

Brazil and cashew nuts intake improve body composition and endothelial health in women at cardiometabolic risk (Brazilian Nuts Study): a randomized controlled trial

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ABSTRACT

Several mechanisms have been proposed for the beneficial effect of nuts on health. However, Brazil and cashew nuts remain the least studied. We aim to evaluate the effect of these nuts within an energy-restricted diet on body weight, body composition, cardiometabolic markers, and endothelial function in cardiometabolic risk women. Brazilian nuts study is a randomized controlled parallel 8-week dietary intervention trial. Forty women were randomly allocated to 1) Control group: Energy-restricted diet without nuts, $n= 19$ or, 2) Brazil and cashew nuts group (BN-Group): Energy-restricted diet containing daily 45 g of nuts (15 g of Brazil nuts + 30g of cashew nuts), $n= 21$. At the beginning and final intervention, anthropometry, body composition, and blood pressure were measured. Fasting blood sampling was obtained to evaluate lipid profile, glucose homeostasis, and endothelial function markers. After 8-week, plasma selenium concentration increased in BN-group ($\Delta = + 31.5 \pm 7.8 \mu\text{g/L}$; $p= 0.001$). Brazil and cashew nuts intake reduced total body fat ($-1.3 \pm 0.4 \%$) parallel to improvement of lean mass percentage in BN-group compared to the control. Besides, the soluble adhesion molecule VCAM-1 decreased ($24.03 \pm 15.7 \text{ pg/mL}$ vs. $-22.2 \pm 10.3 \text{ pg/mL}$; $p= 0.019$) after Brazil and cashew nuts intake compared to the control. However, lipid and glucose profile markers, apolipoproteins, and blood pressure remained unchanged after the intervention. Thus, the addition of Brazil and cashew nuts to an energy-restricted diet can be a healthy strategy to improve body composition, selenium status, and endothelial inflammation in cardiometabolic risk women.

Trial registration: Brazilian Registry of Clinical Trials: RBR-3ntxrm. Registered July 1, 2019, “retrospectively registered”, <http://www.ensaiosclnicos.gov.br/rg/RBR-3ntxrm/>.

Keywords: Brazil nut; cashew nut; obesity; weight loss; VCAM-1; body fat.

INTRODUCTION

Obesity is well-established as a main risk factor for cardiovascular diseases (CVD). The effects of obesity on CVD are associated with other metabolic risk factors such as insulin resistance, hypertension, hypercholesterolemia, and hyperglycemia ^[1]. Recent estimates point out that up to 2025, global obesity prevalence will achieve 18% in men and 21% in women ^[2], which is evidence that women are most vulnerable to the obesity pandemic ^[2,3]

Body weight is the major modifiable independent risk factor for CVD [4]. Weight loss of at least 3% from baseline is clinically relevant since this amount is associated with improvements in multiple cardiometabolic risk markers, such as reducing insulin resistance, triglycerides, LDL, HDL, and non-HDL cholesterol ^[4-7]. Regardless of the cause of obesity, reduction in energy intake and increase of physical activity practice remains among the main cornerstones of obesity treatment ^[8]. However, long-term adherence to an energy-restricted diet is highly challenging, making it difficult to achieve substantial and sustained weight loss ^[9]. For this, foods that promote greater satiety can help reduce energy intake, increase compliance to weight-loss diets, and promote weight loss. Further, some foods' positive effects on managing obesity go beyond weight loss and might drive metabolic benefits ^[8,10].

In this regard, several studies have demonstrated that nuts consumption can modulate lipid profile, glycemic homeostasis, blood pressure, oxidative stress, and food intake ^[11-19]. In subjects with overweight or obesity, sensory and nutritional characteristics of nuts potentially modify the secretion of intestinal hormones, and consequently, the appetite sensation ^[18,19]. Some nutrients from nuts as unsaturated fatty acids, minerals, phytosterols, and fiber, contribute to their health effect. Nevertheless, most of this evidence was not from studies conducted with Brazilian nuts, currently considered poorly studied.

Brazil nut (*Bertholletia excelsa* H.B.K) is a native species to the Amazon considered the main food source of selenium (100-1000 mg of selenium/ g-1) ^[20,21]. Also, this nut contains phytosterols, tocopherols, squalene, and phenolics related to its anti-inflammatory and antioxidant activities ^[20-23]. The cashew tree (*Anacardium occidentale* L.) is native to Central and South America, being Brazil thought its country of origin ^[24-27]. Besides the pleasant taste, cashew nuts have valuable nutritional

properties, such as high lipids content, predominantly MUFA and PUFA, both associated with the reduction of cholesterol, LDL-c, and cardiovascular events ^[26].

At present, few clinical trials have investigated the benefits of Brazil and cashew nuts intake on cardiometabolic risk markers. In all founded studies, these nuts were included in a normal-caloric diet. According to the available studies, the beneficial effect of these nuts on lipid profile and blood pressure is controversial, and no effect on glucose homeostasis has been observed ^[28,29,38,39,30–37]. In order to better investigate the impact of these nuts intake on health, this study aimed to evaluate the effect of Brazil and cashew nuts associated with the traditional approach to the management of obesity and overweight (the energy-restricted diet) on body weight, body composition, cardiometabolic markers, and endothelial function in women at cardiometabolic risk. We hypothesized that an energy-restricted diet parallel to Brazil and cashew nuts intake would potentiate the improvements in evaluated variables.

METHODOLOGY

1.1 Study design, participants, and recruitment

The Brazilian Nuts Study is a randomized, controlled parallel 8-week nutritional intervention trial conducted in free-living conditions with women at cardiometabolic risk. Eligibility criteria included: adult women (20-55 years), with overweight (BMI $\geq 27 \text{ kg/m}^2$ and $<30 \text{ kg/m}^2$), waist circumference $\geq 80 \text{ cm}$, and body fat percentage $\geq 32\%$ associated with at least one another component of metabolic syndrome: triglycerides $\geq 150 \text{ mg/dL}$, high blood pressure arterial ($\geq 130/85 \text{ mmHg}$) or high fasting glucose ($\geq 100 \text{ mg/dL}$); or women with obesity (BMI $\geq 30 \text{ kg/m}^2$), with or without metabolic complications. Non-inclusion criteria were pregnant, lactate, or menopausal women; athletes; vegans; smoking; women with a history of HIV, illness or digestive, liver, kidney, cardiovascular, thyroid, cancer, inflammatory diseases, and eating disorders; history of drug and/or alcohol abuse; aversion or allergy to nuts; infectious episode in the last month; use of anti-inflammatory drugs, corticosteroids, antibiotics, and others that may affect energy appetite and metabolism; body weight instability; regular consumption of nuts greater than 30 g/day; alcohol consumption higher than 21 units (168g) per week; dental problems that interfere with chewing; use of vitamin, mineral and omega 3 supplements.

Advertisements in social media and flyers were the recruitment methods. After an initial screening, the women who met the essential eligibility criteria (age, body weight, height, no pregnancy, menopausal, and medical/supplement use) were invited to a face-to-face visit to evaluate health history, physical activity level, and anthropometry. The study occurred in the Department of Nutrition and Health of Universidade Federal de Viçosa-MG, Brazil, with enrollment between June 2019 and March 2020. The study protocol followed the guidelines of the Helsinki Declaration and was approved by the institutional review board of the Universidade Federal de Viçosa (registration number: CAAE: 92004818.0.0000.5153; N: 2.832.601/ 2018). All participants were informed about objectives and study procedures. Those that accepted the study conditions provided written informed consent. Furthermore, this study is registered on the Brazilian Registers of Clinical Trials – REBEC (protocol: RBR-3ntxrm). The primary outcome measure was the body weight reduction after the intervention period. Body composition, cardiometabolic risk markers, Selenium plasma concentration, and endothelial function markers were not included in the prospective trial registry of the Brazilian Nuts Study but were added post-hoc and were exploratory in nature. Other additional secondary endpoints evaluated in the Brazilian Nuts Study, such as energy metabolism, appetite, satiety, and inflammatory markers will be addressed in future publications.

1.2 Dietary intervention

Before the intervention, a run-in period of seven to ten days was applied to identify and exclude women with a probability of non-compliance to the study protocol. After, women were randomly allocated into two groups: control, which was instructed to consume an energy-restricted diet (-500 kcal/day) without any type of nuts, or Brazil and cashew nuts group (BN-group) that was instructed to follow the energy-restricted diet (-500 kcal/day) containing 45g (30g of cashew plus 15g of Brazil nut) of Brazilian mixed nuts daily. At the beginning and end of the intervention period, the women visited the Laboratory of Energy Metabolism and Body Composition (LAMECC) to fasten blood sample collection, anthropometry, body composition evaluation, and fill out questionnaires about physical activity practice, food intake-behavior, and eating behavior.

For 48-hour before the procedures, all women were asked to avoid caffeine and alcohol and maintain their habitual physical activity levels. Additionally, every fifteen

days, the women attended the LAMECC for face-to-face nutritional advisement visits. On these occasions, body weight, 24-h dietary recalls, and physical activity practice questions were taken to monitoring study compliance (**Figure 1**). Over the study, we checked Brazil and cashew nuts consumption by returning not consumed nut packages. Besides, at the beginning and the end of the study, the plasma selenium concentrations were assessed. All women were asked to maintain their lifestyle during the study and informed of any change in the type or dosage of the medication for continuous use.

1.3 Energy-restriction diet

All women received an eating plan with five nutritionally balanced menus, each with five meals (breakfast, morning snack, lunch, afternoon snack, and dinner). First, the total energy intake was estimated using the Estimated Energy Requirement (EER) for adult women with overweight or obesity^[40]; then, 500 kcal/day was deducted for the dietary prescription. As a result, the average distribution of macronutrients was 22.0%, 32.6%, and 45.4% of daily energy from proteins, lipids, and carbohydrates, respectively, according to AMDR range. For the BN-group, the diets were calculated, including the energy provided by the daily portion of 45 g of mixed nut. In addition, due to the high-fat content of the mixed nut, the control group was asked to consume two tablespoons (twice a day; at lunch and dinner) of a salad dressing based on soy oil and lemon (2:1 ratio, respectively), which was prepared in the Metabolic Kitchen of LAMECC and its calories also included in the total caloric value of control diets. Both nuts and salad dressing were handed out to women fortnightly during the face-to-face nutritional visits.

All dietary advice was individualized and provided by dietitians every two weeks. Participants received instructions to use only soy oil throughout the study period to prepare meals consumed over the day.

1.4 Brazil and cashew nuts composition

The nuts used in the study were donated by Embrapa Agroindustria Tropical - Fortaleza - Ceará (cashew nuts) and Inovam Brasil® (Brazil nuts). After received, all nuts were manually selected to eliminate those that were inadequate for consumption. Then, the chosen nuts were portioned (15g of Brazil nut and 30 g of cashew nut) in laminated packages, vacuum sealed (Selovac Sealer model 200 B), and stored in a freezer at -20°C until distribution to the volunteers.

The number of Brazil nuts on the mixed nuts was defined based on its selenium content to meet daily selenium recommendation; the 30g of cashew nut is supported by previously published studies evaluating tree nuts' cardiovascular benefits ^[41–43]. The selenium content in the Brazil nut was measured by inductively coupled plasma atomic emission spectrometry ^[44]. Each portion of 15g of Brazil nut provides approximately 51µg of selenium. Also, we determined the lipid profile of Brazil and cashew nut by gas chromatography following the protocol proposed by Folch et al. 1957^[45] and Hartman and Lago 1973 ^[46]. Unsaturated fatty acids represent 75.9 % of total fat in Brazil nut and 84.6 % in cashew nut (**Supplemental Table 1**).

1.5 Blood sampling

After overnight fasting (12 h), registered nurses collected venous blood samples from the antebrachial vein using vacuum tubes precoated with EDTA or heparin as an anticoagulant. After, blood samples were centrifugated (1500 g, 15 min, 4 C°), aliquoted, and stored at -80 C° until analysis.

1.6 Cardiometabolic risk markers

Serum LDL, HDL, total cholesterol, triglycerides, and glucose were analyzed by the colorimetric enzymatic method using a commercial kit Bioclin® (Belo Horizonte, MG, Brasil) in the automatic analyzer (BS200 Mindray®, Nanshan, China). Insulin and high-sensitive C-reactive protein were quantified in fasting serum by automated analyzer systems using commercial assay kits. Apolipoproteins were assessed using Immunochemistry Systems MMAGE® (Beckman Coulter, Inc. EUA). The serum very-low-density lipoprotein cholesterol (VLDL) was calculated using the Friedewald equations ^[47]. Non-HDL cholesterol was calculated as total cholesterol – HDL cholesterol. The total-cholesterol:HDL and LDL:HDL ratios were also computed ^[48]. Insulin resistance was evaluated using TyG index, calculated by the formula $\text{Ln} [\text{fasting triglycerides (mg/dl)} \times \text{fasting glucose (mg/dl)} / 2]$ ^[49]. Intra- and inter-assay coefficient variation of biochemical measures could be found in **supplemental Table 2**.

1.7 Endothelial function markers

The adhesion molecules ICAM-1 and VCAM-1 were assessed using commercial ELISA kits following the manufacturer's recommendations (Elabscience Biotechnology Co., Ltd, USA). Nitric oxide (NO) concentration was determined in triplicate using the Griess reagent according to the protocol proposed by Grisham et al. (1996) ^[50].

Before ABI and blood pressure measurement, the women were instructed to remain seated and rest for 10 min. Ankle and brachial systolic blood pressures were measured using a hand-held Doppler machine (MEDMEGA®, DV 610B) and oscillometric blood pressure cuffs. Right and left ABI measurements were calculated by dividing each leg's highest systolic blood pressure by the highest arm pressure. The estimated ABI was classified as normal (1-1.4), borderline (0.91 - 0.99), or at increased cardiovascular risk regardless of the presence of symptoms of Peripheral Arterial Disease (≤ 0.90 and > 1.40) ^[51]. In the first evaluation, blood pressure was measured in both arms. If the measures were different, the arm with the highest value was standardized for the following measurements. Blood pressure was measured using an automatic monitor (Omron Healthcare, Inc., Model OMRON HEM 7200, USA). The average of two additional measurements was used for participants with blood pressure in the hypertensive range.

1.8 Plasma selenium concentrations

A commercial lab determined the plasma selenium level using the inductively coupled plasma mass spectrometry method according to standardized protocols.

1.9 Dietary assessment

Before the study baseline assessments, all women completed one 3-day food record (two nonconsecutive weekdays and one weekend day). During the follow-up, a 24-hour dietary recall was applied by dietitians every fifteen days to estimate the mean intake of energy, macronutrients, and fiber during the intervention period. For both used methods, a dietitian checked all reported food and its respective quantities. The food records and 24-hour dietary recalls were analyzed using REC24h-ERICA software, adapted for the Brazilian population.

1.10 Anthropometry and body composition

Body weight was assessed by a bioelectrical impedance analysis device (Inbody 230, Biospace Corp., Seoul, Korea). Waist circumference was measured using an inelastic tape (precision 0.1 cm). Two measurements were taken at the umbilicus waist at the end of normal expiration, and the mean was calculated. BMI was defined as the ratio between weight in kg and squared height in meters. Hip circumference was also measured twice utilizing an inelastic tape at the maximum posterior extension of the gluteus, and the average was calculated. Additionally, body composition was assessed by dual-energy X-ray absorptiometry (DEXA) (Lunar Prodigy Advance DXA System,

GE Lunar) in a subsample (control group: 73.3%, n=11; BN-group: 71.5%, n=10) due to the equipment schedule availability. The DEXA analyses provided total and regional body fatness, including truncal, android, and gynoid composition. The truncal area included the neck, chest, abdominal, and pelvic regions. The android area is between the ribs and the pelvis, while the gynoid region includes the hips and upper thighs and overlaps the leg and truncal regions ^[52].

1.11 Randomization

The minimization method was employed for randomization to ensure the balance of predefined prognostic factors between groups ^[53]. In the present study, age, BMI, and body fat percentage were considered prognostic factors based on their potential to interfere with the outcome variables. The researchers performed the randomization procedure using the WinPepi software, version 11.65 (Copyright J.H. Abransom, Aug 23, 2016).

1.12 Statistics Analyses

The predetermined primary outcome of this study was the difference in weight loss between groups. We determined a priori that a sample size of ≥ 11 completers per arm would allow detecting a difference clinically meaningful of 2.4 kg of weight loss between groups with a power of 95% and a type 1 error (α) of 5%. We added 30% to compensate for potential dropouts, yielding a sample size of at least fourteen women for each group. To estimate the sample size, we based on a previous study with a similar intervention design ^[54]. We assumed a weight change of -3.68 kg (SD 2.82 kg) to be representative after the intervention with nut (almond) intake in a weight reduction program. The sample size was estimated as proposed by Mera, Thompson, Prasad (1998) ^[55].

In this study, the missing data ratio was 27.5%. Due to nature non-random of the missing data "missing not at random (MNAR)", the use of the multiple imputation method is not recommended ^[56]. Therefore, the intention to treat analyses (ITT) was not possible. All statistical analyses used the Statistical Package for the Social Sciences software Version 23.0 for Windows (SPSS, Chicago, IL, USA), and a p-value < 0.05 was considered statistically significant. Figures displaying statistical analysis were produced using Prism 6 (GraphPad, La Jolla, CA, USA). The database was made after double data entry to identify and correct possible failures. We also used the Shapiro-

Wilk normality test to check for the normal distribution of the data and the Levene test to assess the homoscedasticity of the variances. To determine the effect of time on treatments, we performed the paired t-test or Wilcoxon test to the between-group evaluation, t-test, or Mann-Whitney U test to independent-samples comparisons. When appropriate, the One-way ANCOVA adjusted by baseline value was used to compare means between groups. Data are expressed as mean \pm SEM.

RESULTS

Forty women were randomized, and twenty-nine concluded the study. The drop out in the follow-up was higher in the BN group (25.9%) than in the control group (22.2%). The main explanations were "personal reasons" (72.8%), "side effects" (fullness gastric sensation and itching skin - 18.1%), and "noncompliance to study protocol" (9.1%) (**Supplemental Figure 1**). There was no difference for prognostic factors (BMI, age, and body fat percentage) between women that completed and those who did not complete the study (data not shown). Women included in the study had 31.4 ± 1.6 years and 33.4 ± 0.7 kg/m². At baseline, there was no significant difference between groups for cardiometabolic risk markers, endothelial function markers, anthropometric and body composition variables, and plasma selenium concentrations.

After the 8-week dietary intervention, body weight, BMI, waist and hip circumference, waist-to-height, and waist-to-hip ratio showed significant reductions compared with baseline, but no difference between groups (**Table 1; Figure 2**). In contrast, the BN-group women exhibited body composition improvement compared to the control group. Brazil and cashew nuts intake significantly promoted a reduction in body fat (%) parallel to a rise in lean mass (%) and free fat mass (%). Besides, for body regions, truncal lean mass (kg and %) and free fat mass (kg and %) increase in the BN-group compared to the control group. However, the android fat mass was higher in the BN-group than in the control group (**Supplemental Table 3**). Regarding cardiometabolic risk factors, both groups showed a similar reduction in total cholesterol, LDL-c, and systolic blood pressure. Interestingly, VCAM-1 significantly reduced after Brazil and cashew nuts consumption compared to the control group (**Table 2**).

Basal plasma selenium was 57.4 ± 3.8 μ g/L and 57.6 ± 4.1 μ g/L in control and mixed nut, respectively. All women presented low plasma selenium (<100 μ g/L) before this study ^[57]. After intervention, BN-group showed a higher increase in plasma

selenium ($\Delta = +35.4 \pm 7.2 \mu\text{g/L}$; $p = 0.001$) in comparison to control group ($\Delta = +8.9 \pm 7.3 \mu\text{g/L}$; $p = 0.157$). Furthermore, 86.7% ($n = 13$) in the control group and 57.2% ($n = 8$) in the BN-group remained with plasma selenium $<100 \mu\text{g/L}$ (**Table 2**).

Regarding food intake, compared with baseline, the energy intake decreased similarly, $-201.9 \pm 206.6 \text{ kcal}$ and $-287.7 \pm 107.3 \text{ kcal}$ in control and BN-group, respectively (**Table 3**). Cholesterol intake was reduced during the intervention on both groups, without a difference between them. Carbohydrate, protein, total fat, and fiber intake remained comparable between groups after intervention. As expected, at 8-week, MUFA intake was higher in the BN-group than in the control group ($\Delta -1.0 \pm 3.9 \text{ g}$ vs. $\Delta 1.9 \pm 2.1 \text{ g}$; $p = 0.009$), while the PUFA intake was the opposite ($\Delta 6.6 \pm 1.8 \text{ g}$ vs. $\Delta -1.1 \pm 1.4 \text{ g}$; $p = <0.001$).

DISCUSSION

In this dietary intervention trial, the Brazil and cashew nuts intake within an energy-restricted diet for 8-weeks promoted improvements in body composition and ICAM-1 reduction, suggesting improvement of endothelial inflammation and enhanced plasma selenium concentrations in women at cardiometabolic risk.

Weight control is a primary strategy to reduce the cardiovascular disease burden [58]. At the same time, body fat reduction contributes to ameliorates metabolic alterations associated with overweight and obesity [59]. Therefore, for the first time, an RCT evaluated the effect of Brazil and cashew nuts intake within an energy-restricted diet. After the intervention, women allocated in the BN-group had a lower total fat mass (%) and the most preserved total lean and free fat mass than the control group. According to available trials, Brazilian nuts have never been part of an approach to body weight reduction. However, there was no impairment in weight maintenance in studies with regular diets, including cashew [42] or Brazil nuts [28,29] in free-living conditions.

The effect of nuts on adiposity might be associated with the high content of unsaturated fatty acids. MUFA and PUFA are possibly more quickly oxidized and have a higher thermogenic effect than saturated fatty acids, carrying less fat accumulation [60,61]. Also, Moussavi et al. (2008) proposed that the consumption of a MUFA-rich diet could reduce body fat due to the energy expenditure enhancement, mediated, at least in part, through activation of the sympathetic nervous system [62]. Additionally, we should

mention that the lipids provided by nuts are not wholly bioaccessible ^[63–66]. Some studies have shown that the cellular wall of nuts restricts access to their lipid content, resulting in a high proportion of the lost fat in the feces, becoming unavailable for energy metabolism ^[67–69]. However, the nuts effect on body composition, especially on fat content, remains a topic for further studies.

Although the Brazil and cashew nuts intake has promoted reduction in the total body fat mass (%), no effect on metabolic markers evaluated was observed in the present study. Few clinical trials have investigated the effect of Brazil and cashew nuts on lipid profiles. In a previous study, 16-week of Brazil nut supplementation (15–25g/day) in adolescents with obesity reduced total cholesterol and LDL but did not affect HDL and triglycerides. In dyslipidemia and hypertension subjects, the consumption of partially defatted Brazil nut flour for 12 weeks reduced the total cholesterol but did not change LDL, HDL, and triglycerides (Carvalho et al., 2015). Concerning the four RCTs with cashew nuts, supplementation of these nuts (30–108g/day, for 8–12 weeks) did not affect total cholesterol, LDL, HDL, triglycerides, or VLDL ^[36–38,70], while only Mah et al. (2017) found a decrease in LDL and total cholesterol in dyslipidemic adults after ten weeks of cashew nut intake (32–64g/day). The evidence about Brazil and cashew nuts effect on lipid profile is still controversial. In the present study, 8-week intervention with these nuts containing high proportions of MUFA did not change total cholesterol, LDL, HDL, VLDL, and triglycerides compared to the control group, which was supplemented with polyunsaturated fat. Perhaps, the lipid profile markers at the normal range at baseline may have influenced the effect of Brazil and cashew nuts intake on these markers.

Apolipoproteins are structural and functional proteins of the lipoprotein particles that conduct the lipids to the organism's target organs and tissues ^[71]. Our investigation showed that 8-week mixed nut intake did not affect the concentrations of Apo AI, Apo B or Apo E, and Apo B/Apo AI ratio. This result supports the no effect observed on lipid profile markers. The lack of significant changes in apolipoproteins has also been observed in previous human studies with Brazilian nuts. The cashew nut consumption for 12 weeks did not modify Apo AI, Apo AII, and Apo B in healthy individuals ^[38]. The other two studies did not observe changes in apolipoproteins after 45g of Brazil nut daily or 15g of partially defatted Brazil nut flour for 15 days and 12 weeks, respectively ^[28,33]. Furthermore, the literature suggests that for different mechanisms, some

components of nuts - magnesium, fiber, α -linolenic acid, L-arginine, antioxidants, and MUFA - may protect against insulin resistance. However, like the apolipoproteins result, glucose homeostasis markers remained unchanged after daily Brazil and cashew nuts intake ^[72].

Nuts are complex food matrices that contain macro and micronutrients previously associated with blood pressure regulation and endothelial function improvement. Unsaturated fatty acids have well-established macro and microvascular functions and can regulate blood pressure ^[73,74]. Nutrients and bioactive components in nuts, such as alpha-linolenic acid, L-arginine, fiber, and polyphenols, may modulate inflammation and the development of endothelial dysfunction ^[72]. Besides, micronutrients of nuts, such as magnesium, potassium, and calcium, may involve several blood pressure regulation mechanisms ^[75-77]. Regardless of the benefits attributed to their nutrients, we observed no changes in NO plasma concentration and systolic or diastolic blood pressure after Brazilian nuts intake. To our knowledge, only two clinical trials investigated the effect of Brazil nut on blood pressure, and similarly, no difference was observed ^[33,34]. For cashew nut, two studies ^[38,70] observe no effect on blood pressure regulation, and one ^[42] showed a reduction only in systolic blood pressure. Additionally, the most recent meta-analyses evaluating 61 RCTs about tree nuts effect on blood pressure showed no effect ^[78]. Thus, in the dietary context, merely the tree nuts intake for 8-weeks seems not to be enough to promote blood pressure benefits. Additionally, it should be noted that all self-declared high blood pressure volunteers used antihypertensive drugs at baseline. Therefore, as well as lipid profile, the mean of SBP and DBP were at the normal range at baseline, which might have minimized the effect of nuts intake.

Concerning endothelial function, two recent meta-analyses showed a positive effect of tree nuts consumption on flow-mediated dilation (FMD), a traditional indicator of endothelial function ^[79,80]. However, the no effect on biomarkers of endothelial dysfunction, such as soluble cellular adhesion molecules (ICAM-1, VCAM-1), indicates a lack of consistent evidence for the effects of nut consumption on endothelial inflammation ^[79]. For Brazilian nuts, only one trial ^[38] evaluated the effect of cashew nut on ICAM-1 and VCAM-1, and no effect was verified. Regarding the Brazil nut, no available study assessed soluble adhesion molecules. Herein, we observe a significant reduction of 24.3% VCAM-1 concentration after Brazil and cashew nut intake.

Complementary to our results, the most comprehensive available metaanalysis about the nuts effect on inflammatory markers also verifies, in its subgroup analyses, a significant reduction in VCAM-1 levels after nut interventions, suggesting that nut consumption may have beneficial effects on endothelial function ^[81]. However, the mechanisms through which nuts consumption may modulate the endothelial function have not been evident. Preclinical trials suggest that oleic acid, the major fatty acid in the nuts mixture provided, may inhibit the expression of adhesion molecules in endothelial cells ^[82,83]. Similarly, selenium may also reduce the expression of ICAM-1, VCAM-1, and E selectin in endothelial cell surfaces ^[84]. While human studies are not available, the above-mentioned preclinical studies support, at least in part, the beneficial effect of Brazil and cashew nuts on VCAM-1 levels and consequently on endothelial inflammation.

The ABI was initially proposed as a noninvasive diagnostic method for lower-extremity peripheral artery disease. Later, it came to be used as an indicator of atherosclerosis, serving as a prognostic marker for cardiovascular events and functional impairment, even in the absence of symptoms ^[51,85]. This study is the first to evaluate the Brazilian nuts intake on ABI, but we did not find a significant ABI change in both groups. However, all women showed normal values for ABI at baseline, suggesting no peripheral arterial impairment to suffer modulation by the dietary intervention.

The beneficial effect of Brazil nut intake on selenium status has been demonstrated for all studies that could be found ^[28,29,89–91,30–34,86–88]. Toward the optimal activity of selenium-dependent proteins, such as the glutathione peroxidases and selenoprotein P, serum selenium values should be between 100 and 130 µg/L ^[92]. In our investigation, all women did have low plasma selenium levels at baseline. However, at 8-week, plasma selenium was significantly improved in the BN-group, although 57.2% of these women have remained with plasma Se below the expected values. This result can be explained by the Se quantity provided in the mixed nut (66 µg by portion), which was below the amount commonly provided by the long-term studies with Brazil nut (at least 200 µg of Se/day). Regarding the potential cardiovascular benefits of selenium, evidence show selenoproteins prevent oxidative modification of lipids, inhibit platelet aggregation, and reduce inflammation ^[93]. Thus, it is plausible to suggest that any plasma selenium improvement already contributes to reducing cardiovascular risk.

Despite the attempts to control the women's food intake, they did not achieve the planned energy restriction (-500 kcal/day), according to the evaluation of the basal and final food intake by 24-hour food record. Nevertheless, the energy restriction was similar between groups after follow-up. The free live condition can explain this result since variations in food available might interfere with energy intake control, despite closely nutritional monitoring over the study. However, the decreases in cholesterol intake demonstrate a diet modification with a potentially positive effect on cardiometabolic risk, independent of energy restriction.

This study has some limitations. First, there was a high percentage of lost follow-up, leading to missing data with a non-random nature (final assessments), making it impossible to carry out the ITT analysis. Additionally, besides the statistical analysis, the small sample size investigated and short-term intervention limits our conclusions. Also, due to the not blinded design, we have to consider that some changes in diet, of which we were not aware, might have happened. Finally, it is essential to note that the mean of all investigated metabolic risk markers showed values at the normal range at baseline, explaining, at least in part, the lack of impact of Brazil and cashew nuts intake on these markers. On the other hand, the present study's strength was the close control of lifestyle throughout the intervention period, which minimizes its influence on the outcomes. Furthermore, this clinical trial represents the first scientific evidence about the effects of a mixed with Brazilian nuts with an energy-restriction diet on body composition, traditional cardiometabolic risk factors, and endothelial function in cardiometabolic risk women.

CONCLUSION

In this 8-week dietary intervention study, Brazil and cashew nut intake within an energy-restricted diet improves body composition, reduces the VCAM-1, an endothelial inflammation marker, and enhances selenium status. Thus, Brazilian nuts intake can potentially improve dietary strategies for obesity control and CVD prevention. However, more studies should be carried out to investigate the present results.

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Conflict of interest

The authors declare no conflict of interest.

Authorship

A.P.S.C, D.M.U.P.R, H.H.M.H, and J.B. designed the experiment. A.P.S.C, D.M.U.P.R, conducted the research and collected data. A.P.D, A.P.S.C, and D.M.U.P.R. analyzed the data. A.P.S.C. wrote the manuscript. A.P.S.C, D.M.U.P.R, H.H.M.H, and J.B. edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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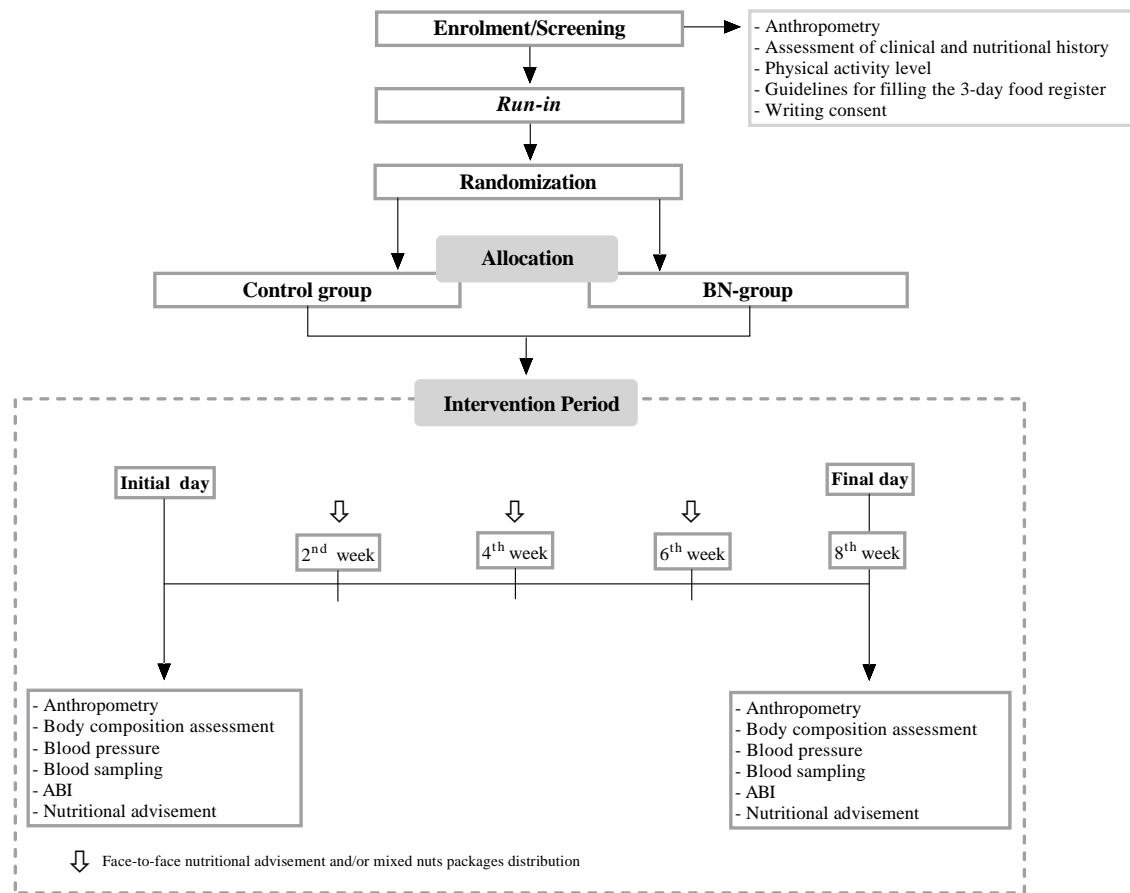


Figure 1. Study design schematic. ABI, Ankle-brachial index.

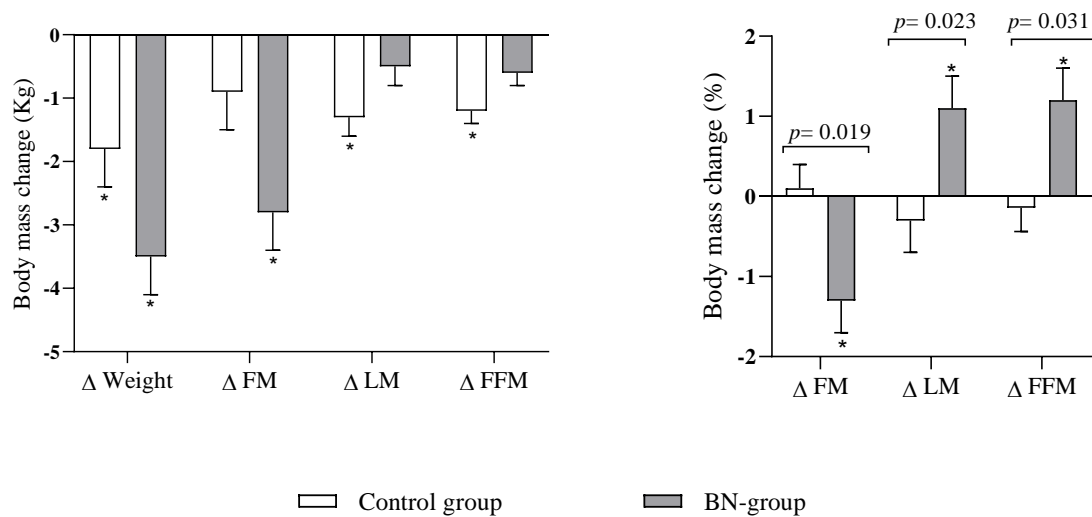
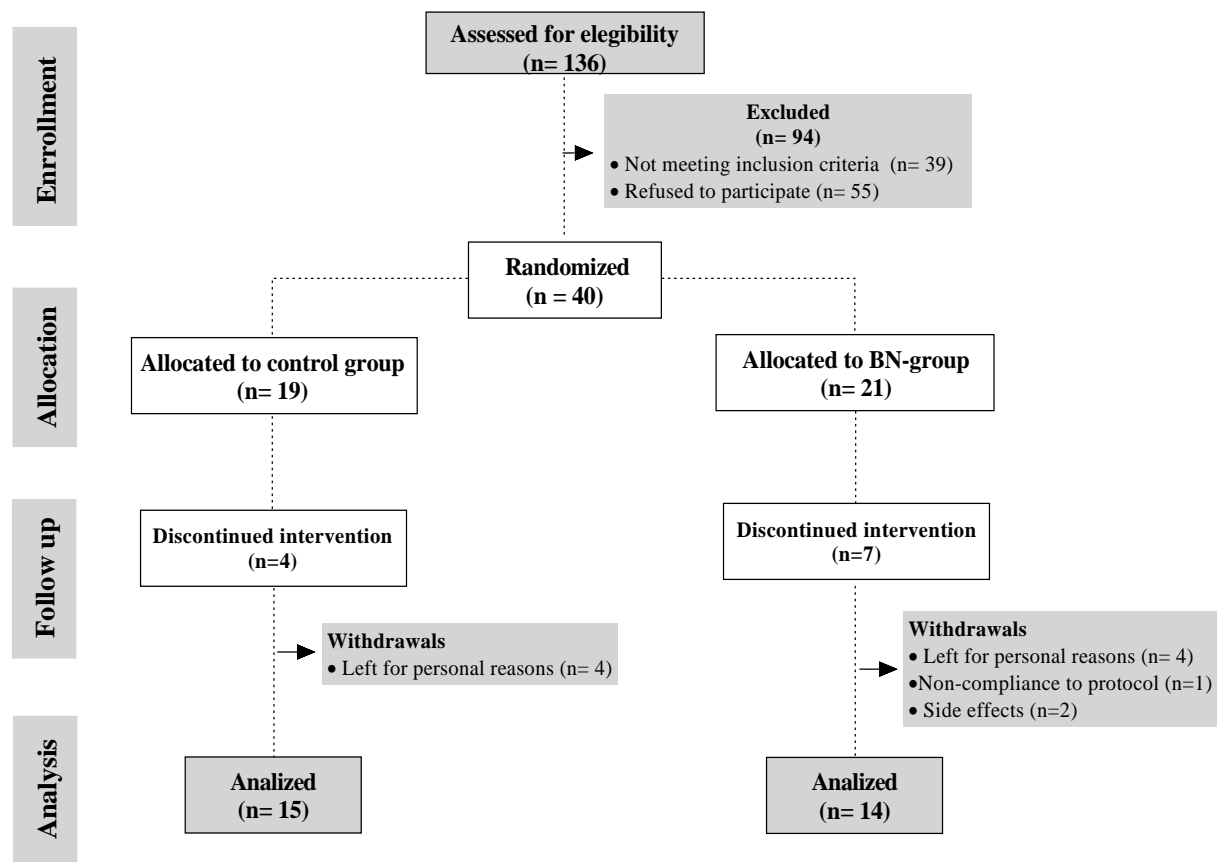


Figure 2. Body mass changes. Values are mean \pm SEM. Δ = final – baseline assessment; FM, fat mass; LM, lean mass; FFM, free fat mass. *Significant difference within-group ($p < 0.05$; paired t-test or Wilcoxon test). p-values refer to between-groups comparison (independent-samples t-test or Mann-Whitney U test).



Supplemental Figure 1. CONSORT flow diagram.

Table 1. Effect of 8-week intervention on anthropometric and body composition characteristics according to the diet groups.

	Control (n= 15)		BN-group (n= 14)		Δ <i>p</i> -values
	Baseline	Δ	Baseline	Δ	
<i>Anthropometry</i>					
Age (years)	31.6 \pm 2.1	-	31.2 \pm 2.7	-	
Body weight (kg)	87.9 \pm 3.9	-1.8 \pm 0.6*	90.5 \pm 3.8	-3.5 \pm 0.6**	0.073
BMI (kg/m ²)	33.0 \pm 1.0	-0.6 \pm 0.2*	33.8 \pm 1.2	-1.3 \pm 0.2**	0.071
Waist (cm)	107.7 \pm 2.6	-2.9 \pm 0.6**	107.7 \pm 2.8	-5.5 \pm 1.1**	0.104
Hip (cm)	117.2 \pm 2.6	-1.9 \pm 0.6*	116.7 \pm 2.8	-1.9 \pm 0.8*	0.961
Neck (cm)	36.8 \pm 0.8	-1.2 \pm 0.4*	36.9 \pm 0.6	-0.6 \pm 0.2*	0.282
WHtR	0.6 \pm 0.01	-0.01 \pm 0.004**	0.65 \pm 0.01	-0.034 \pm 0.007**	0.064
WHR	0.9 \pm 0.01	-0.01 \pm 0.004*	0.92 \pm 0.01	-0.032 \pm 0.01*	0.073
<i>Body composition (DEXA) [§]</i>					
Total fat mass (kg)	42.04 \pm 2.4	-0.9 \pm 0.6	43.9 \pm 2.7	-2.8 \pm 0.6*	0.065
Total body fat (%)	48.08 \pm 1.3	0.1 \pm 0.3	48.7 \pm 1.0	-1.3 \pm 0.4*	0.019
Total lean mass (kg)	41.9 \pm 1.8	-1.3 \pm 0.3*	42.5 \pm 1.3	-0.5 \pm 0.3	0.106
Total lean mass (%)	48.6 \pm 1.3	-0.3 \pm 0.4	47.9 \pm 1.0	1.1 \pm 0.4*	0.023
Total fat free mass (kg)	44.8 \pm 1.8	-1.2 \pm 0.2*	45.4 \pm 1.3	-0.6 \pm 0.2	0.142

Total fat free mass (%)	51.9 ± 1.3	-0.14 ± 0.3	51.2 ± 1.0	1.2 ± 0.4*	0.031
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Values are mean ± SEM. Δ = final – baseline assessment. * $p \leq 0.05$ or ** $p \leq 0.001$ are significant differences within-group (paired t-test or Wilcoxon test). Δp -values refer to the comparison between groups (independent-samples t-test or Mann-Whitney U test). BMI, body mass index; n.s, non-significant. WHtR, Waist-to-height ratio; WHR, Waist-to-hip ratio. [§] Subsample analyze (control $n=10$; mixed nut $n=11$).

Table 2. Effect of 8-week intervention on cardiometabolic risk markers and endothelial function according to the diet groups.

	Control (n= 15)		BN-group (n= 14)		Δ <i>p</i> -values
	Baseline	Δ	baseline	Δ	
<i>Cardiometabolic risk markers</i>					
Total cholesterol (mg/dL)	173.9 \pm 8.8	-7.4 \pm 3.03*	172.8 \pm 7.3	-8.3 \pm 2.6*	0.828
Triglycerides	128.5 \pm 26.8	4.1 \pm 10.0	109.2 \pm 14.4	-4.64 \pm 14.1	0.377
LDL-c (mg/dL)	89.4 \pm 6.08	-4.8 \pm 1.6*	87.8 \pm 6.3	-5.5 \pm 2.3*	0.787
HDL-c (mg/dL)	49.6 \pm 3.2	-1.4 \pm 2.3	55.7 \pm 3.8	-3.7 \pm 2.5	0.524
VLDL-c (mg/dL)	25.7 \pm 5.3	0.8 \pm 2.01	21.8 \pm 2.8	-0.9 \pm 2.8	0.377
Non-HDL-c	124.3 \pm 6.81	-6.0 \pm 2.8*	117.1 \pm 8.6	-4.6 \pm 3.4	0.914
Total cholesterol:HDL-c	3.6 \pm 1.6	-0.02 \pm 0.1	3.2 \pm 0.2	0.01 \pm 0.1	0.826
LDL-c:HDL-c	1.8 \pm 0.1	-0.03 \pm 0.08	1.7 \pm 01	-0.01 \pm 0.06	0.870
Fasting glucose (mg/dL)	97.7 \pm 2.5	0.2 \pm 2.3	94.0 \pm 2.4	0.3 \pm 2.5	0.979
Insulin (μ UI/mL)	11.3 \pm 1.2	-0.7 \pm 1.2	14.4 \pm 2.8	-0.16 \pm 1.6	0.621
TyG index	8.5 \pm 0.1	-0.009 \pm 0.09	8.4 \pm 0.1	-0.04 \pm 0.1	0.822
Apo AI (mg/dL)	128.5 \pm 7.2	-2.4 \pm 5.2	131.2 \pm 7.09	-2.7 \pm 3.2	0.960
Apo B (mg/dL)	82.0 \pm 3.9	-2.4 \pm 1.2	79.6 \pm 4.2	-4.1 \pm 2.06	0.492
Apo E (mg/L)	42.1 \pm 4.9	-0.9 \pm 2.0	38.5 \pm 3.9	0.22 \pm 2.8	0.743
ApoB/ApoA	0.6 \pm 0.03	0.009 \pm 0.02	0.6 \pm 0.05	-0.02 \pm 0.02	0.477

hs-PCR (mg/dL)	5.1 ± 0.8	0.1 ± 0.8	4.4 ± 0.7	-0.6 ± 0.4	0.447
<i>Selenium status marker</i>					
Se (µg/L)	57.4 ± 3.8	8.9 ± 7.3	57.6 ± 4.1	35.4 ± 7.2**	0.010
<i>Endothelial function markers</i>					
DBP (mmHg)	80.3 ± 1.4	-4.2 ± 1.4*	80.2 ± 1.7	-4.0 ± 1.3*	0.920
SBP (mmHg)	117.2 ± 2.2	-2.4 ± 2.1	119.7 ± 2.7	-3.8 ± 2.7	0.688
ABI	1.1 ± 0.03	0.02 ± 0.03	1.07 ± 0.04	-0.02 ± 0.02	0.374
NO (µM/mL)	27.7 ± 15.9	1.3 ± 2.05	15.0 ± 1.8	0.9 ± 2.9	0.913
ICAM-1 (pg/mL)	3,538.7 ± 207.1	35.3 ± 366.2	3,106.6 ± 311.2	79.5 ± 449.7	0.939
VCAM-1 (pg/mL)	85.0 ± 7.5	24.3 ± 14.6	92.6 ± 8.4	-25.8 ± 10.4*	0.010

Values are mean ± SEM. Δ = final – baseline assessment * $p \leq 0.05$ or ** $p \leq 0.001$ are significant differences within-group (paired t-test or Wilcoxon test). Δ p -values refer to the comparison between groups (independent-samples t-test or Mann-Whitney U test). ABI, ankle-brachial index; Apo A, Apolipoprotein A; Apo B, Apolipoprotein B; Apo E, Apolipoprotein E; hs-PCR, high sensitivity protein C reactive; HDL, high-density lipoprotein; ICAM-1, intercellular adhesion molecule-1; LDL, low-density lipoprotein; n.s, non-significant; VLDL-c, very low-density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; VCAM-1, Vascular cell adhesion molecule-1.

Table 3. Dietary compliance of women who completed the study according to diet groups.

Daily intake	Control group (n= 15)		BN-group (n= 14)		Δ p-values
	Baseline	Δ	Baseline	Δ	
Total energy intake (kcal)	1,747.6 \pm 183.3	-201.9 \pm 206.6	1,810.3 \pm 109.6	-287.7 \pm 107.3*	0.339
Carbohydrate (g)	230.3 \pm 22.1	-44.0 \pm 23.9	224.0 \pm 20.3	-51.9 \pm 17.0*	0.794
Protein (g)	78.9 \pm 8.8	-5.6 \pm 10.0	78.6 \pm 6.0	-3.0 \pm 7.8	1.000
Total fat (g)	58.2 \pm 7.8	0.6 \pm 9.6	68.2 \pm 5.0	-6.4 \pm 5.8	0.551
MUFA (g)	19.04 \pm 3.1	-1.0 \pm 3.9	23.1 \pm 1.9	1.9 \pm 2.1*	0.009[†]
PUFA (g)	10.7 \pm 1.8	6.6 \pm 1.8*	13.2 \pm 1.3	-1.1 \pm 1.4	<0.001[†]
SFA (g)	20.8 \pm 2.5	-4.0 \pm 3.5	23.6 \pm 1.7	-4.9 \pm 2.0*	0.843
Cholesterol (mg)	322.4 \pm 40.7	-96.8 \pm 37.3*	370.4 \pm 35.0	-113.7 \pm 29.3*	0.713
Fiber (g)	20.7 \pm 2.9	1.5 \pm 3.5	16.4 \pm 1.9	2.9 \pm 1.8	0.786

Values are mean \pm SEM. Δ = final – baseline assessment * $p \leq 0.05$ significant differences within-group (paired t-test or Wilcoxon test). Δ p-values refer to the comparison between groups (independent-samples t-test or Mann-Whitney U test). [†] One-way ANCOVA adjusted by baseline value. MUFA, monounsaturated fatty acid; n.s, non-significant; PUFA, polyunsaturated fatty acid; and SFA, saturated fatty acid.

Supplemental Table 1. Fatty acid profile of the Brazil nuts and Cashew nuts (% of total fatty acids)

Fatty Acids	Brazil nut	Cashew nut
Palmitic acid (C16:0)	14.0	8.19
Stearic acid (C18:0)	10.0	7.23
Oleic acid (C18:1n9)	36.3	67.0
Linoleic acid (C18:2n6)	39.5	17.5
Alpha-linolenic acid (C18:3n3)	0.1	0.07
Total SFA	24.0	15.4
Total MUFA	36.3	67.0
Total PUFA	39.6	17.6

Values are mean of triplicate

Supplemental Table 2. Intra and inter-assay coefficient variation of biochemical measures.

Biochemical measures	Intra-assay CV (%)	Inter-assay CV (%)
LDL-c	1.40	1.43
HDL-c	1.50	0.11
Total cholesterol	1.08	2.75
TG	0.61	0.46
Glucose	3.61	0.52
hs-CRP	2.81	5.9
ApoA	5.0	7.5
ApoB	5.0	7.5
ICAM-1	<10%	<10%
VCAM-1	5.20	4.34

CV, coefficient variation.

Supplemental Table 3 Effect of 8-week intervention on body composition according to diet groups.

	Control (n= 15)		BN-group (n= 14)		Δ <i>p</i> -value
	Baseline	Δ	Baseline	Δ	
<i>Body composition (DEXA) [§]</i>					
Gynoid lean mass (kg)	6.3 ± 0.3	-0.17 ± 0.06*	6.6 ± 0.2	-0.2 ± 0.1	0.561
Gynoid lean mass (%)	44.2 ± 1.4	-0.3 ± 0.4	45.03 ± 1.2	0.6 ± 0.6	0.203
Gynoid fat mass (kg)	7.9 ± 0.5	-0.2 ± 0.1	8.03 ± 0.5	-0.3 ± 0.1*	0.543
Gynoid fat mass percentage (%)	55.8 ± 1.2	-0.8 ± 1.4	56.3 ± 1.1	-5.4 ± 1.7*	0.057
Gynoid fat free mass (kg)	6.6 ± 0.3	-0.1 ± 0.06*	6.9 ± 0.2	-0.2 ± 0.1	0.508
Gynoid fat free mass (%)	46.1 ± 1.4	-0.2 ± 0.4	47.0 ± 1.2	0.7 ± 0.6	0.216
Android lean mass (kg)	3.01 ± 0.1	-0.2 ± 0.04*	3.01±0.1	-0.1 ± 0.05	0.206
Android lean mass (%)	43.2 ± 1.2	0.2 ± 0.6	42.6 ± 1.1	1.2 ± 0.5*	0.263
Android fat mass (kg)	3.9 ± 0.2	-0.2 ± 0.06*	4.07 ± 0.3	-0.3 ± 0.1*	0.794
Android fat mass (%)	49.5 ± 1.7	4.1 ± 1.4*	48.3 ± 1.6	8.5± 1.5*	0.045
Android fat free mass (kg)	3.0 ± 0.1	-0.2 ± 0.04*	3.08 ± 1.5	-0.1± 0.05	0.262
Android fat free mass (%)	44.1 ± 1.2	0.4 ± 0.6	43.6 ± 1.1	1.2 ± 0.5	0.382
Truncal lean mass (kg)	20.0 ± 0.9	-1.2 ± 0.3*	19.8± 0.7	0.08 ± 0.2	0.004
Truncal lean mass (%)	47.2± 1.4	-1.0 ± 0.7	45.2 ± 1.1	1.9 ± 0.7*	0.010
Truncal fat mass (kg)	21.6 ± 1.2	-0.7 ± 0.4	23.2 ± 1.4	-1.5 ± 0.4*	0.218
Truncal fat mass e (%)	42.6 ± 1.8	9.8 ± 2.0*	44.1 ± 2.0	6.5 ± 2.0*	0.272

Truncal fat free mass (kg)	20.9 ± 0.9	-1.2 ± 0.3**	20.8 ± 0.7	0.03 ± 0.2**	0.003
Truncal fat free mass (%)	49.4 ± 1.4	-0.7 ± 0.6	47.6 ± 1.1	1.8 ± 0.6*	0.013

Values are mean ± SEM. Δ = final – baseline assessment. Significant differences within-group are expressed by * $p \leq 0.05$ or ** $p \leq 0.001$ (paired t-test or Wilcoxon test). Δ p -values refer to the comparison between groups (independent-samples t-test or Mann-Whitney U test). BMI, body mass index; WHtR, Waist-to-height ratio; WHR, Waist-to-hip ratio. [§] Subsample analyze (control $n=10$; mixed nut $n=11$).