rad Benchmark Microplate Reader (Nippon Bio-Rad, Tokyo) [18].

## 2.8. Selenium analysis

The selenium concentration in the plasma was determined through atomic absorption spectrometry, using a HITACHI model Z-5000 spectrometer (Hitachi High-Technologies

Corp., Tokyo) with generation of hydrides coupled with the cell of quartz (HGQTAAS), as described previously [19].

## 2.9. Statistical analysis

Results are expressed as means and standard deviations (mean ± SD). Differences of  $P < .05$  were considered significant. All variables were compared using paired Student  $t$  test. Sigma Stat 3.11 for Windows software (Systat Software Inc, Calif) was used in the statistical calculations.

## 3. Results and discussion

Table 1 shows that, according to the dietary inquiry, energy intake increased by about 18% after the introduction of the Brazil nut supplementation. This was due to the increase in the ingestion of MUFA, PUFA, and SFA contained in the nuts.

Table 2 shows that the participant body weight did not change at the end of the supplementation period and the concentration of selenium in the serum increased 4-fold. Table 2 also shows that the nut supplementation did not alter HDL or LDL cholesterol and triacylglycerol concentration. Apolipoprotein A-I concentration, which together with HDL cholesterol is a marker for the HDL levels in the plasma, also remained unchanged. There was, however, a trend for increased apo B but did not attain statistical significance ( $P = .07$ ).

As shown in Table 3, neither the HDL average particle diameter nor the activity of the antioxidative enzyme PON 1 was affected by the nut supplementation. The ability of HDL to simultaneously receive (normal lipoprotein metabolism for lipid transfer enzymes) the 4 major lipid classes from the donor nanoemulsion is also shown in Table 3. Nut supplementation resulted in increased reception by HDL of cholesteryl esters ( $P < .05$ ) but did not alter the reception of phospholipids, free cholesterol, or triacylglycerols (Table 3).

During the study, the participants did not complain of symptoms associated with selenosis, such as gastrointestinal disturbances, rashes, fatigue, irritability, and nervous system alterations.

Table 2

Body weight and serum measurements of the 15 study subjects immediately before and on the last day of the 15-day period of Brazil nut consumption

Parameters	Before Brazil nut consumption	Last day of Brazil nut consumption
Body weight (kg)	67.4 ± 11.5	66.3 ± 11.3
Selenium (μg/L)	56 ± 9	208 ± 55 **
Cholesterol (mg/dL)		
Total	166 ± 39	170 ± 36
LDL	84 ± 32	88 ± 30
HDL	62 ± 17	63 ± 17
Triacylglycerols (mg/dL)	102 ± 62	96 ± 66
Apo A-I (g/L)	1.72 ± 0.4	1.73 ± 0.3
Apo B (g/L)	0.70 ± 0.2	0.74 ± 0.2 *

Results are expressed as means ± SD (n = 15; 10 women and 5 men).

\*  $P = .07$ .

\*\*  $P < .0001$  compared to baseline (paired  $t$  test).

Table 3

High-density lipoprotein particle size, PON1 activity, and ability of HDL to receive lipids from the artificial nanoemulsion in normolipidemic subjects immediately before and on the last day of the 15-day period of Brazil nut consumption

Parameters	Before Brazil nut consumption	Last day of Brazil nut consumption
HDL diameter (nm)	9 ± 0.8	9 ± 0.6
PON 1 (nmol mL <sup>-1</sup> min <sup>-1</sup> )	73 ± 38	74 ± 39
Lipid transfer (%)		
Triacylglycerols	4.0 ± 1.1	4.7 ± 2.5
Cholesterol	8.6 ± 2.1	8.7 ± 3.0
Cholesteryl esters	4.0 ± 1.0	4.7 ± 1.0*
Phospholipids	26.1 ± 2.0	27.7 ± 3.5

Results are expressed as means ± SD (n = 15; 10 women and 5 men).

\*  $P < .05$ , paired  $t$  test.

High MUFA and PUFA content in foodstuffs is potentially beneficial in terms of fat composition compared to the SFA [20]. Daily ingestion of equivalent amounts of other nuts, such as almonds [2], walnuts [3], pecans [4], pistachios [5], and peanuts [6], has led to the improvement of the plasma lipid profile, with diminished atherogenic LDL cholesterol. The increase in HDL cholesterol, which is a protective factor, occurred less frequently under the ingestion of those nuts [2-6,11]. In this study, the lack of effectiveness of Brazil nut feeding in reducing LDL cholesterol might be attributed to the relatively greater SFA content of this nut compared with other nuts [20].

As expected, Brazil nut consumption pronouncedly increased the plasma selenium concentration. Some studies have found a positive correlation between plasma selenium and HDL cholesterol [21,22]. The plasma levels of selenium attained in our study,  $208 \pm 55 \mu\text{g/L}$ , are above the recommended concentrations for this micronutrient, ranging from 53 to 161  $\mu\text{g/L}$  [23]. However, in our study, the high levels attained were not harmful to the participants because of the short, 15-day supplementation period and the fact that in Brazil nut selenium is mainly found in the less toxic selenomethionine form [24]. Selenium intoxication was described after consumption excesses that lead to a higher than 1000  $\mu\text{g/L}$  concentration in the plasma for prolonged, long-term periods in areas of endemic selenosis [24].

In this study, in addition to the determination of HDL cholesterol and apo A-I, some fundamental properties of HDL were also evaluated. Because of the many antiatherogenic functions of the lipoprotein, the importance of functional approaches has been highlighted. The ability to receive lipids, mediated by transfer proteins such as cholesteryl ester transfer protein and phospholipid transfer protein, is a fundamental property of HDL because the metabolism and function of this lipoprotein in the reverse cholesterol transportation depends heavily on lipid exchanges [25,26].

The single alteration in the lipid transfers to HDL elicited by nut consumption, that is, the increased influx of cholesteryl esters into the HDL fraction may be interpreted

as beneficial. The transfer of cholesterol into the HDL pool can be considered an antiatherogenic mechanism because it would facilitate cholesterol elimination into bile while avoiding arterial deposition that may occur with cholesterol transported in apo B-containing lipoproteins [27].

The lack of effect of nut consumption on the size of the HDL fraction suggests that Brazil nuts did not change the HDL subclass profile. In respect to PON1, this enzyme is related to the antioxidant functions of HDL, and the fact that no alterations of the enzyme activity were found under nut consumption suggests that, at least in part, HDL antioxidative function was unaffected.

As a limitation of the study, we should point out the short period of nut consumption was adopted because of the potential toxicity of high-selenium consumption for long periods [24] and the small study sample. However, as the participants were employees of the institution, a strict day-to-day control of the dietary protocol was possible.

In summary, the consumption of 45 g/d of Brazil nuts by normolipidemic subjects during a short period, at the daily amount range tested for other nuts, did not affect the established atherosclerosis lipid and apolipoprotein markers. The only alteration in HDL functional parameters measured herein was the increase in cholesteryl ester reception by the lipoprotein, which may be a protective mechanism against atherosclerosis.

## Acknowledgments

This study was supported by the Fundação do Amparo à Pesquisa do Estado de São Paulo, São Paulo, Brazil. Maranhão is a Research Awardee of the Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brasília, Brazil.

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