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

Ana Paula Silva Caldas[1]*, Daniela Mayumi Usuda Prado Rocha[1], Ana Paula Dionísio[2], Helen Hermana Miranda Hermsdorff[1] and Josefina Bressan[1]

[1]*Universidade Federal de Viçosa, Viçosa, Minas Gerais, 36570-900, Brazil*
[2]*Embrapa Agroindústria Tropical, Fortaleza, Ceará, 60511-110, Brazil*

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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 randomised 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 + 30 g 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 Se concentration increased in BN-group ($\Delta = +31{\cdot}5$ (SEM 7·8) μg/l; $P = 0{\cdot}001$). Brazil and cashew nuts intake reduced total body fat (−1·3 (SEM 0·4) %) parallel to improvement of lean mass percentage in BN-group compared with the control. Besides, the soluble adhesion molecule VCAM-1 decreased (24·03 (SEM 15·7) pg/ml *v.* −22·2 (SEM 10·3) pg/ml; $P = 0{\cdot}019$) after Brazil and cashew nuts intake compared with 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, Se status and endothelial inflammation in cardiometabolic risk women.

Key words: **Brazil nut: Cashew nut: Obesity: Weight loss: VCAM-1: Body fat**

Obesity is well established as a main risk factor for CVD. The effects of obesity on CVD are associated with other metabolic risk factors such as insulin resistance, hypertension, hypercholesterolaemia and hyperglycaemia[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, TAG, LDL-cholesterol and non-HDL-cholesterol[4–7]. Regardless of the cause of obesity, reduction in energy intake and increase of physical activity practice remain 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, glycaemic 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 fibre 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 Se

Abbreviations: ABI, ankle-brachial index; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1.

* **Corresponding author**: Ana Paula Silva Caldas, email paulacaldas06@hotmail.com

(100–1000 mg of Se/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-cholesterol 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-energetic 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[28–39]. 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 hypothesised that an energy-restricted diet parallel to Brazil and cashew nuts intake would potentiate the improvements in evaluated variables.

Methodology

Study design, participants and recruitment

The Brazilian Nuts Study is a randomised, 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 ≥ 27 kg/m^2 and < 30 kg/m^2), waist circumference ≥ 80 cm and body fat percentage ≥ 32 % associated with at last one another component of metabolic syndrome: TAG ≥150 mg/dl, high blood pressure arterial (≥ 130/85 mmHg) or high fasting glucose (≥ 100 mg/dl) or women with obesity (BMI ≥ 30 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 > 30 g/d; alcohol consumption higher than twenty-one units (168 g) per week; dental problems that interfere with chewing and use of vitamin, mineral and *n*-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, Se 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.

Dietary intervention

Before the intervention, a run-in period of 7 to 10 d 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/d) containing 45 g (30 g of cashew plus 15 g 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 to fasten blood sample collection, anthropometry, body composition evaluation and fill out questionnaires about physical activity practice, food intake-behaviour and eating behaviour.

For 48-hour before the procedures, all women were asked to avoid caffeine and alcohol and maintain their habitual physical activity levels. Additionally, every 15 d, the women attended the Laboratory of Energy Metabolism and Body Composition 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 (Fig. 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 Se 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.

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 for adult women with overweight or obesity[40]; then, 500 kcal/d 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

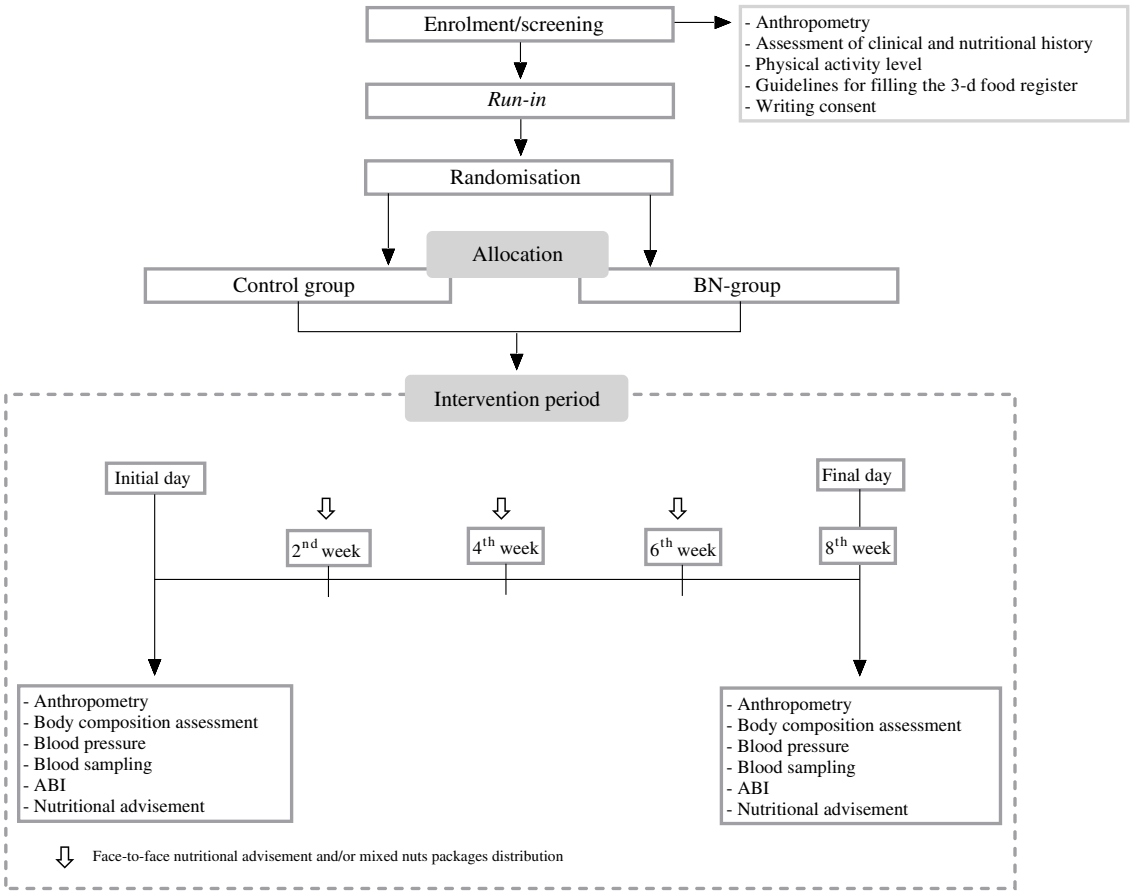

Fig. 1. Study design schematic. ABI, ankle-brachial index.

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 soya oil and lemon (2:1 ratio, respectively), which was prepared in the Metabolic Kitchen of Laboratory of Energy Metabolism and Body Composition and its energies also included in the total energetic 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 individualised and provided by dietitians every two weeks. Participants received instructions to use only soya oil throughout the study period to prepare meals consumed over the day.

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 (15 g 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 Se content to meet daily Se recommendation; the 30 g of cashew nut is supported by previously published studies evaluating tree nuts' cardiovascular benefits[41–43]. The Se content in the Brazil nut was measured by inductively coupled plasma atomic emission spectrometry[44]. Each portion of 15 g of Brazil nut provides approximately 51 μg of Se. Also, we determined the lipid profile of Brazil and cashew nut by GC following the protocol proposed by Folch *et al.*[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 (online Supplemental Table 1).

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.

Cardiometabolic risk markers

Serum LDL-cholesterol, HDL-cholesterol, total cholesterol, TAG and glucose were analysed by the colorimetric enzymatic method using a commercial kit Bioclin® (Belo Horizonte) in

the automatic analyser (BS200 Mindray®, Nanshan). Insulin and high-sensitive C-reactive protein were quantified in fasting serum by automated analyser systems using commercial assay kits. Apolipoproteins were assessed using Immunochemistry Systems MMAGE® (Beckman Coulter, Inc. EUA). The serum very-low-density lipoprotein cholesterol was calculated using the Friedewald equations[47]. Non-HDL-cholesterol was calculated as total cholesterol – HDL-cholesterol. The total-cholesterol:HDL-cholesterol and LDL-cholesterol:HDL-cholesterol ratios were also computed[48]. Insulin resistance was evaluated using TyG index, calculated by the formula Ln [fasting TAG (mg/dl) × fasting glucose (mg/dl)/2][49]. Intra- and inter-assay coefficient variation of biochemical measures could be found in online Supplemental Table 2.

Endothelial function markers

The adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) were assessed using commercial ELISA kits following the manufacturer's recommendations (Elabscience Biotechnology Co., Ltd). Nitric oxide concentration was determined in triplicate using the Griess reagent according to the protocol proposed by Grisham *et al.*[50].

Before ankle-brachial index (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 ($\leq$ 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 standardised for the following measurements. Blood pressure was measured using an automatic monitor (Omron Healthcare, Inc., Model OMRON HEM 7200). The average of two additional measurements was used for participants with blood pressure in the hypertensive range.

Plasma selenium concentrations

A commercial lab determined the plasma Se level using the inductively coupled plasma MS method according to standardised protocols.

Dietary assessment

Before the study baseline assessments, all women completed one 3-d food record (two nonconsecutive weekdays and one weekend day). During the follow-up, a 24-h dietary recall was applied by dietitians every 15 d to estimate the mean intake of energy, macronutrients and fibre during the intervention period. For both used methods, a dietitian checked all reported food and its respective quantities. The food records and 24-h dietary recalls were analysed using REC24h-ERICA software, adapted for the Brazilian population.

Anthropometry and body composition

Body weight was assessed by a bioelectrical impedance analysis device (Inbody 230, Biospace Corp.). 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 utilising 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 (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 dual-energy X-ray absorptiometry 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].

Randomisation

The minimisation method was employed for randomisation 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 randomisation procedure using the WinPepi software, version 11·65 (Copyright J.H. Abramsom, 23 August 2016).

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 $\geq$ 11 completers per arm would allow detecting a difference clinically meaningful 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 programme. The sample size was estimated as proposed by Mera *et al.* (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', the use of the multiple imputation method is not recommended[56]. Therefore, the intention to treat analyses were not possible. All statistical analyses used the Statistical Package for the Social Sciences software Version 23.0 for Windows (SPSS), and a *P*-value $<$ 0·05 was considered statistically significant. Figures displaying statistical analysis were produced using Prism 6 (GraphPad, La Jolla). 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 ± SEM.

Results

Forty women were randomised, 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 %) (online Supplemental Fig. 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 (SEM 1·6) years and 33·4 (SEM 0·7) kg/m^2. At baseline, there was no significant difference between groups for cardiometabolic risk markers, endothelial function markers, anthropometric and body composition variables and plasma Se 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; Fig. 2). In contrast, the BN-group women exhibited body composition improvement compared with 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 with the control group. However, the android fat mass was higher in the BN-group than in the control group (online Supplemental Table 3). Regarding cardiometabolic risk factors, both groups showed a similar reduction in total cholesterol, LDL-cholesterol and systolic blood pressure. Interestingly, VCAM-1 significantly reduced after Brazil and cashew nuts consumption compared with the control group (Table 2).

Basal plasma Se was 57·4 (SEM 3·8) μg/l and 57·6 (SEM 4·1) μg/l in control and mixed nut, respectively. All women presented low plasma Se (<100 μg/l) before this study[57]. After intervention, BN-group showed a higher increase in plasma Se (Δ = + 35·4 (SEM 7·2) μg/l; $P = 0{\cdot}001$) in comparison with control group (Δ= +8·9 (SEM 7·3) μg/l; $P = 0{\cdot}157$). Furthermore, 86·7 % (*n* 13) in the control group and 57·2 % (*n* 8) in the BN-group remained with plasma Se < 100 μg/l (Table 2). Regarding food intake, compared with baseline, the energy intake decreased similarly, −201·9 (SEM 206·6) kcal and −287·7 (SEM 107·3) 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 fibre intake remained comparable between groups after intervention. As expected, at 8 weeks, MUFA intake was higher in the BN-group than in the control group (Δ −1·0 (SEM 3·9) g *v.* Δ 1·9 (SEM 2·1) g; $P = 0{\cdot}009$), while the PUFA intake was the opposite (Δ 6·6 (SEM 1·8) g *v.* Δ −1·1 (SEM 1·4) g; $P =< 0{\cdot}001$).

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,

Table 1. Effect of 8-week intervention on anthropometric and body composition characteristics according to the diet groups (Mean values with their standard errors of the mean)

	Control (*n* 15)				BN-group (*n* 14)				
	Baseline		Δ		Baseline		Δ		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Δ *P*-values
Anthropometry									
Age (years)	31·6	2·1	–		31·2	2·7	–		
Body weight (kg)	87·9	3·9	−1·8	0·6*	90·5	3·8	−3·5	0·6**	0·073
BMI (kg/m^2)	33·0	1·0	−0·6	0·2*	33·8	1·2	−1·3	0·2**	0·071
Waist (cm)	107·7	2·6	−2·9	0·6**	107·7	2·8	−5·5	1·1**	0·104
Hip (cm)	117·2	2·6	−1·9	0·6*	116·7	2·8	−1·9	0·8*	0·961
Neck (cm)	36·8	0·8	−1·2	0·4*	36·9	0·6	−0·6	0·2*	0·282
WHtR	0·6	0·01	−0·01	0·004**	0·65	0·01	−0·034	0·007**	0·064
WHR	0·9	0·01	−0·01	0·004*	0·92	0·01	−0·032	0·01*	0·073
Body composition (DEXA)†									
Total fat mass (kg)	42·04	2·4	−0·9	0·6	43·9	2·7	−2·8	0·6*	0·065
Total body fat (%)	48·08	1·3	0·1	0·3	48·7	1·0	−1·3	0·4*	0·019
Total lean mass (kg)	41·9	1·8	−1·3	0·3*	42·5	1·3	−0·5	0·3	0·106
Total lean mass (%)	48·6	1·3	−0·3	0·4	47·9	1·0	1·1	0·4*	0·023
Total fat free mass (kg)	44·8	1·8	−1·2	0·2*	45·4	1·3	−0·6	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

Δ = final – baseline assessment. *$P \leq 0{\cdot}05$ or **$P \leq 0{\cdot}001$ 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). WHtR, waist-to-height ratio; WHR, waist-to-hip ratio.
† Subsample analyse (control *n* 10; mixed nut *n* 11).

British Journal of Nutrition

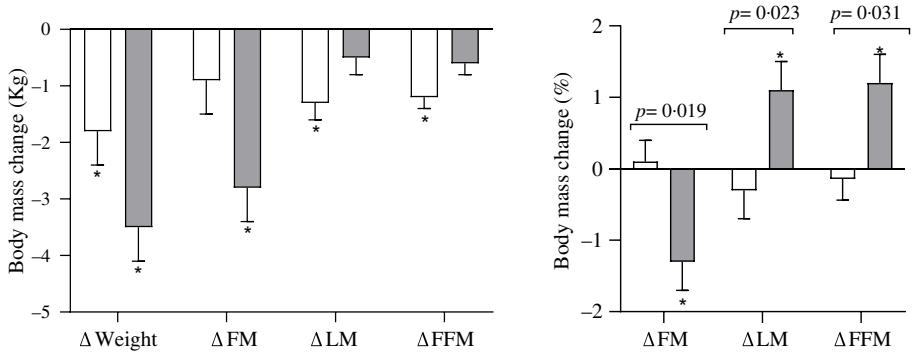

Fig. 2. Body mass changes. Values are mean ± SEM. Δ = final – baseline assessment; FM, fat mass; LM, lean mass; FFM, free fat mass. *Significant difference within-group ($P < 0·05$; paired *t* test or Wilcoxon test). *P*-values refer to between-groups comparison (independent-samples *t* test or Mann–Whitney *U* test).

Table 2. Effect of 8-week intervention on cardiometabolic risk markers and endothelial function according to the diet groups (Mean values with their standard errors of the mean)

	Control (*n* 15)				BN-group (*n* 14)				
	Baseline		Δ		Baseline		Δ		
Cardiometabolic risk markers	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Δ *P*-values
Total cholesterol (mg/dl)	173·9	8·8	–7·4	3·03*	172·8	7·3	–8·3	2·6*	0·828
TAG	128·5	26·8	4·1	10·0	109·2	14,4	–4·64	14·1	0·377
LDL-cholesterol (mg/dl)	89·4	6·08	–4·8	1·6*	87·8	6·3	–5·5	2·3*	0·787
HDL-cholesterol (mg/dl)	49·6	3·2	–1·4	2·3	55·7	3·8	–3·7	2·5	0·524
VLDL-cholesterol (mg/dl)	25·7	5·3	0·8	2·01	21·8	2·8	–0·9	2·8	0·377
Non-HDL-cholesterol	124·3	6·81	–6·0	2·8*	117·1	8·6	–4·6	3·4	0·914
Total cholesterol:HDL-cholesterol	3·6	1·6	–0·02	0·1	3·2	0·2	0·01	0·1	0·826
LDL-cholesterol:HDL-cholesterol	1·8	0·1	–0·03	0·08	1·7	01	–0·01	0·06	0·870
Fasting glucose (mg/dl)	97·7	2·5	0·2	2·3	94·0	2·4	0·3	2·5	0·979
Insulin (μUI/ml)	11·3	1·2	–0·7	1·2	14·4	2·8	–0·16	1·6	0·621
TyG index	8·5	0·1	–0·009	0·09	8·4	0·1	–0·04	0·1	0·822
Apo AI (mg/dl)	128·5	7·2	–2·4	5·2	131·2	7·09	–2·7	3·2	0·960
Apo B (mg/dl)	82·0	3·9	–2·4	1·2	79·6	4·2	–4·1	2·06	0·492
Apo E (mg/l)	42·1	4·9	–0·9	2·0	38·5	3·9	0·22	2·8	0·743
ApoB/ApoA	0·6	0·03	0·009	0·02	0·6	0·05	–0·02	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
Se status marker									
SE (μg/l)	57·4	3·8	8·9	7·3	57·6	4·1	35·4	7·2**	0·010
Endothelial function markers									
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)	3538·7	207·1	35·3	366·2	3106·6	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

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; ICAM-1, intercellular adhesion molecule-1; VLDL-cholesterol, very low-density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; VCAM-1, vascular cell adhesion molecule-1.

suggesting improvement of endothelial inflammation and enhanced plasma Se concentrations in women at cardiometabolic risk.

Weight control is a primary strategy to reduce the CVD 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 randomised controlled trial 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 oxidised and have a higher thermogenic effect than SFA, 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

Table 3. Dietary compliance of women who completed the study according to diet groups (Mean values with their standard errors of the mean)

	Control group (*n* 15)				BN-group (*n* 14)				
	Baseline		Δ		Baseline		Δ		
Daily intake	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Δ *P*-values
Total energy intake (kcal)	1747·6	183·3	−201·9	206·6	1810·3	109·6	−287·7	107·3*	0·339
Carbohydrate (g)	230·3	22·1	−44·0	23·9	224·0	20·3	−51·9	17·0*	0·794
Protein (g)	78·9	8·8	−5·6	10·0	78·6	6·0	−3·0	7·8	1·000
Total fat (g)	58·2	7·8	0·6	9·6	68·2	5·0	−6·4	5·8	0·551
MUFA (g)	19·04	3·1	−1·0	3·9	23·1	1·9	1·9	2·1*	0·009†
PUFA (g)	10·7	1·8	6·6	1·8*	13·2	1·3	−1·1	1·4	<0·001†
SFA (g)	20·8	2·5	−4·0	3·5	23·6	1·7	−4·9	2·0*	0·843
Cholesterol (mg)	322·4	40·7	−96·8	37·3*	370·4	35·0	−113·7	29·3*	0·713
Fibre (g)	20·7	2·9	1·5	3·5	16·4	1·9	2·9	1·8	0·786

Values are mean ± SEM. Δ = final − baseline assessment.
* $P \leq 0{\cdot}05$ 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.

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 (15–25 g/d) in adolescents with obesity reduced total cholesterol and LDL-cholesterol but did not affect HDL-cholesterol and TAG. In dyslipidaemia and hypertension subjects, the consumption of partially defatted Brazil nut flour for 12 weeks reduced the total cholesterol but did not change LDL-cholesterol, HDL-cholesterol and TAG (Carvalho et al., 2015). Concerning the four randomised controlled trial with cashew nuts, supplementation of these nuts (30–108 g/day, for 8–12 weeks) did not affect total cholesterol, LDL-cholesterol, HDL-cholesterol, TAG or very-low-density lipoprotein cholesterol[36–38,70], while only Mah *et al.* (2017) found a decrease in LDL-cholesterol and total cholesterol in dyslipidaemic adults after ten weeks of cashew nut intake (32–64 g/d). 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-cholesterol, HDL-cholesterol, very-low-density lipoprotein cholesterol and TAG compared with 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 45 g of Brazil nut daily or 15 g of partially defatted Brazil nut flour for 15 d and 12 weeks, respectively[28,33]. Furthermore, the literature suggests that for different mechanisms, some components of nuts – Mg, fibre, 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 α-linolenic acid, L-arginine, fibre and polyphenols, may modulate inflammation and the development of endothelial dysfunction[72]. Besides, micronutrients of nuts, such as Mg, potassium and Ca, 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 metanalyses evaluating sixty-one randomised controlled trial 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 minimised the effect of nuts intake.

Concerning endothelial function, two recent meta-analyses showed a positive effect of tree nuts consumption on flow-mediated dilation, 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 metanalysis 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, Se 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 Se status has been demonstrated for all studies that could be found[28–34,86–91]. Towards the optimal activity of Se-dependent proteins, such as the glutathione peroxidases and selenoprotein P, serum Se values should be between 100 and 130 μg/l[92]. In our investigation, all women did have low plasma Se levels at baseline. However, at 8-week, plasma Se 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 last 200 μg of Se/d). Regarding the potential cardiovascular benefits of Se, evidence shows that selenoproteins prevent oxidative modification of lipids, inhibit platelet aggregation and reduce inflammation[93]. Thus, it is plausible to suggest that any plasma Se 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/d), according to the evaluation of the basal and final food intake by 24-h 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 (at final assessments), making it impossible to carry out the intention to treat analysis. Additionally, besides the statistical analysis, the small sample size investigated and short-term intervention limit 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 minimises its influence on the outcomes. Furthermore, this clinical trial represents the first scientific evidence about the effects of a mixed with Brazilian nuts within 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 and endothelial inflammation marker and enhances Se 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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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. analysed 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.

The authors declare no conflict of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S000711452100475X

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