Intake of partially defatted Brazil nut flour reduces serum cholesterol in hypercholesterolemic patients- a randomized controlled trial

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Abstract

Objective: Thyroid hormones can lower levels of atherogenic lipoproteins, and selenium is important in thyroid hormone homeostasis. We aimed to investigate the effects of a healthy diet associated with the Brazil nut (Bertholletia excelsa) in dyslipidemic and hypertensive patients.

Methods: This study was a randomized, placebo-controlled, double-blind trial. Seventy-seven dyslipidemic and hypertensive patients already receiving lipid-lowering drugs received either a dietary treatment associated with partially defatted Brazil nut flour (13 g/day providing 227.5 μg of selenium/day), or with dyed cassava flour as a placebo. All patients received a personalized dietary guideline with nutritional recommendations for dyslipidemia and hypertension and were followed for 90 days.

Results: The Brazil nut group showed reductions in total cholesterol (−20.5 ± 61.2 mg/dL, P = 0.02), non HDL-cholesterol (−19.5 ± 61.2 mg/dL, P = 0.02) and Apo A-1 (−10.2 ± 26.7 mg/dL, P = 0.03) without significant alterations in the Apo B/Apo A-1 ratio. The placebo group showed a reduction in FT3 levels (−0.1 ± 0.4, P = 0.03) and increased Lp(a) levels (5.9 ± 18.0 mg/dL, P = 0.02). There were no statistical differences in blood pressure and serum lipids between Brazil nut and placebo group.

Conclusions: Supplementation with Brazil nuts seems to favor the maintenance of FT3 levels and contributes to lipemia reduction in hypercholesterolemic and euthyroid patients.

Trial registration: ClinicalTrials.gov Identifier NCT01990391

Keywords: Brazil nut, Selenium, Serum lipids, Thyroid hormones, Dyslipidemia

Introduction

Atherosclerotic cardiovascular disease is a high worldwide prevalence and mortality [1]. Hyperlipidemia increases the incidence and risk of this disease, as confirmed by several large-population studies [1, 2].

It has been described that thyroid hormones and thyromimetic drugs has LDL cholesterol (C) lowering effect [3–7] and also reduce levels of other atherogenic lipoproteins such as triglycerides [5, 7], apolipoprotein B (apo B) [6] and lipoprotein (a) (Lp(a)) [5–7], although they also decrease levels of non-atherogenic lipoproteins, such as apolipoprotein A-1 (apo A-1) [3]. These effects may be caused by stimulation of LDL receptors, which increases hepatic clearance and cholesterol elimination, and presumably promotes reverse cholesterol transport, even in individuals without subclinical hypothyroidism [6, 8, 9].
Selenium is an essential micronutrient for the metabolism of thyroid hormones, playing a major role in the conversion of the more active form of triiodothyronine because the activity of some deiodinases is dependent on selenium [10, 11]. The thyroid gland contains the highest amount of selenium [10], and selenoprotein glutathione peroxidase can protect the thyroid gland from the oxidative damage produced during thyroid hormone synthesis [12]. Selenium also plays an important antioxidant role in cardiovascular disease and studies have shown that serum and urinary levels of selenium are lower in patients who suffered acute myocardial infarcts or ischemic cardiomyopathy [13].

The Brazil nut (Bertholletia excelsa, family Lecythidaceae) originates from the Amazon region and is the richest known food source of selenium [14]. Most selenium content in this nut is present in protein fraction therefore, the cake protein has the highest amount of selenium presented as selenomethionine and selenocysteine [15]. The high concentration of selenium in the Brazil nut may improve serum lipoprotein, due to its stimulation of the metabolically active form of thyroid hormones.

The present study investigated the effects of a healthy diet associated with defatted Brazil nut flour on thyroid hormones and in serum lipoproteins in dyslipidemic and hypertensive patients.

Patients and methods

Ethical considerations

The Ethics Committee for Clinical Research of the National Cardiology Institute approved this study (protocol number 316/2011) in Rio de Janeiro, Brazil. This study was also registered at ClinicalTrials.gov (NCT01990391). All patients were informed about the procedures and gave their written informed consent.

Study patients

Eighty-nine dyslipidemic and hypertensive patients (49 men and 40 women) were recruited at the National Institute of Cardiology at the Dyslipidemia and Atherosclerosis Outpatient Clinic. Eligible patients were males and females aged 40 to 80 years old, with referred diagnoses of dyslipidemia and hypertension who were taking medications for both conditions for at least 3 months before being included in the study, and were diagnosed with euthyroidism. Euthyroidism was defined as thyroid stimulating hormone (TSH) (reference range, 0.45 – 4.50 μU/mL) and free thyroxine (FT4) (reference range, 0.70 – 1.48 ng/dL) within the normal reference range [16].

Patients with history of thyroid disease, thyroid medication use, chronic renal failure with glomerular filtration rate < 60 mL/min/1.73 m² [17] were excluded. As well as those currently or previously having ingested supplements containing > 20 μg Se/day, or presenting excessive consumption of Brazil nuts in the past year, having plasma selenium levels above 125 μg/L [18], being current smokers [19], and having been in a rigorous exercise/weight-reduction program within the 3 months before entering the study.

Experimental design

This study was a 90-day randomized, placebo-controlled, double-blind clinical trial. Eligible patients were randomly assigned to one of two groups: diet + partially defatted Brazil nut flour (n = 35) or diet + placebo (artificially flavored dyed cassava flour) (n = 42).

Patients were randomized using the method of computer generated random list restricted in blocking of 10 patients and sequentially numbered labels were inserted in sealed containers with Brazil nut or placebo. The researcher that generated the random allocation sequence was not the same that enrolled participants and assigned participants to interventions.

Patients were assessed before and during the 90-day trial, with monthly blood test to measure thyroid hormones, lipid profile, and plasma selenium levels. In addition, food intake was analyzed through patients’ dietary anamnesis whereas anthropometric data were evaluated through measures of waist circumference (WC) and body weight and height, for calculation of body mass index (BMI). At each visit, patients were given a sealed opaque flask containing either 450 g of Brazil nut flour or placebo, and standard measuring spoons. Plasma selenium levels were used as markers of compliance to Brazil nut consumption.

Self-reported questionnaires were used to determine physical activity [20], dietary habits, and medications. Physical exercise was taken into account when patients exercised at least once a week, and was reported as Metabolic Equivalent of Task (MET), expressed in kcal/day [21]. Patients were asked to maintain their normal amount of physical activity and dose of lipid-lowering medication. This was verified by analyzing questionnaires filled out by patients and by examining the medical record containing prescription information of each patient visit.

Nutritional intervention

Patients received either 13 g/day of partially defatted Brazil nut flour providing 64.4 kcal, 0.17 g carbohydrate, 3.4 g protein, 5.56 g total fat, 2.58 g dietary fiber and 227.5 μg of selenium/day (Ouro Verde Amazônia® – Mato Grosso, Brazil) or 11 g/day of artificially flavored dyed cassava flour (Mane of Brazil Industry and Commerce Ltda – Rio de Janeiro, Brazil). The nutritional composition 11 g of placebo is: 36.5 kcal, 8.92 g carbohydrate, 0.12 g protein, 0.03 g total fat, 0.65 g dietary fiber [22] and 0.07 μg selenium [23]. The volume of Brazil nut and placebo was different because of the total volume of the opaque flask used to store the supplements. Since placebo was less dense, it requires a higher volume and more flasks, so it could difficult the double blinding of the study.
Partially defatted granulated Brazil nut was used in this study rather than the Brazil nut kernel because the granulated Brazil nut has a higher Se content than the Brazil nut kernel (227.5 μg vs. 249.21 μg [22, 23], respectively) and they have similar centesimal compositions except for less total fat and fewer calories. In addition, granulated Brazil nut allowed for blinding the study and is already commercialized.

The amount of selenium present in the Brazil nut flour was measured by flame atomic absorption spectrometry. The energy value of Brazil nut was 59 kcal/day while the placebo group was 46.8 kcal/day.

The dietary plan was prescribed in accordance to the volunteers’ dietary habits and nutritional recommendations according to guidelines for dyslipidemia and hypertension [24, 25]. Participants also received a weekly menu and healthy recipes. Dietary compliance was verified monthly at the check-up via 24 h diet reminders that were analyzed with the Food Processor software, version 12 (EshaResearch, Salem, USA, 1984).

**Blood collection and biochemical evaluation**

Blood samples were collected following 12 h of overnight fasting and analyzed at the Clinical Analyses Laboratory of National Cardiology Institute (Rio de Janeiro, Brazil) using an automated method (ARCHITECT ci8200, Abbott ARCHITECT®, Abbott Park, IL, USA) and commercial kits (Abbott ARCHITECT c8000™, Abbott Park, IL, USA).

Serum TSH and thyroid hormones free triiodothyronine (FT3) and free thyroxine (FT4) were measured by immunochemiluminescence of microparticle [26, 27] with analytical sensitivity of 1 pg/mL for FT3, 0.4 ng/dL for FT4, and 0.0025 μU/mL for TSH.

Serum concentrations of triglycerides, total cholesterol, and HDL-c were assayed by enzymatic-colorimetric methods: glycerol phosphate oxidase/peroxidase [28], cholesterol oxidase/peroxidase [29], and direct detergent [30], respectively. Non-HDL cholesterol was calculated as follows: total cholesterol – HDL-c [31]. LDL-c values were obtained according to the Friedewald formula [32]. Serum levels of Apo A-1 and Apo B were measured with immunoturbidimetry [33]. The Apo B / Apo A-1 ratio was obtained using the conventional values. To determine plasma Lp(a) levels, collecting was carried out with tubes containing ethylenediaminetetraacetic acid (EDTA), and analyses were conducted by particle-enhanced turbidimetric immunoassay [34].

Plasma selenium was analyzed in samples collected in NH Trace Element tubes with sodium heparin (VACUETTE®) and were maintained at −70 °C until they were analyzed with atomic absorption spectrometry using an inductively coupled plasma mass spectrometer (NexION™ 300 ICP-MS, PerkinElmer, Massachusetts, USA) [35]. This analysis was conducted at the LABSPECTRO Laboratory of the Pontifical Catholic University (Rio de Janeiro, Brazil).

**Statistical analyses**

The estimated sample size was based on detection of a significant mean increase in T3 levels of 0.07 ± 0.2 nmol/l, with selenium supplementation at a dosage of 200 μg/d during three months, as one of the objectives of this study. It was used a two-sided type I error of less than 5 %. Thus, 70 patients was required for a statistical power of 80 %.

All statistical analyses were conducted using the Statistical Package Social Sciences (SPSS) software, version 16. Results were reported as mean ± SD. Data normality was tested by Shapiro-Wilk W test and the chi-square test to assess the differences in the qualitative variables between groups. To evaluate the effect of Brazil nut consumption on the investigated variables, we used the Mann-Whitney U test to analyze between-group differences and a Wilcoxon Signed Ranks test to assess changes in each group for non-normal data. Finally, for normal data, the Independent-Simple Student t-Test was used to compare means between groups and the Paired-Simple Student t-Test was used to assess differences in each group throughout the study. Significant differences were assumed at *P* < 0.05.

**Results**

Of the 89 patients randomized, 12 did not complete the study (Fig. 1). No adverse effects were reported by any of the participants. One patient discontinued consuming Brazil nut due to pleural effusion, but this condition was unrelated to the supplementation, as this patient had a history of coronary artery disease. Seventy-seven patients completed the study; 35 in the diet + Brazil nut group and 42 in the diet + placebo group. The patients who did not complete the study had the same investigated characteristics as those who completed it (Table 1).

At baseline, the groups did not differ in terms of age, sex, BMI, diabetes mellitus diagnosis, plasma selenium levels, physical activity, and medications (Table 2). Likewise, there was no statistically significant difference between the groups in terms of daily dietary components (Table 3) and the variables studied (Table 4) at baseline.

Patients kept their usual levels of physical activity during the study, thus maintaining their caloric expenditure. There were no significant changes in BMI (Brazil nut group: Δ *T* = −0.2 ± 0.7 kg/m²; placebo group: Δ *T* = −0.1 ± 0.9 kg/m²) and WC (Brazil nut group: Δ *T* = −0.6 ± 2.5 cm; placebo group: Δ *T* = −0.5 ± 3.3 cm) within and between groups.

Plasma selenium concentrations increased significantly in the Brazil nut group at all time-points (Δ *T* =
In the placebo group, on the other hand, those concentrations decreased significantly at T30 and T60 ($\Delta T_{60} - T_0 = -17.2 \pm 23.4 \mu g/L, P < 0.001$).

Selenium levels differed significantly between groups at all time-points, except at baseline ($T_{90}$: Brazil nut = 169.5 ± 46.5 µg/L versus placebo = 92.7 ± 16.8 µg/L, $P < 0.001$) (Fig. 2).

Due to the nutritional recommendations, energy intake decreased significantly in both the Brazil nut ($P = 0.03$) and placebo ($P < 0.001$) groups, while selenium intake increased significantly in the Brazil nut group ($P < 0.001$) (Table 3).

Thyroid hormone profiles are presented in Table 4. There were no statistical differences in TSH and FT4 levels within and between groups, while FT3 decreased significantly only in the placebo group ($P = 0.03$) at $T_{90}$, without alterations between groups.

Serum lipoprotein levels are presented in Table 4. There were no significant differences within or between groups in LDL-c, HDL-c, triglycerides, Apo B and the Apo B / Apo A-1 ratio during the study. After 90 days, total cholesterol ($P = 0.02$), non-HDL cholesterol ($P = 0.02$) and Apo A-1 ($P = 0.03$) decreased significantly only in the Brazil nut group, however there was no difference between groups. The placebo group presented an increase in Lp(a) levels ($P = 0.02$). Figure 3 presents the changes in total cholesterol between groups during the study.

**Discussion**

In this study, we analyzed the effects of a personalized balanced diet associated with defatted Brazil nut flour on the lipid levels and thyroid hormones of dyslipidemic and hypertensive patients.
Recent reports indicated that food sources naturally or artificially containing high amounts of selenium improve lipid profiles, with significant reductions in total cholesterol and non-HDL cholesterol levels [36, 37], which is supported by the present study. Moreover, Maranhão et al. [37] showed a significant reduction in LDL-c levels. Other authors who used selenium supplements in doses greater than 290 μg/day did not observe significant reduction in atherogenic lipoproteins, even though they did observe increase in HDL-c levels [36, 38].

These beneficial effects in the lipid profile resulting from Brazil nut consumption may be due to selenium’s ability to maintain the metabolically active form of thyroid hormones, as recent studies have shown that using thyroid hormone analogues such as eprotirome (KB2115) favors reduction in serum lipoproteins in euthyroid patients [6, 39].

Decreases in the non-atherogenic lipoprotein Apo A-1 levels observed in the current study are in line with previous studies in which patients displayed lower Apo A-1 levels after receiving thyromimetic medications [6, 39] and in hyperthyroidism [39]. On the other hand, we found no changes in the Apo B / Apo A-1 ratio, indicating no deleterious effect on reverse cholesterol transport, as a significant reduction of non-HDL cholesterol was observed.

Lp(a), one of the most atherogenic lipoproteins, is greatly determined (> than 90 %) by genetic factors and is little influenced by diet and lifestyle. Most lipid-lowering medications have no significant influence on Lp(a) [40]. It is well-established that many hormones have a strong effect on Lp(a) metabolism, and the thyroid hormone T₃ seems to provide a significant reduction in plasma Lp(a) levels [5, 7]. Thus, the plasma Lp(a) level increases observed in the placebo group could be explained by the serum reduction of FT₃ levels.

In the current study, a significant reduction in serum FT₃ levels was observed only in the placebo group. These hormonal alterations could be explained by the effect of energy intake reduction, as previous studies have shown that these alterations can be diet-induced [41, 42]. On the other hand, the Brazil nut group maintained FT₃ levels, which may be explained by the fact that selenium favors increases in the more metabolically active form of thyroid hormones [43–47].

The selenium status at baseline, in the sample population of the present study, was below the normal range.
of plasma selenium levels required to reach maximum glutathione peroxidase activity, established as 90 to 125 μg/l [18]. We suggest this occurs because of the increased oxidative stress in dyslipidemia [48].

The reduction in plasma selenium concentrations in the placebo group could be due to the nutritional recommendations for individuals with dyslipidemia, as the main food sources of this trace element are offal, seafood, meats, and cereals and grains [22]. The amount of selenium provided in this study (~200 μg) can be obtained by consuming 03 units of Brazil nut (102 Kcal) daily.

The present study showed that a small restriction in energy intake might not influence an individual’s lipid profile; however, the consumption of Brazil nut in combination

### Table 3 Dietary data for the two study groups

| Dietary component (intake/day)     | Brazil nut (n = 35) ΔT<sub>90</sub>−T<sub>0</sub> | Placebo (n = 42) ΔT<sub>90</sub>−T<sub>0</sub> | P value intragroup<sup>a</sup> | P value intragroup<sup>b</sup> | P value intergroup<sup>c</sup> |
|-----------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Energy intake (kcal)              | -232.3 ± 153.7                              | -390.1 ± 534.0                              | 0.030<sup>*</sup>               | <0.001<sup>*</sup>               | NS                              |
| Lipids (%)                        | 0.2 ± 5.4                                   | -0.6 ± 7.4                                  | NS                              | NS                              | NS                              |
| Saturated FA (%)                  | -0.4 ± 2.4                                  | -0.7 ± 3.3                                  | NS                              | NS                              | NS                              |
| Monounsaturated FA (%)            | 0.3 ± 3.0                                   | -0.6 ± 4.1                                  | NS                              | NS                              | NS                              |
| Polyunsaturated FA (%)            | 0.2 ± 1.6                                   | 0.2 ± 1.3                                   | NS                              | NS                              | NS                              |
| n-3 polyunsaturated FA (g)        | -0.1 ± 0.3                                  | -0.1 ± 0.4                                  | NS                              | NS                              | NS                              |
| Carbohydrates (%)                 | −2.4 ± 11.1                                 | 2.5 ± 12.3                                  | NS                              | NS                              | NS                              |
| Proteins (%)                      | 1.8 ± 9.2                                   | 1.0 ± 10.0                                  | NS                              | NS                              | NS                              |
| Selenium (μg)                     | 231.1 ± 35.3                                | −8.2 ± 44.0                                 | <0.001<sup>*</sup>              | NS                              | <0.001<sup>*</sup>              |

Mean ± SD. FA, fatty acids; NS, non-significant

* Significant differences were assumed at P < 0.05

<sup>a</sup> Statistical differences in Brazil nut group compared to baseline

<sup>b</sup> Statistical difference in placebo group compared to baseline

<sup>c</sup> Statistical differences between groups compared at T90

### Table 4 Thyroid hormones, blood pressure and serum lipoproteins at baseline and after the 90-day trial

| Variables                      | Brazil nut (n = 35) ΔT<sub>90</sub>−T<sub>0</sub> | Placebo (n = 42) ΔT<sub>90</sub>−T<sub>0</sub> | P value intragroup<sup>a</sup> | P value intragroup<sup>b</sup> | P value intergroup<sup>c</sup> |
|--------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------|---------------------------------|---------------------------------|
| FT<sub>3</sub> (pg/mL)         | 0.1 ± 1.1                                   | −0.1 ± 0.4                                  | NS                              | 0.030<sup>*</sup>               | NS                              |
| FT<sub>4</sub> (ng/dL)         | 0.1 ± 0.6                                   | −0.1 ± 0.1                                  | NS                              | NS                              | NS                              |
| TSH (μUI/mL)                   | 0.2 ± 1.8                                   | −0.2 ± 0.9                                  | NS                              | NS                              | 0.06                            |
| SBP (mmHg)                     | 3.5 ± 18.0                                  | −4.0 ± 30.7                                 | NS                              | NS                              | NS                              |
| DBP (mmHg)                     | −1.5 ± 13.6                                 | −5.0 ± 15.2                                 | NS                              | NS                              | 0.020<sup>*</sup>               |
| Total cholesterol (mg/dL)      | −20.5 ± 61.2                                 | −7.4 ± 44.5                                 | 0.020<sup>*</sup>               | NS                              | NS                              |
| LDL-cholesterol (mg/dL)        | −6.4 ± 57.9                                 | −6.5 ± 39.5                                 | NS                              | NS                              | NS                              |
| HDL-cholesterol (mg/dL)        | −1.0 ± 5.0                                  | 0.8 ± 7.1                                   | NS                              | NS                              | NS                              |
| Triglycerides (mg/dL)          | −49.6 ± 198.2                               | −0.05 ± 104.1                               | NS                              | NS                              | NS                              |
| Non-HDL cholesterol (mg/dL)    | −19.5 ± 61.2                                 | −8.2 ± 44.5                                 | 0.020<sup>*</sup>               | NS                              | NS                              |
| Apolipoprotein A-1 (mg/dL)     | −10.2 ± 26.7                                 | −7.9 ± 27.8                                 | 0.040<sup>*</sup>               | NS                              | NS                              |
| Apolipoprotein B (mg/dL)       | −5.2 ± 25.9                                 | −5.0 ± 23.4                                 | NS                              | NS                              | NS                              |
| ApoB/ApoA-1 ratio              | 0.02 ± 0.2                                  | −0.06 ± 0.3                                 | NS                              | NS                              | NS                              |
| Lp(a) (mg/dl)                  | −0.5 ± 23.7                                 | 5.9 ± 18.0                                  | NS                              | 0.02<sup>*</sup>               | NS                              |

Mean ± SD. FT<sub>3</sub>, free triiodothyronine; FT<sub>4</sub>, free thyroxine; TSH, thyroid stimulating hormone; SBP, systolic blood pressure; DBP, diastolic blood pressure, ApoB, apolipoprotein B; ApoA-1, apolipoprotein A-1; Lp(a), lipoprotein (a)

* Significant differences were assumed at P < 0.05

<sup>a</sup> Statistical differences in Brazil nut group compared to baseline

<sup>b</sup> Statistical difference in placebo group compared to baseline

<sup>c</sup> Statistical differences between groups compared at T90

For normally distributed variables (FT<sub>3</sub> and FT<sub>4</sub>), we used a Student’s t test for independent samples and a paired-samples t test to investigate the differences within and between groups, respectively. For non-normally-distributed variables, we used a Mann–Whitney U test and a Wilcoxon Signed Ranks test to assess the differences within and between groups, respectively.
with the diet could significantly improve the serum lipids profile. Nevertheless, there were not found statistical differences in blood pressure and serum lipids between groups of intervention, possibly because the nutritional recommendation favored improves in these parameters in both groups and due the complexity of these patients with regard to disease duration, medication use and heterogeneity.

Strengths and limitations
To our knowledge, this is the first randomized, double-blind, placebo-controlled study to investigate the effects of a personalized balanced diet associated with Brazil nut consumption in dyslipidemic and hypertensive patients. The present study has limitations that may have influenced the findings, including the group of participants who did not complete the study. Considering the potential public health implications of our results, there is a need for more randomized studies with larger patient groups, conducted in populations with a wider range of plasma selenium concentrations, and no medication use. In addition, in vitro studies are needed to evaluate the fractional $T_3$ that would be affected by selenium supplements.

Conclusion
The reduction in energy intake associated with the consumption of partially defatted Brazil nut flour (200 µg of selenium/day) contributed to a reduction in serum total cholesterol and non-HDL cholesterol levels in dyslipidemic and hypertensive patients undergoing drug treatment, without altering thyroid hormone concentrations.
Competing interests
The authors declare that they have no competing interests.

Authors' contributions
Conception and design of the study: RFC, ASBM, GMMO, GR; generation, collection, assembly, analysis and interpretation of data: RFC, GVBH, RRL, ASBM, GMMO, GR; drafting and revision of the manuscript: RFC, GVBH, ASBM, GMMO, GR; approval of the final version of the manuscript: RFC, GVBH, ASBM, GMMO, GR.

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