#### **ORIGINAL CONTRIBUTION**



## Acute consumption of a shake containing cashew and Brazil nuts did not affect appetite in overweight subjects: a randomized, cross-over study

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#### Abstract

**Purpose** Evidence from epidemiological and clinical studies suggests that nut consumption provides satiety and may contribute to the management of obesity. However, the effect of acute intake of nuts on appetite responses remains unclear. The objective of this study was to evaluate the acute effect of a shake containing 30 g of cashew nuts (*Anacardium occidentale L.*) and 15 g of Brazil nuts (*Bertholletia excelsa H.B.K*) on appetite responses in overweight subjects.

**Methods** This was a clinical, randomized, controlled, single-blind, cross-over, pilot study. On two non-consecutive test days, 15 subjects received a shake containing nuts, and a shake absent of nuts matched for energy and macronutrient content. Subjective appetite sensation was evaluated by visual analogue scales (VAS). Food intake was measured by weighing the lunch served at the end of each morning-test, which subjects ate ad libitum. Total energy intake was estimated by food records. This study is registered on the Brazilian Registers of Clinical Trials—ReBEC (protocol: U1111-1203-9891).

**Results** We observed no significant difference in subjective appetite sensations between the groups. Food intake at lunch, as well as energy intake throughout the day also did not differ between the treatments.

**Conclusion** Our results suggest that the acute intake of a shake containing nuts was not able to enhance satiety, compared to a shake matched for energy and macronutrient content. Further studies are warranted to elucidate the satiety mechanisms of nuts intake.

Keywords Brazil nuts · Cashew nuts · Obesity · Satiety · Hunger · Food intake

## Introduction

The prevalence of obesity almost tripled between 1975 and 2016 [1], becoming one of the main public health concerns worldwide [2]. Obesity is a major risk factor for non-communicable chronic diseases, including type 2 diabetes, cardiovascular diseases, dyslipidemia, and some forms of cancer [1, 3].

The etiology of obesity is complex and multifactorial, however, it is often considered the result of an imbalance between energy intake and energy expenditure [4]. Diet plays an essential role in weight control; therefore, the consumption of specific foods may be a useful strategy in the management of obesity [1].

Nuts, a group in which cashew and Brazil nuts are included, are rich in unsaturated fatty acids, fiber, vitamins, minerals, and antioxidant compounds [5–7]. Due to this composition, several studies have demonstrated the benefits associated with regular consumption of nuts on health, including reduced risk of coronary heart disease and metabolic syndrome as well as a positive impact on oxidative stress, inflammation, and blood pressure [8, 9]. Brazil nut (*Bertholletia excelsa H.B.K*) is a native species from the Amazon, considered the main food source of selenium (100–1000 mg of selenium/g<sup>-1</sup>) [6, 10]. Brazil nut also contains phytosterols, tocopherols, and phenolics

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associated with health benefits [6, 10-12]. Cashew nut (*Anacardium occidentale L.*) is native to Central and South America, being Brazil its country of origin [13, 14]. Besides its pleasant taste, cashew nut has a high lipid content, predominantly monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), which are associated with reduction of cholesterol, low-density lipoprotein concentrations, and cardiovascular events [13].

On the other hand, nuts are energy-dense foods [5], which could increase daily energy intake leading to weight gain. Despite that, regular nut consumption has been inversely associated with obesity, body mass index, and adiposity [15-18]. Some hypotheses try to explain the relation between nuts consumption and weight management. It is likely that the high content of protein, fiber, and even phenolic compounds present on nuts, in conjunction with a low glycemic index when consumed with other foods [19, 20], promotes a satiety effect that is extended until the next meal [17, 21, 22]. Hence, there is a dietary compensation, leading to a decreased calorie intake from other foods [17, 18]. In addition, some studies suggest that nuts consumption may affect satiety through the modulation of gut hormones such as glucagon-like peptide 1 (GLP-1) [23, 24], glucose-dependent insulinotropic polypeptide (GIP), glucagon, and insulin [24]. The form that the nuts are consumed (whole or processed), demanding or not mastication process, might activate neural and endocrine mechanisms that stimulate postprandial hormones, influencing hunger and satiety sensations [23, 25].

Another possible explanation involving nuts consumption and weight management regards to the lipid profile of nuts. Some studies suggest that their high-unsaturated fat content increases resting metabolic rate (RMR), leading to greater energy expenditure [26–28]. Lastly, there is evidence that the lipids provided by nuts are not highly bioaccessible [29–32]. Some studies have shown that the cellular wall of nuts limits digestion and, as a result, a high proportion of the fat is excreted in the feces becoming unavailable for energy metabolism [23, 33, 34].

In the most recent meta-analysis about the effect of nuts on energy intake, hunger, and fullness, the authors observed that nuts consumption was associated with a greater energy intake in individuals with overweight/obesity and suppression of hunger sensation overall, although no significant effect on the sense of fullness was noted. The authors also highlighted the limited evidence about the nuts effect on hunger and satiety [21]. Therefore, we aimed to evaluate the effects of acute intake of a shake containing cashew nuts (*Anacardium occidentale L.*) and Brazil nuts (*Bertholletia excelsa H.B.K*) compared with a shake absent of nuts (control), matched for energy and macronutrient content, on appetite responses in overweight and obese subjects. We hypothesized that test shake

would provide greater satiety, due to the nutritional composition of nuts.

## Subjects and methods

#### Subjects

Subjects were recruited by advertisements in the local community and social media. After answering an online questionnaire, those who met inclusion criteria were invited to a screening at the laboratory, which included anthropometric measurements and questions about their nutritional, medical, and familiar history. We included subjects aged between 18 and 59 years, non-smokers, with a body mass index (BMI) between 26 and 39 kg/m<sup>2</sup>, body fat > 25% for men and > 33% for women, and waist circumference > 88 cm for women and > 102 cm for men. We did not include individuals with a known allergy to cashew or Brazil nuts; on regular medication that might affect appetite and/or glucose, lipid, or energy metabolism; those who presented infectious or allergic episodes 30 days before the study; individuals undertaking weight-loss diets; those who did not present stable weight  $(\pm 3 \text{ kg})$  during the previous 90 days and those drinking more than 168 g of alcohol a week.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the Human Research Ethics Committee of the Universidade Federal de Viçosa (UFV) (registration no. 2.205.393). Written informed consent was obtained from all subjects. This study is registered on the Brazilian Clinical Trials Registry (ReBEC), available at: < http://www.ensai osclinicos.gov.br > (registration no. U1111-1203-9891).

#### Study design

This was a clinical, randomized, controlled, single-blind, cross-over, pilot study.

On the night before each experimental day, participants were asked to consume a standard dinner, which included a chicken sandwich and 200 mL of grape juice (total calories: 670 kcal; carbohydrate: 75 g; protein: 44 g; total lipid: 17 g; fiber: 4.3 g). The standard dinner was provided by the researchers—which participants picked up at the laboratory to consume at home—and the amount consumed was reported on the experimental day. Participants were advised to consume the same quantities on both days. All participants were instructed to avoid physical exercise and alcohol consumption as well as caffeine, dried fruits, nuts, and thermogenic foods 48 h before the test to reduce within-subject variability.

The subjects attended the laboratory following overnight fasting (12 h). Bodyweight, body composition, and anthropometry measures were assessed. Appetite and energy metabolism were also measured at fasting. Then, participants were asked to consume within 15 min a control (absent of nuts) or test shake (with nuts). After a washout period, from 7 to 14 days, the subjects were allocated to the other group. The order of shakes consumption was defined by randomization at <www.random.org> and the participants were blinded to the allocated treatment. Following shake consumption, participants remained for the next 4 h at the laboratory. They were not allowed to consume any food during that period. Once completed the study protocol, subjects were invited to consume an ad libitum standard lunch (Fig. 1).

## **Composition of the shakes**

The control and test shakes were isocaloric and matched for macronutrient content (Table 1). Ingredients of both shakes were mixed with 250 mL of water and 150 mL of ice in a

blender. Nuts were crushed in a food processor before being mixed with the other ingredients to get a homogeneous beverage with a similar texture to the control shake. In addition, hazelnut essence and food coloring were added to the control shake to turn its sensory aspects similar to the test shake. Therefore, we ensure the blinding of the study by presenting beverages with similar taste and appearance.

Visual analogue scales (VAS), which are 100 mm lines with opposite feelings at each end, were used to evaluate the control and test shakes. Questions included the visual appeal, taste, after taste, smell, and palatability of the shakes [35]. Answers varied from "good" to "bad" at each extreme of the scale, and participants were instructed to mark a line at the point that reflected their opinion about each aspect.

## Appetite and energy metabolism assessment

Subjective appetite sensations were also analyzed through VAS. Questions evaluated hunger, satiety, fullness, desire

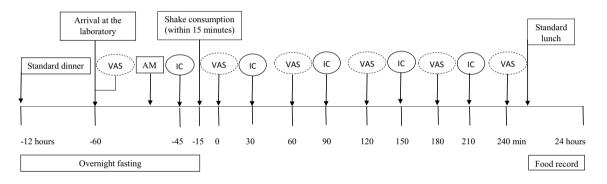


Fig. 1 Experimental protocol. AM anthropometric measurements, IC indirect calorimetry, VAS Visual Analogue Scale

Ingredients	Total (g)	Energy (kcal)	CHO (g)	PTN (g)	LIP (g)	SFA (g)	MUFA (g)	PUFA (g)	Fiber (g)
Control (500 mL)									
Skim milk powder	40.0	143.7	21.2	13.8	0.3	0.24	0.08	0	0
Soybean oil	22.7	204.3	0	0	22.7	3.45	5.28	13.62	0
Sugar	10.0	39.9	9.9	0	0	0	0	0	0
Whey protein	7.0	27.5	1.6	4.2	0.4	0.30	0.07	0	0
Corn starch	6.0	21.0	5.2	0	0	0	0	0	0
Total	85.7	436.4	37.9	18.0	23.4	3.99	5.43	13.62	0
Test (500 mL)									
Cashew nuts	30.0	182.1	8.7	5.5	13.8	2.74	8.19	2.35	1.0
Brazil nuts	15.0	103.4	2.2	2.1	9.5	2.29	4.11	3.15	1.1
Skim milk powder	30.0	107.8	15.9	10.4	0.3	0.18	0.06	0	0
Sugar	11.0	43.9	10.9	0	0	0	0	0	0
Total	86.0	437.2	37.7	18.0	23.6	5.21	12.36	5.50 2	2.1

 Table 1
 Nutrient profile and composition of the control and test shakes

CHO carbohydrates, PTN protein, LIP total lipid, SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids

to eat something salty, sweet, fatty or savory, and prospective of food consumption [35]. Therefore, subjects were instructed to mark a line at the point that corresponded to their feeling at that moment. Quantification of each scale was done by measuring the distance from the left end to the marked point. VAS was recorded at fasting, immediately after shake consumption, and hourly for the subsequent 4 h.

Consumption was evaluated by weighing the quantity of food consumed at lunch. As mentioned, it was served an ad libitum meal at the end of each morning-test, which included approximately 1000 g of pasta with Bolognese sauce and 200 mL of grape juice. The meals were weighed before and after serving to determine the amount consumed, by using a digital scale (Exact Basic<sup>®</sup>, model BS-3000A).

Appetite was also assessed by measuring food consumption over day. On the test days, subjects were asked to fill out a food record from the time they left the laboratory until the end of the day. They were previously instructed by a dietitian on how to register the food consumption adequately. All food records were analyzed using a computer software (Avanutri 2.0.0, Avanutri, Equipamentos de Avaliação Ltda).

Our primary outcome also included the measurement of energy expenditure (EE) by indirect calorimetry. Following anthropometric measurements and the first VAS, at fasting, participants were instructed to rest for 15 min in a horizontal position. Then, the fasting resting energy expenditure (REE) and the substrate oxidation rates (SOR) were assessed by indirect calorimetry (Carefusion Vmax® Series, California, USA). Each EE measurement lasted 30 min. At postprandial state, EE assessment started at 30 min and finished at 60 min after the shake consumption. Then, participants were required to fill out a new VAS. This procedure was repeated hourly for the subsequent 4 h. During each EE measurement, participants remained awake and were instructed to be the most immobile as possible. Due to technical issues during the study, we could not evaluate all participants. However, it did not modify our protocol since the other measurements were performed following the same intervals previously established.

#### Anthropometric assessment

Anthropometric measurements were collected at the screening and on the two test days to detect any possible variation that could occur in weight and/or body composition. Anthropometric assessments and body composition were measured while subjects were barefoot, wearing light clothes. Bodyweight and body composition were measured by a tetrapolar bioelectrical impedance analysis device (InBody<sup>®</sup>, model 230, BiospaceCo). Height was measured using a fixed stadiometer (SECA model 206, Hamburg, Germany). Waist circumference was measured at the midpoint between the lower margin of the least palpable rib and the top of the iliac crest. Hip circumference was measured around the widest portion of the buttocks, with the tape parallel to the floor. For both measurements, we used a stretch-resistant tape [36, 37].

#### Sample size calculation and statistical analyses

The sample size was based on a previous study by Tapsell et al. [38] that evaluated diet-related energy expenditure and substrate oxidation rates in a crossover design study.

The statistical analyses were performed by using SPSS 20 software for Windows (SPSS, Inc., Chicago, IL, USA). Data were expressed as mean  $\pm$  SEM. The Shapiro–Wilk test was performed to verify the normality of the continuous variables. The analysis of variance (ANOVA) of repeated measures was applied to evaluate the effect of time, shake consumption, and interaction of both factors on the satiety scales. Bonferroni correction was applied for the variable time in the ANOVA analysis since only the time effect was observed. The Greenhouse-Geisser correction was performed when the sphericity was violated. The trapezoidal method was used to calculate the incremental area under the curve (iAUC) of subjective satiety sensations by using GraphPad Prism software (Version 6; GraphPad Software Inc). The iAUC was used due to the baseline differences for some subjective appetite sensations in the test and control group. The paired t test was performed to compare total energy intake and macronutrient content, palatability of the shakes and iAUC for subjective appetite sensations between control and test groups. Statistical significance criterion ( $\alpha$ ) of 0.05 was set for all analyses.

## Results

Fifteen participants (10 women and 5 men) completed the study (Fig. 2). Participants were between 20 and 45 years old and had a mean BMI of  $30.9 \pm 0.9$  kg/m<sup>2</sup> (Table 2). Only a few participants reported that they did not consume the entire standard dinner and were advised to maintain the same dietary pattern on the next experimental day.

Regarding sensations of hunger and satiety, the feeling of hunger and prospective of food consumption decreased, while fullness and satiety increased immediately after shakes consumption, and then gradually changed in the following 240 min, as expected. We did not observe any significant difference between groups or interaction between time and group for hunger, satiety, fullness, prospective of food consumption, and desire to eat something sweet, salty, savory, or fatty (Fig. 3). When iAUC was performed for the same ratings, the control and test groups also did not differ significantly from each other (Table 3).

We also measured satiety directly via food intake, by weighing the lunch served at the end of each morning-test,

# **Fig. 2** Flowchart of study participants

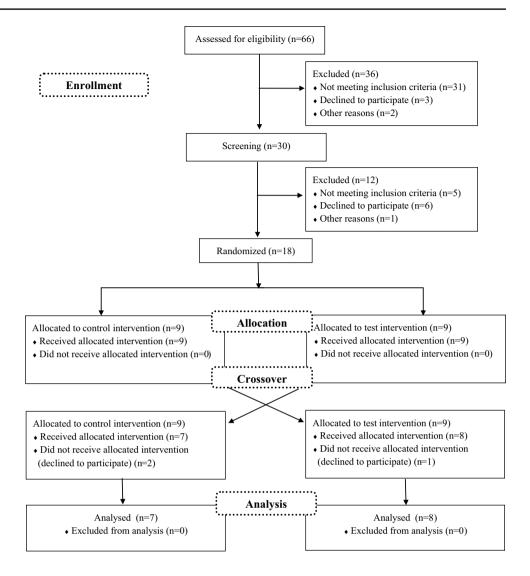


 Table 2
 Anthropometric characteristics of the subjects at baseline

Anthropometric measurements	Mean $\pm$ SEM
Age (y)	$29.0 \pm 1.9$
BMI (kg/m <sup>2</sup> )	$30.9 \pm 0.9$
Body fat (%)	$39.2 \pm 1.4$
Waist circumference (cm)	$100.7 \pm 3.1$
Waist-hip ratio	$0.92 \pm 0.01$
SBP (mmHg)	$124.8 \pm 3.0$
DBP (mmHg)	$81.1 \pm 2.2$

Total n = 15 (female = 10; male = 5)

*BMI* Body Mass Index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure

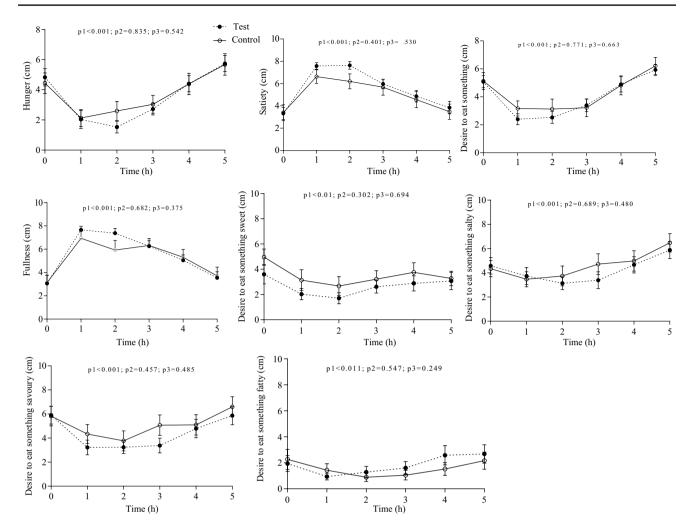
which subjects ate ad libitum. There was no significant difference in consumption between treatments. Moreover, we estimated total energy intake through food records, considering the moment in which participants left the laboratory until the end of each test day. Results were analyzed by investigating whether total energy intake differed in response to control or test shakes consumption. As shown, no significant difference was observed between groups (Fig. 4).

Regarding palatability of the shakes, there was no significant difference in VAS scores for visual appeal, taste, after taste, smell, and palatability between the groups (Fig. 5).

Three participants completed the energy expenditure assessment. For this reason, the data was not analyzed since this sample is insufficient to detect any alteration in energy metabolism.

## Discussion

Since the prevalence of overweight and obesity has taken epidemic proportions worldwide, it is necessary to understand the mechanisms that regulate eating behavior [39, 40]. Satiety, the feeling of fullness that persists until the next



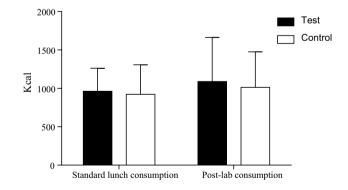
**Fig.3** Subjective appetite sensations and desire to eat specific types of food after an overnight fasting (time 0); immediately after shake consumption (time 1); and on the following 4 h (time 2-5). Solid lines: control shake; dashed lines: test shake. Values

are means  $\pm$  SEM. *p*1: *p* value to time effects; *p*2: *p* value to shake effects; *p*3: *p* value to interaction effects according to ANOVA of repeated measures with Bonferroni correction. *n* = 15

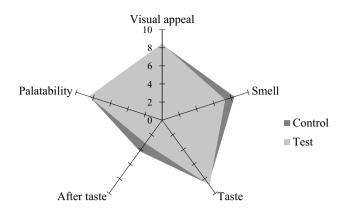
 Table 3
 iAUC values for subjective appetite sensations and desire for specific foods

iAUC (cm h)	Control shake	Test shake	p value
Salty food	$10.84 \pm 2.36$	$8.34 \pm 1.87$	0.341
Sweet food	$10.94 \pm 2.32$	$7.36 \pm 2.23$	0.076
Savory food	$11.69 \pm 2.68$	$9.94 \pm 1.80$	0.462
Fatty food	$5.67 \pm 2.34$	$4.35 \pm 1.55$	0.486
Satiety	$11.56 \pm 1.61$	$12.68 \pm 1.51$	0.610
Hunger	$8.51 \pm 1.43$	$11.09 \pm 1.28$	0.157
Fullness	$13.53 \pm 1.76$	$15.15 \pm 1.58$	0.487
Prospective of food consumption	$8.71 \pm 1.27$	$9.01 \pm 1.34$	0.881

Values are means  $\pm$  SEM. n = 15. p values according to paired t test



**Fig. 4** Energy intake on the test and control days. Standard lunch consumption: total calories from pasta and grape juice consumed at the end of each morning test. Post-lab consumption: total calories estimated through food records, filled out from the time that participants left the laboratory until the end of the day. Values are means  $\pm$  SEM and did not differ between groups according to the paired *t* test (*p* > 0.05)



**Fig.5** Palatability of the control and test shakes. Values are means  $\pm$  SEM and did not differ between groups according to the paired *t* test (*p* > 0.05)

meal, is an important factor in regulating food intake by suppressing overconsumption. In this sense, foods that promote satiety have a positive impact on energy balance and consequently, on weight management [40, 41].

Several studies have reported the satiating properties of nuts, mainly due to their nutritional content that is high in fat, fiber, and protein [18, 42, 43]. However, most results were attributable to the regular consumption of nuts [18, 44]. To our knowledge, this is the first study that investigated the acute effect of cashew and Brazil nuts on appetite responses. We expected that the presence of nuts on the test shake would exert a greater impact on satiety and lower energy intake. The results of this study, however, are not consistent with our hypothesis. Subjective and objective appetite measurements, assessed by VAS, ad libitum meal, and food records, did not indicate a significant difference in satiety response and energy intake between the treatments.

In our study, the control and test shakes were isocaloric and matched in macronutrient distribution. The main difference between them was the unsaturated fat content since the test shake provided mostly monounsaturated fatty acids (MUFA) and the control, polyunsaturated fatty acids (PUFA). Some studies demonstrated that saturated fatty acid (SFA) was associated with greater fullness and lower prospective of food consumption when compared with MUFA and PUFA [45, 46]. On the other hand, other studies that measured satiety hormones showed that both unsaturated fatty acids, PUFA and MUFA, exerted a greater satiety response and greater hunger suppression when compared to SFA [47, 48]. Taken together, these results suggest that, when comparing unsaturated fatty acids with SFA, it is possible to find some difference in the satiety response. Otherwise, comparing MUFA and PUFA the difference is not so evident, which may explain our results.

Regarding the period of the study, some authors have noted no impact on satiety after acute nut consumption [44,

49, 50]. In a randomized, double-blind, and cross-over study, walnut consumption increased the participants' satiation only after the third day [51]. Similarly, in another cross-over study, acute walnut consumption did not differ from an iso-caloric meal in terms of satiety response [24]. Both studies measured hormones related to hunger and satiety, including CCK, GLP-1, PYY, ghrelin, insulin, and leptin, and did not find any difference between acute nuts consumption and the control meal. It suggests that the mechanism by which nuts enhance satiety may not occur over a short period [24, 51], corroborating our results.

Another important point is the form of how nuts are consumed, which may be a determining factor in satiety response. Although we standardized control and test as liquid meals to avoid the bias of particle size of food and nutrient bioaccessibility, mastication plays a crucial role in satiety, mainly because of its consequences on neural and endocrine mechanisms [25, 42]. Indeed, the oral effort and time required during mastication of whole nuts have been associated with important effects on satiety, meal fat availability, and stimulation of postprandial hormones such as insulin, ghrelin, CCK, PYY, and GLP-1 [23, 25]. McArthur et al. [25] investigating the effect of nuts mastication on pre-swallowing particle size and, consequently, on digestion, observed an increase of fullness sensation after the mastication of whole walnuts compared to walnut butter, although gut peptide concentrations remained unchanged. The particle size of the nuts can also influence postprandial lipemia [52] since larger particles compromise lipid bioaccessibility [34, 53]. The consumption of whole almonds was associated with reduced hunger and desire to eat [18] as well as a greater feeling of fullness along the day when compared to almond butter [54]. These results suggest, at least in part, that oral processing of nuts through mastication can affect aspects related to satiety sensation. Although the isolated effect of mastication on satiety is still unclear [25], skipping this step may have affected our results.

The long intervals between the measurements of subjective appetite sensations should also be considered. In literature, there are some studies in which VAS were administrated at intervals of 60 min [55]. However, in most of the studies, VAS are applied at the first 30 min after meal intake, since the sensations of appetite change rapidly [24, 44, 56]. Opting for this methodology may have influenced our results.

In a recent systematic review and meta-analysis [21], nuts consumption was associated with increased daily energy intake in overweight and obese individuals (BMI  $\geq$  25 kg/m<sup>2</sup>), but not in individuals with normal weight (BMI 18.5–24.9 kg/m<sup>2</sup>). Despite that, no effect on body weight was observed. Nuts consumption also suppressed a sense of hunger regardless of BMI. Sense of fullness increased in individuals with normal weight and decreased in individuals with overweight/obesity,

although this difference was not significant. The results of this meta-analysis reinforce the negative correlation between nuts consumption and weight gain [17, 18]. On the other hand, the authors suggest that weight status may influence the effect of nuts on satiety since individuals with overweight or obesity tended to present less suppressed hunger, less sense of fullness, and increased energy intake following nuts consumption when compared to normal-weight individuals. Perception of hunger and satiety can be altered in individuals with obesity due to several reasons, including hormonal [57, 58] and genetic conditions [59]. However, due to the lack of studies in this area, the findings are still inconclusive.

Our study has several strengths. First, the cross-over design reduced individual variability by evaluating the same subjects. The control and test groups were matched in energy and macronutrient content, as well as portion size. Moreover, since control and test were standardized as liquid meals, there was no bias of mastication, which could have influenced our results by affecting the particle size of the food. All these factors are key determinants of satiety [26, 44, 60]. Finally, it must be highlighted that we evaluated appetite response directly and indirectly, through three different methods. The main limitation of this study is the small sample size. It should also be mentioned that one of our primary aims was to evaluate energy expenditure, however, due to technical issues, it was not possible.

Future randomized clinical studies should be carried out focusing on key questions about the mechanisms involved in nuts satiety (e.g. energy availability of nuts, nuts processing, and the impact of diet-induced thermogenesis). Mastication may have a strong influence on satiating properties of nuts due to two main reasons: first, the role of mastication on neural and endocrine mechanisms, secreting hormones involved in suppression of hunger and also enhancement of satiety [25]. Second, the act of chewing ruptures the cell wall of nuts, releasing lipid content and making it available for energy metabolism [25, 34]. The resistance of these cell walls to digestion in the gut could influence satiety, but this question remains unclear. Finally, there is evidence that weight status may impact the satiety response of nuts since obese subjects can be less sensitive to recognize signals of hunger and satiety [21]. A possible alternative is to assess satiety by several methods (biological, such as hormone measurements, and behavioral methods that evaluate appetite sensations), choosing those that are more adequate for the study. Further studies are warranted to confirm these hypotheses.

## Conclusion

In this study, the acute intake of a shake containing Brazil and cashew nuts did not differ from a shake absent of nuts on subjective and objective satiety responses, neither on subsequent energy intake over the day in overweight subjects. We reinforce that the long intervals between the measurements of subjective appetite sensations and the small differences of nutrients content between the beverages may have influenced our results. Further studies are necessary to elucidate the mechanisms involved in the satiating properties of nuts, especially regarding short-term food intake.

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Author contributions MACC, HHMH, APSC, DMUPR, and JB: contributed to the conception and design of the project, data interpretation, and analysis, writing and review of the final manuscript. AS and LLO: contributed to data analysis and interpretation, as well as writing and review of the final manuscript. All authors have approved the final article.

#### Declarations

Conflict of interest The authors declare no conflict of interest.

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