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Consumption of Brazil nuts with high selenium levels increased inflammation biomarkers in obese women: A randomized controlled trial

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ABSTRACT

Objective: Increased inflammatory response is an important factor in the pathophysiology of obesity. The mineral selenium (Se), of which one of the main food sources is the Brazil nut, has important antioxidant and anti-inflammatory functions through the action of selenoproteins. Thus, the evaluation of the influence of this micronutrient in this context is of great relevance. The aim of this study was to evaluate the effects of Brazil nut intake with high Se concentrations on inflammatory biomarkers and its relation to Se status in obese women.

Methods: A randomized controlled clinical trial was carried out with 55 women recruited at Clinical Hospital in São Paulo, Brazil. Patients were randomly assigned to either the Brazil nut group (BN) or the control group (CO) and followed up for 2 mo. The BN group consumed 1 unit/d of Brazil nuts (~ 1261 μg/Se); the CO group did not receive any intervention. At baseline and after 2 mo, analysis of biochemical parameters related to Se status, oxidative stress, and inflammatory biomarkers were performed.

Results: At baseline, both groups did not present Se deficiency. In the BN group, a significant increase ($P < 0.05$) in all Se biomarkers and in gene expression of several proinflammatory parameters (interleukin-6, tumor necrosis factor-α, and Toll-like receptors 2 and 4) were observed after the intervention period. No changes were observed for the CO group.

Conclusion: Although there were no changes in plasma inflammatory biomarkers levels, a significant increase in gene expression may be an indication of a proinflammatory stimulus in obesity, induced by the consumption of Brazil nuts with high Se levels.

Introduction

The relationship between inflammation and obesity was described by Hotamisligil et al. in 1993 [1], and since then many studies have associated this process with metabolic complications implicated in this disease [2,3]. The inflammatory response to obesity is characterized by a chronic, systemic, and low-intensity reaction called *metabolic inflammation* or *meta-inflammation*, which is different from the classic inflammatory response. The development of meta-inflammation is associated with a wide and integrated

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network of intracellular signaling pathways that can induce the synthesis of inflammatory mediators in different cell types [4,5].

Adipose tissue produces a variety of proinflammatory adipokines such as tumor necrosis factor (TNF)-α, monocyte chemoattractant protein (MCP)-1, interleukin (IL)-1 β, and IL-6. Adipose tissue is considered the main source of this systemic inflammatory response. In this context, obese individuals present an increase of proinflammatory and a decrease of anti-inflammatory biomarkers [6,7]. The factors related to chronic low-grade inflammation include macrophage infiltration, endothelial cell activation, the presence of hyperplastic and hypertrophic adipocytes, and oxidative stress [8–10]. In this context, two important inflammatory signaling pathways are involved: the c-Jun-N-terminal kinase (JNK) and the nuclear factor (NF)-κB, which can be activated by TNF-α, lipopolysaccharides (LPS), and saturated fatty acids [7]. It should be noted that some nutrients such as vitamins and minerals can modulate some processes related to the inflammatory process [11].

Selenium (Se) is an important micronutrient for human health and exerts its biological functions in the form of the amino acid selenocysteine (Sec) by its incorporation in selenoproteins [12]. Brazil nut (*Bertholletia excelsa H.B.K*) is considered an excellent food source of Se because of its high concentration of this mineral and its bioavailability [13,14]. However, some studies have shown that high blood Se concentration has been associated with an increased risk for type 2 diabetes [15], metabolic syndrome [16], adverse blood lipid profile [17], and increased mortality [18].

In addition to its important antioxidant functions, Se also has anti-inflammatory properties [19,20]. In response to Se intake, the increase of glutathione peroxidase (GPx) expression can reduce reactive oxygen species (ROS) levels and inhibit the phosphorylation of Inhibitor of κB(IkB)-α, preventing the translocation of NF-κB to the nucleus and its activation, leading to attenuation of the inflammatory response [20,21].

Several in vitro and in vivo studies have shown that Se deficiency implies an increase in inflammatory response, and the supplementation of this mineral is able to attenuate this process [22–25]. However, the potential mechanisms of action of Se on inflammatory pathways in humans have not yet been thoroughly explored. Thus, the present study aimed to evaluate the effects of Brazil nut intake with high Se levels on inflammatory biomarkers and its relation to Se status in obese individuals.

Participants and Methods

Study sample

This study is part of a randomized controlled trial in which the calculated sample size was 56 women (28 per group). However, to compensate for potential dropouts, the sample was increased by 30% [26], yielding a total of 72 women (36 per group), recruited at the Division of Endocrinology and Metabolism from the Clinical Hospital (School of Medicine, University of São Paulo, São Paulo, Brazil) between July and December 2015. Seventy-two participants were included in the study according to the following criteria:

- between 18 and 55 y of age,
- body mass index 28 to 39.9 kg/m^2;
- not pregnant or participating in another clinical trial;
- not using vitamin-mineral supplements;
- not smoking or abusing alcohol;
- not engaging in intense physical activity;
- not allergic to nuts or reporting a regular consumption of Brazil nuts.

The criteria for exclusion were presence of recent infections, high-grade inflammation, chronic cardiovascular and gastrointestinal diseases (inflammatory bowel disease, celiac disease), and cancer. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all the procedures involving the patients were approved by Ethics Committee of the Faculty of Pharmaceutical Sciences at the University of São Paulo. Written informed consent was obtained from all patients before the interview.

Experimental protocol

Patients were randomly assigned to either the ingestion of Brazil nuts (BN) group or the control (CO) group and were followed for 2 mo. The treatment group (BN) consumed one Brazil nut daily and the CO group avoided the intake of any food containing the nut for the same period of time. All patients were instructed to maintain their usual diet. The randomization was done with a 1:1 allocation, concealed in opaque and sealed envelopes, organized at random containing the phrases "nut" or "without nut." On the day of the first blood collection (T0), each participant randomly chose an envelope, thus defining her respective group. The participants in the BN group received a pot with the required number of Brazil nuts at no cost, to be consumed during the study. Nutritional information and the correct instructions for storage and consumption of this food were available on the label of the pots. The nut consumption was followed up and controlled by means of telephone contact during the 2 mo. At the end of the study (T1), it was requested that participants who, for some reason, forgot to eat any Brazil nuts returned them in the pot that was delivered at the beginning of the study, so intake could be verified. The CO group was instructed to return after 60 d for a new evaluation. At baseline and after 2 mo of follow-up, blood collection and anthropometric evaluation were performed.

Centesimal composition and Se content of Brazil nut

A random sample of Brazil nuts (harvest/2013, Amazon region, Brazil) was used to determine the centesimal composition and Se concentration. Humidity, ash, lipids, and protein were analyzed according to the Association of Official Analytical Chemists (AOAC). Total carbohydrates were calculated by difference: 100 − total grams of humidity, ash, lipid, and protein. Se concentration was determined by inductively coupled plasma mass spectrometry (NexION 2000 ICP Mass Spectrometer, Perkin Elmer, Waltham, MA, USA) according to Nardi et al. [27]. Quality control of data was acquired by the analysis of two standard reference materials from the National Institute of Standards and Technology (Whole Egg Powder RM 8415, Rice Flour SRM 1568 a). Measured values were always in good agreement (*t* test 95%) with certified values.

Blood sample collection

Fasting blood samples were drawn by venipuncture into 5 mL EDTA tubes and 8 mL tube without anticoagulant. The total volume of blood samples collected was 25 mL for obtaining serum, plasma, erythrocyte, and mononuclear cells (isolation procedure described later). Plasma and serum were separated from whole blood by centrifugation at 3000*g* for 15 min at 4°C. The erythrocyte pellet obtained from whole blood was washed three times with 5 mL sterile 9 g/L NaCl solution, slowly homogenized by inversion and centrifuged at 10 000*g* for 10 min at 4°C, and the supernatant was discarded. Aliquots of serum, plasma, and erythrocyte were stored in trace element–free tubes at −80°C until analysis was performed.

Peripheral blood mononuclear cells isolation

Peripheral blood mononuclear cells (PBMCs) were isolated from 10 mL of whole blood using Ficoll reagent (Histopaque-1077, Sigma-Aldrich, St. Louis, USA) according to the manufacturer's instructions with some modifications. First, the whole blood was diluted 1:1 with 1 × phosphate-buffered saline and then layered slowly and carefully over Ficoll (20 mL of diluted blood for 15 mL of Ficoll) in a sterile 50 mL centrifuge tube and centrifuged at 400*g* for 40 min at room temperature. Next, the PBMCs were harvested from the interface between Ficoll and the sample layers and placed in another sterile 50 mL centrifuge. This layer was then washed with 1 × phosphate-buffered saline at 1500*g* for 10 min at 15°C. After the isolation, an aliquot of the cells was removed for counting in an automated counter (TC20, BioRad, Hercules, CA, USA). Lastly, the PBMCs (minimum number of 5×10^6 cells) were stored in sterile tubes with trizol reagent (TRIzol Reagent, Invitrogen, Carlsbad, CA, USA) at −80°C. All steps of PBMC isolation were performed in laminar flow.

Biomarkers of Se status

The plasma and erythrocyte Se levels were determined by inductively coupled plasma mass spectrometry (Perkin Elmer DRC II) as described previously [28]. Samples were diluted 1:50 into a 15-mL polypropylene tube with a solution containing 0.01% (v/v) Triton X-100, 0.5% (v/v) nitric acid, and 10 μg/L of each one of the internal standards. The certified reference material Seronorm Trace Elements Serum (SERO AS, Billingstad, Norway) was used for the quality control assessment. Erythrocyte GPx activity was performed according to the method of Paglia and Valentine [29] using a commercial kit (Ransel 505, Randox Laboratories, Crumlin, UK) in an automated biochemical analyzer. GPx activity was expressed in units per gram of hemoglobin (U/gHb) level. Plasma selenoprotein P (SELENOP) concentration was determined by enzyme-linked immunosorbent assay (ELISA) using a

commercial kit (Abexxa, Ltd, Dallas/Fort Worth, TX, USA) on a microplate reader (Biotech Synergy H1, Biotek, Winooski, VT, USA).

Biochemical analysis

Serum triacylglycerol, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and glucose levels were measured with commercially available kits (Labtest, Minas Gerais, Brazil) in an automated biochemical analyzer (Labmax 240, Labtest, Minas Gerais, Brazil). Homeostatis model of resistance with insulin resistance (HOMA-IR) was calculated using the following equation [30]:

$$\text{HOMA}-\text{IR} = \text{fasting glucose (mmol)} \times \text{insulin } (\mu\text{U/mL})/22.5$$

The analysis of plasma inflammatory biomarkers (MCP-1, IL-10, IL-13, IL-2, IL-6, IL-1 β, TNF-α, IFN-γ, C-reactive protein [CRP]), fibrinogen, LPS, leptin, and insulin levels were performed by Multiplex technique using a commercial kit (Milliplex, Merck Millipore, Darmstadt, Germany).

Gene expression

Total RNA from PBMC samples were extracted using Direct-zol KNA kit (Zymo Research, CA, Irvine, USA), and the final concentration was measured in a Nanodrop ND 1000 spectrophotometer (NanoDrop ND 1000, Thermo Scientific, Wilmington, DE, USA). The cDNA was synthesized by reverse transcription polymerase chain reaction (PCR) using High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). Gene expression analysis was performed by real-time quantitative PCR (qRT-PCR) on StepOne Plus equipment (Life Technologies, Carlsbad, CA, USA) using TaqMan Gene Expression Assays for *GPx1, SELENOP, TNF-α, IL-6, IL-1 β, IL-10*, Toll-like receptor (*TLR*)*2* and *TLR4* (Applied Biosystems, Foster City, CA, USA). The reference gene used for these analyses was the glyceraldehyde phosphate dehydrogenase (*GAPDH*). The fold-change calculation was performed using the following formula $2^{-\Delta\Delta Ct}$ [31], considering:

$$\Delta\Delta Ct = (Ct_{\text{after-intervention}} - Ct_{\text{reference}}) - (Ct_{\text{baseline}} - Ct_{\text{reference}})$$

Statistical analysis

Statistical analysis was carried out using SPSS version 22 (SPSS, Chicago, IL, USA) and GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). The presence of outliers was verified, and when appropriate, we opted for sample exclusion. The Shapiro–Wilk W test was used to verify the normality of the continuous variables. Comparison of the numerical variables between the groups was performed using the Student's *t* test when data presented normal distribution and Mann–Whitney for the non-parametric data. To evaluate the total variation of parameters throughout the study, the delta (Δ) was calculated by subtracting the values of postintervention (T1) and the baseline (T0). Subsequently, to investigate the effect of the time and intervention, analysis of variance repeated measures analyses were performed. Two post hoc analyses were performed: a Spearman correlation to verify an association between the variation (Δ) of Se biomarkers and the fold-change of inflammatory parameters, and a Mann–Whitney test to evaluate the difference of plasma LPS concentration according to plasma Se tercile. The significance level adopted for the statistical tests was 5% ($P < 0.05$).

Results

Seventy-two obese women were enrolled in this study in year 2015. During the study period (2 mo), there were dropouts in the segment for the reasons described in Figure 1. Twenty-nine participants from the BN group and 26 from the CO group completed the trial.

Brazil nut nutritional composition and patient characteristics

The results of centesimal composition and Se concentration in Brazil nuts are shown in Supplementary Table 1. In relation to Se, considering an average weight of 5 g, each nut provided about 1261.4 μg/Se. Concerning the recommendation for Se intake, this value is higher than that established by the Estimated Average Requirement for adults (45 μg/d) and exceeded the tolerable upper intake level of 400 μg/d and the lowest observed adverse effect level of 900 μg/d [32]. However, during the experimental protocol, no signs or symptoms of Se toxicity were observed in the study participants.

The mean age (SD) of BN and CO groups were 40.4 (9) and 39.4 y (9.5; $P = 0.714$), respectively. In relation to body composition and biochemical analysis, both groups presented similar characteristics and were not different at baseline (Table 1). After 8 wk, only fasting glucose and total cholesterol showed a significant variation (Supplementary Table 2). However, this variation was expressive only in the CO group, not reflecting the Brazil nut intake.

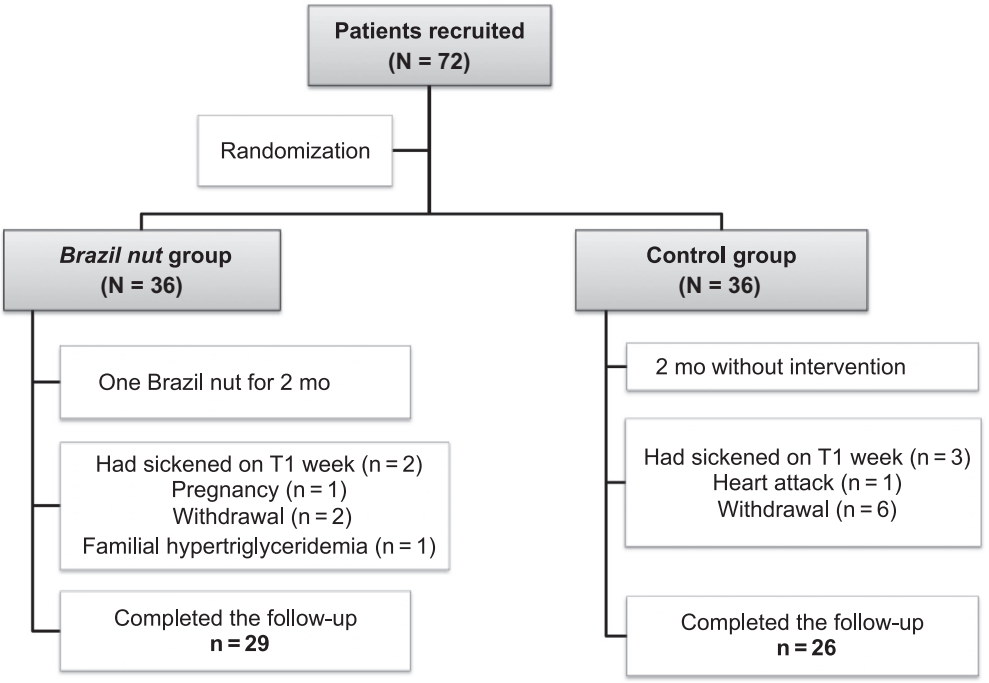

Fig. 1. Participant flowchart.

Table 1
Characteristics of BN and CO groups at baseline (T0)*

Parameters	BN group (n = 29)	CO group (n = 26)	*P*-value
Weight (kg)	90.3 (85.3–101.6)	88.6 (81.7–103.5)	0.654†
BMI (kg/m^2)	34.6 (30.8–37.4)	34.8 (33.1– 40.2)	0.418‡
BF (%)	44.8 (39.9–50.9)	45.2 (41.9–50.7)	0.328†
WC (cm)	100.5 (94–104.4)	99.8 (91–108.1)	0.780†
Fasting glucose (m/dL)	94 (83.8–104.2)	82 (75.9–88)	0.332‡
Insulin (μU/mL)	7.1 (5.3–8.9)	6.8 (4.5–9.1)	0.853‡
HOMA-IR	1.4 (1–2.5)	1.6 (0.9–3.8)	0.807‡
Total cholesterol (mg/dL)	177.6 (162.9 –192.3)	188.4 (175.2–201.6)	0.204†
HDL-C (mg/dL)	52.8 (48.5–56.9)	48.3 (43.8–52.9)	0.116‡
LDL-C (mg/dL)	116 (103–128.3)	123.6 (112.1–134.9)	0.296†
Non-HDL-C	124.9 (111.6–138.2)	140.0 (126.2–153.2)	0.073†
Triacylglycerol (mg/dL)	109.9 (85.2–134.5) §	108.2 (81.2–135.2)	0.931‡
AST (mg/dL)	26 (21.7–30.3)	22.9 (20.6–25.2)	0.633‡
ALT (mg/dL)	22.6 (16–29.3)	20.5 (16.6–24.4)	0.886‡
Albumin (mg/dL)	4.4 (4.3– 4.4)	4.4 (4.3– 4.5)	0.993‡

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BF, body fat; BMI, body mass index; BN, Brazil nut; CO, control; WC, waist circumference.
*Values are median and interquartile range.
†Student *t* test for independent samples.
‡Mann–Whitney test.
§n = 28 (after outlier exclusion).

Assessment of Se biomarkers before and after Brazil nut intake

Plasma and erythrocyte Se levels, GPx1 activity, and plasma SELENOP levels are described in Table 2. At baseline (T0), only plasma Se concentration differed between the two groups. However, neither group was Se deficient according to plasma and erythrocyte concentration of this mineral [33]. After 2 mo (T1), Brazil nut consumption resulted in a significant increase of these biomarkers ($P < 0.001$) with values above the normal range [33]. A significant increase in plasma SELENOP concentration and GPx1 erythrocyte activity ($P = 0.030$; $P = 0.001$, respectively) was observed in the BN group after 8 wk of intervention. No change was observed in the CO group. These results are indicative of the participants' good adherence to the experimental protocol and the high bioavailability of Se present in Brazil nuts.

Effects of Brazil nut intake on inflammatory biomarkers

Table 3 shows the plasma concentrations of inflammatory biomarkers. After 8 wk of intervention, no significant changes were observed in these parameters in both groups. Considering the high Se content in Brazil nuts, we sought to evaluate correlations between the biomarkers related to Se status and inflammation. At baseline (T0), a significant negative correlation was observed between plasma SELENOP and the following inflammatory markers: IL-2 ($r = -0.373$; $P = 0.011$), IL-6 ($r = -0.333$, $P = 0.024$) and TNF-α ($r = -0.305$, $P = 0.040$). After the intervention with Brazil nut (T1), significantly negative correlations were found between plasma Se and IL-6 ($r = -0.679$, $P < 0.001$) and IFN-γ ($r = -0.621$; $P = 0.001$). mRNA expression of genes related to inflammatory response and selenoproteins were evaluated in PBMCs of obese women. Figure 2 shows the fold-change of the selected genes. *IL-1*β mRNA expression was the only one that did not show any significant difference between the groups. In relation to other genes, a significant increase was observed in the BN group, except for *GPx1* mRNA. For *GPx1*, gene expression was lower in the BN group than in the CO group. As an exploratory posthoc analysis, a Spearman correlation was performed between the variation (Δ) of Se biomarkers and the fold-change of the evaluated genes (Table 4). The results showed a positive relation between Se and the gene expression of almost all inflammatory biomarkers, except for *IL-1* β.

Table 2
Concentration of Se status biomarkers in obese women before and after 8 wk

	BN group			CO group			
Biomarker	T0	T1	$\Delta_{(T1-T0)}$	T0	T1	$\Delta_{(T1-T0)}$	*P*-value
Plasma Se (μg/L)*	87.1 (82– 97.7)†	244 (226–278)	157.1	65.7 (61.7–71.3)†	63.2 (60.6–67.7)	−2.5	<0.001
Erythrocyte Se (μg/L)‡	125 (122–143)	351 (309–480)	225.9	125 (120–141)	130.2 (116–146)	4.7	<0.001
GPx1 activity (U/gHb)	48.7 (37.5–57.6)	57.2 (45.8–67.5)	8.5	47.2 (41–57.1)	49.7 (43.5–58.5)	2.5	0.030
SELENOP (ng/mL)	37.7 (16.1–51.9)	55.5 (37.1–150.6)	17.8	28.5 (12.5–52.1)	23.6 (16–32.6)	−4.9	0.001

ANOVA, analysis of variance; BN, Brazil nut; CO, Control; GPx, glutathione peroxidase; Se, selenium; SELENOP, selenoprotein P
Values are median and interquartile range. $\Delta_{(T1-T0)}$ represents the mean variation of the parameters evaluated after the intervention. *P*-value calculated by analysis of variance for repeated measurements (ANOVA repeated measures)
*BN group: n = 26 and CO group: n = 23.
†Significant difference between groups at baseline ($P < 0.001$). Reference values: Plasma Se = 60–120 μg/L; Erythrocyte Se = 90–190 μg/L [33].
‡BN group: n = 28 and CO group: n = 25.

Table 3
Plasma inflammatory biomarkers levels of BN and CO groups before (T0) and after (T1) the intervention period

	BN group (n = 29)		CO group (n = 26)		
Biomarkers	T0	T1	T0	T1	*P*-value
CRP (mg/dL)	2.3 (1.1–3.5)	2.1 (1.4–2.8)	1.8 (0.7–3)	2 (1–2.9)	0.585
MCP-1 (pg/mL)	266.4 (229–303.9)	296 (240.7–351.3)	296.2 (228.6–363.9)	319.7 (256.2–383.1)	0.808
IL-6 (pg/mL)	2.4 (1.7–3)	2.7 (1.8–3.5)	2.7 (1.8–3.6)	2.9 (2–3.8)	0.349
IL-10 (pg/mL)	3.6 (2.6– 4.7)	4 (2.6– 5.4)	3 (1.7– 4.3)	3 (2– 3.9)	0.124
IL-1 β (pg/mL)	2.5 (1.3–3.8)	2.4 (1.3–3.5)	1.5 (1–2)	1.6 (1–2.1)	0.808
TNF-α (pg/mL)	2.1 (1.6–2.7)	2.4 (1.7–3)	2.1 (1.6–2.5)	2.2 (1.6–2.8)	0.773
IFN-γ (pg/mL)	11.4 (9.6–13.2)	11.5 (9.3–13.7)	11.0 (8.9–13.1)	11 (9–12.9)	0.489
Fibrinogen (g/L)	1.5 (1.5–1.6)	1.5 (1.5–1.6)	1.5 (1.4–1.6)	1.5 (1.5–1.6)	0.409

ANOVA, analysis of variance; BN, Brazil nut; CO, control; CRP, C-reactive protein; IFN, interferon; IL, interleukin; MCP, monocyte chemoattractant protein; Se, selenium; TNF, tumor necrosis factor
Values are geometric means (95% CI). *P*-value calculated by analysis of variance for repeated measurements (ANOVA repeated measures)

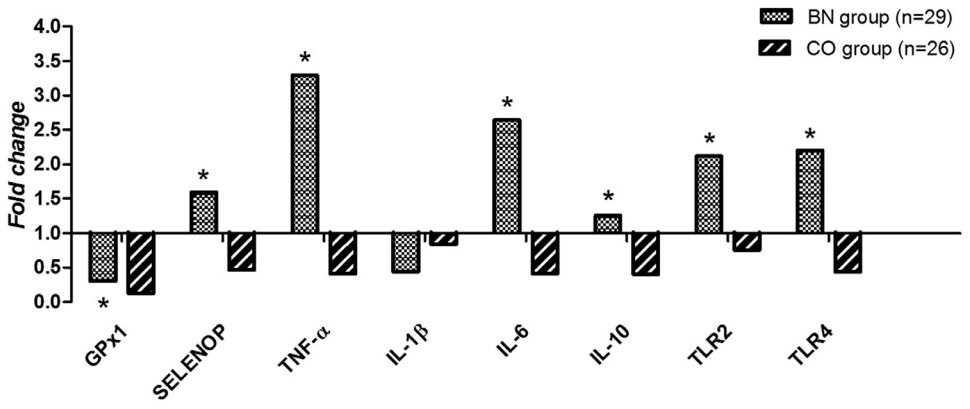

Fig. 2. Fold-change (comparison of postintervention with baseline) of *GPx1, SELENOP, TNF-α, IL-1 β, IL-6, IL-10, TLR2,* and *TLR4* genes. BN, Brazil nut; CO, control; GPx1, glutathione peroxidase 1; SELENOP, selenoprotein P; TNF, tumor necrosis factor; IL, interleukin; TLR, Toll-like receptor. *$P < 0.05$ (Mann–Whitney test).

Table 4
Spearman correlation between the variation (Δ) of Se biomarkers and the mRNA fold-change of *TNF-α, IL-1β, IL-6, IL-10, TLR2,* and *TLR4* genes.

Gene	Δ plasma Se (*P*-value)	Δ erythrocyte Se (*P*-value)	Δ SELENOP (*P*-value)
TNF-α	**0.579 (<0.001)**	**0.564 (<0.001)**	**0.433 (0.003)**
IL-1β	−0.147 (0.313)	−0.122 (0.376)	0.032 (0.833)
IL-6	**0.625 (<0.001)**	**0.647 (<0.001)**	**0.636 (<0.001)**
IL-10	**0.567 (<0.001)**	**0.437 (0.001)**	**0.389 (0.008)**
TLR2	**0.662 (<0.001)**	**0.561 (<0.001)**	**0.321 (0.037)**
TLR4	**0.753 (<0.001)**	**0.698 (<0.001)**	**0.421 (0.004)**

IL, interleukin; Se, selenium; SELENOP, selenoprotein P; TLR, Toll-like receptor; TNF, tumor necrosis factor
Δ = variation of the parameters evaluated (postintervention − baseline). Correlation coefficient and *P*-value in parentheses. **Bold** values indicate a significant correlation ($P < 0.05$)

Evaluation of plasma Se and LPS concentration

Another exploratory post hoc analysis was performed to evaluate the study participants according to the tercile of plasma Se and LPS concentration. Figure 3 shows that at baseline and after 8 wk, plasma LPS concentrations were higher according to the increase of plasma Se. After 8 wk, a positive and significant correlation was found between these parameters ($r = 0.317$; $P = 0.026$).

Discussion

Because of the Se levels in the soil in some regions of Brazil, some Brazil nuts can reach Se values between 158.4 Se and 512.0 μg/g [34,35]. Then, only one sample can easily exceed 1000 μg of Se content. In this study, the consumption of Brazil nuts for 2 mo was effective in improving Se biomarkers as demonstrated in various studies conducted in Brazil [36–39]. These data support the evidence that Se content in this nut is highly bioavailable.

SELENOP acts as the primary Se transporter for peripheral tissues and is considered an excellent biomarker of Se status [40]. The daily intake of Brazil nuts with lower Se concentration (300 μg/Se) than that used in the present study (1261.4 μg/Se) was also able to increase plasma SELENOP levels. This is the first study to evaluate the effect of Brazil nut intake on this biomarker in obese individuals. It is important to consider that this result agrees with those observed for plasma Se.

Brazil nut consumption did not promote significant changes in plasma concentrations of inflammatory biomarkers in the present study. However, the consumption of 20 or 50 g of Brazil nuts by healthy adults evaluated in different periods of time resulted in a reduction of IL-6, TNF-α, and IFN-γ serum concentration after 24 h and an increase of IL-10 after 9 h. After a washout period (30 d) these parameters remained stable [41]. Supplementation with Se yeast (200 μg) significantly reduced serum CRP in women with

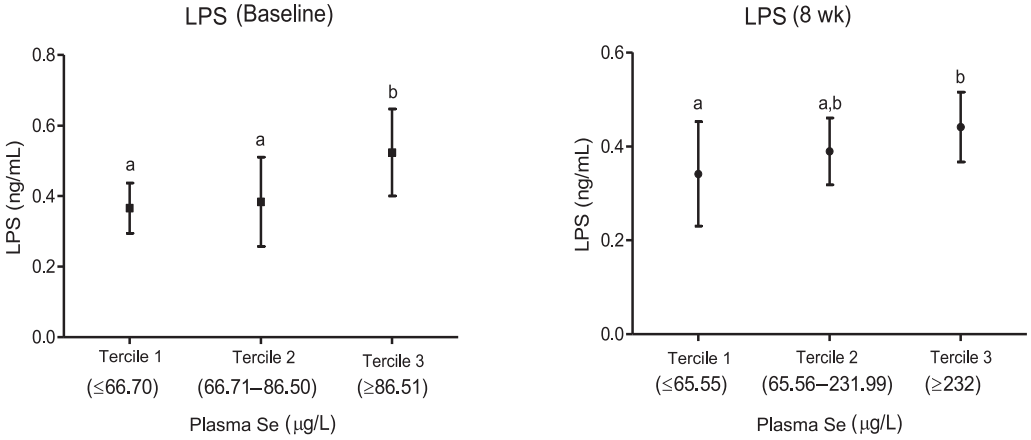

Fig. 3. Plasma concentration of LPS of obese women at baseline and after 8 wk according to plasma Se tercile. LPS, lipopolysaccharide; Se, selenium. Values are geometric means (95% CI). Different letters represent a significant difference between the groups ($P < 0.05$ by Mann–Whitney test).

gestational diabetes mellitus [42] and in patients with congestive heart failure [43], but the same was not observed for patients diagnosed with diabetic nephropathy [44]. In the context of obesity, a supplementation of antioxidants containing Se (50 μg) as Se yeast for 4 mo was able to reduce oxidative stress but not the inflammatory markers in obese children and adolescents [45]. In this context, there are few studies that evaluated in humans the effects of Se supplementation using its inorganic form on inflammation parameters. One study observed a reduction on serum CRP levels in patients with coronary artery disease after oral supplementation with zinc and Se as selenite (150 μg) in association with rosuvastatin [46].

Gene expression analysis in PBMC was used in several studies because these cells can migrate through the bloodstream and infiltrate a variety of tissues that are exposed to both endogenous and exogenous stimuli. Thus, these cells can reflect not only the immune and metabolic responses of adipocytes and hepatocytes for example, but also the responses to dietary interventions, such as increased Se supply at the gene expression level [47, 48].

Evidence has shown that Brazil nut consumption can modulate gene expression of selenoproteins by increasing the availability of Se [49,50]. In the present study, there was an increase in SELENOP and a decrease in *GPx1* gene expression in the BN group. In this case, it is suggested that the increase of Se supply was being used for the synthesis of SELENOP, whereas *GPx1*, having already reached its plateau of enzymatic activity, would receive a smaller amount of substrate.

After Brazil nut intake by obese women with no Se deficiency, a significant increase in gene expression of proteins (TNF-α, IL-6, TLR2, and TLR4) involved with proinflammatory pathways was observed, and a positive correlation was demonstrated between these results and Se biomarkers. However, a concomitant increase in SELENOP and *IL-10* gene expression was observed, which may indicate a mechanism of compensatory anti-inflammatory response. The mechanism by which Se is involved in the inflammatory response is related to the NF-κB pathway as described previously [20].

Consumption of a high-fat diet is associated with dysbiosis in obese individuals and has been shown to increase intestinal permeability and LPS translocation from the intestinal lumen to the bloodstream. This process, called metabolic endotoxemia, is associated with increased gene expression of inflammatory markers through the activation NF-κB pathway via TLR4 [7]. However, evidence also has shown that the inhibition NF-κB activity in non-immune cells, such as intestinal epithelial cells, can trigger chronic inflammation and lead to an imbalance of physiologic immune homeostasis. Chronic inhibition of NF-κB in the intestinal epithelium may lead to increased death of these intestinal cells and decreased expression of antimicrobial peptides, which results in a compromised epithelial barrier and allows the invasion of commensal bacteria in the colonic mucosa and lead to increased intestinal permeability contributing to metabolic endotoxemia [51,52]. Considering the present results, it can be suggested that the increased expression of proinflammatory genes in obese women may be related to increased LPS translocation. This mechanism could be attributed to the increase of Se supply that has resulted in increased expression of selenoproteins acting indirectly in the inhibition of NF-κB through pathways related to oxidative stress. In the present study, a relation between the increase of plasma Se and LPS concentration was verified, suggesting that there may be a connection between high blood Se levels and the process of metabolic endotoxemia. However, to date, there is no evidence to prove this connection, and therefore more studies in this area are necessary to investigate and clarify this hypothesis.

In terms of recommendations, the Se content of one Brazil nut in the present study is almost 23 times greater than the value established as recommended daily allowance (55 μg/d) for adults and was reflected in a significant increase of this mineral in blood concentrations. This fact is relevant because, as previously mentioned, it may contribute to the increased risk for some chronic diseases. This information is important because this nut is not only consumed in the national market but also exported to other countries of North America and Europe. Thus, it is important to provide information about the Se concentration of Brazil nuts and, in case of a recommendation of intake or supplementation, to consider Se status and the metabolic condition of the individuals to ensure the safe consumption of this nut and to avoid possible adverse effects.

The limitations of this study were its small sample size and the number of dropouts after the intervention period, which influenced the final sample size and may have influenced the statistical power to detect slight effects of some analysis. In addition, it is also important to separately evaluate the effect of Se in these outcomes to better understand the mechanistic role in this context. We cannot infer precisely that the effects were caused only by the high Se intake.

Conclusion

The consumption of Brazil nuts with high Se concentrations for 2 mo by obese women resulted in alterations in molecular response related to inflammation. In this way, the consumption of Brazil nuts with high Se concentrations for prolonged periods could increase this proinflammatory response and lead to metabolic complications in obese women.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2019.02.009.

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