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# *SEPP1* polymorphisms modulate serum glucose and lipid response to Brazil nut supplementation

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**Abstract**

*Purpose* The consumption of Brazil nuts has been associated with benefits to lipid metabolism and reductions in total cholesterol and LDL levels. They are the richest natural source of selenium, which has essential functions in human physiology. Genetic polymorphisms in Selenoprotein P could impair lipid and glucose metabolism. The aim of this work was to verify the influence of polymorphisms in genes for selenoproteins on blood lipid levels after dietary supplementation with Brazil nuts in healthy adults. *Methods* The study included 130 healthy volunteers selected at the University of São Paulo, Brazil. They were supplemented with one nut a day for eight weeks, followed by eight weeks without intervention. The following analyses were performed: anthropometric measurements, serum fasting glucose, lipid profile, C-reactive protein and plasma MDA levels. The volunteers were genotyped for SNPs rs1050450, rs3811699, rs1800699, rs713041, rs3877899, rs7579, rs34713741, and rs5845 in genes for selenoproteins. *Results* The concentrations of total cholesterol and fasting glucose levels decreased after eight weeks of supplementation ($p < 0.05$). Glucose levels were modulated by rs3877899 in *SEPP1*, with significantly lower levels observed for individuals with the GA+AA genotype ($p = 0.025$). In addition, rs7579 was associated with cholesterol concentrations, which were significantly lower for individuals with the GG genotype ($p = 0.053$). *Conclusions* Supplementation with one Brazil nut a day for eight weeks reduced total cholesterol and glucose levels. Furthermore, our results suggest that rs3877899 might be associated with glucose concentrations and rs7579 with cholesterol concentrations. Therefore, the effect of genetic variations should be considered in future nutritional interventions evaluating the response to Brazil nut supplementation.

Key words: Brazil nuts, lipid profile, polymorphisms, nutrigenetics

## Introduction

The consumption of tree nuts (e.g. peanuts, almonds, hazelnuts, walnuts, Brazil nuts, pistachios, cashews, macadamias) has been associated with decreased risk of cardiovascular disease in human trials [1–4]. The benefits of tree nut consumption are probably associated with their nutritional composition: rich in monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), magnesium, copper, selenium, vitamin E, folic acid and other bioactive compounds such as phytosterols and phenolic acids [3, 5, 6]. The analysis of two human trials, the Nurse's Health Study (NHS) (female subjects) and The Health Professionals Follow-up Study (HPFS) (male subjects), both performed in the USA, revealed that the intake of tree nuts was associated with lower risk of cardiovascular disease [7]. Comparable results were found in the analysis of the National Health and Nutrition Examination Survey (NHANES) from 2005 to 2010 [3].

There are some mechanisms that could explain the positive effect of tree nuts on cardiovascular health. The most accepted is the lipid-lowering effect, since tree nuts have a high content of PUFA that could explain the reduction of total cholesterol and LDL-c levels, observed for almonds, peanuts, walnuts and pecan nuts [4]. The lipid-lowering effect of Brazil nuts has been investigated in several studies. The supplementation of one Brazil nut per day during 18 weeks in obese females reduced cardiovascular risk by increasing HDL-c levels [8]. The increase in HDL-c and the decrease in LDL-c were observed in healthy adults after an ingestion of 4 units of Brazil nuts for 4 weeks [9] and a decrease in total cholesterol and LDL-c was observed in obese female adolescents after the ingestion of 3 to 4 units per day during 16 weeks [10]. Brazil nut supplementation did not affect HDL-c and LDL-c levels in another study with healthy adults after ingestion of 10 units of nuts per day during 2 weeks,

however it did affect the transfer of cholesteryl esters into HDL pool, which is important to reverse cholesterol transport and its elimination into bile. Overall, the increase of cholesteryl esters into HDL pool after consumption of Brazil nuts can be considered as an antiatherogenic effect [11].

Brazil nuts are the richest source of selenium (Se) known in nature [12]. As the amino acid selenocysteine (Sec), Se is inserted into selenoproteins, which have important functions in the antioxidant system, lipid peroxidation, immune function, brain function, diabetes risk, among others [13]. The insertion of Sec into selenoproteins occurs during translation and it requires the presence of a specific structure in the 3'untranslated region (3'UTR) of the mRNA, a specific RNA for Sec ($tRNA^{[Ser]Sec}$), and other structures [14].

In contrast to other selenoproteins, Selenoprotein P (SePP) has ten Sec residues, which are expressed primarily in liver but also in other tissues such as brain, gut, heart, and kidneys [15]. In Se-deficient conditions, SePP is preferentially directed to the brain and testis, which implies the presence of SePP receptors APOER2 in these two tissues [15]. Plasma Se and SePP concentrations are the most used biomarkers of Se status. SePP concentration and mRNA expression have been associated with insulin resistance. A higher concentration of plasma SePP was found in people with type 2 diabetes and in rodent models of type 2 diabetes, hepatic mRNA of *SEPP1* was elevated [16]. In addition, treatment of mice with two intraperitoneal injections of 1 mg/kg body weight of purified human SePP induced glucose intolerance and insulin resistance [17]. Se and selenoprotein levels have been related to lipid and carbohydrate metabolism [18] and also to diabetes risk, where patients with type 2 diabetes have higher plasma concentrations of SePP [16]. Moreover, plasma SePP was positively associated with carotid intima media thickness [16]. In animal studies, Se supplementation either with

selenite or selenate decreased plasma triglyceride levels, however, only selenate decreased plasma cholesterol and suppressed the gene expression of gluconeogenic enzymes in the liver. It is noteworthy that high levels of selenate were necessary to achieve these effects; therefore, its use in clinical practice is not applicable [18].

SePP is encoded by the gene *SEPP1* located in chromosome 5p12. It has two functional single nucleotide polymorphisms (SNPs) [19, 20]. Both are a G to A substitution, the first one is located in the coding region of the gene changing the amino acid Alanine to Threonine at position 234 of the protein (rs3877899). This polymorphism modulates plasma Se levels in healthy adults in response to supplementation with sodium selenite [20]. The second one (rs7579) is located in the 3´UTR, important for Sec insertion, and it modulates plasma SePP concentrations [20]. Genetic variants in *SEPP1* have not been previously associated with lipid profile or glucose levels in response to supplementation with Brazil nuts. Therefore, due to the evidence associating SePP with glucose and lipid metabolism, the hypothesis of this study was that functional polymorphisms in selenoprotein genes would influence the response of serum lipids and glucose after supplementation with a high-Se and high-lipid nut such as the Brazil nut.

## Methods

### Study population and supplementation protocol

One hundred and thirty unrelated healthy adults (males and females) aged 20 to 60 years old were selected at University of São Paulo, Brazil. Volunteers taking multivitamins and mineral supplements, anti-inflammatory drugs, with excessive alcohol consumption, athletes, obese (BMI > 30) and with chronic diseases such as cancer, diabetes and cardiovascular disease were not included in the study. The

participants were invited by electronic correspondence and personal communication when the protocol was explained.

The Supplementation with Brazil Nuts study (SU.BRA.NUT study) was an eight-week dietary intervention with one Brazil nut a day in healthy subjects, followed by eight more weeks without intervention. At the beginning of the study, 20 mL of blood sample were drawn and subsequently the volunteers took a daily supplement of one Brazil nut for eight weeks. At the end of four and eight weeks of supplementation, another 20 mL blood sample was taken, and then two more blood samples were taken at four week intervals during eight weeks without intervention. (Figure 1). Volunteers were asked to complete a control calendar and mark with an “x” when they consumed each nut throughout the intervention period. For all participants, weight and height were measured and the body mass index (BMI) was calculated. Written informed consent was signed by all volunteers before blood sampling. The protocol was approved by Faculty of Pharmaceutical Sciences Ethical Committee (CAE: 00961112.3.0000.0067) and was conducted according to the Declaration of Helsinki.

**Centesimal Composition and Se content of Brazil nuts**

The centesimal composition of a random sample of Brazil nuts representative of the four batches used in the study was analysed according to the methods proposed by the Association of Official Analytical Chemists (AOAC, 1990). The Se content of Brazil nuts was determined using hydrid generation flame atomic absorption spectrometry (HGFAAS), as described previously [8].

**Blood sampling**

Fasting blood samples (20 mL) were drawn by venipuncture into four 5-mL EDTA tubes. An aliquot of 1.5 mL of EDTA whole blood was used for DNA extraction

and subsequent genotyping. Another 5 mL of blood were collected in a tube without anticoagulant to obtain serum. Plasma was separated by centrifugation at 3,000 *rpm* for 15 min at 4 °C. The erythrocyte pellet was washed three times with 5 mL sterile 9 g/L NaCl solution, slowly mixed by inversion, and centrifuged at 10,000 *rpm* for 10 min (Eppendorf, C5408) at 4 °C, and the supernatant fluid was discarded. Aliquots of whole blood, serum, plasma and erythrocytes were frozen at -80 °C in sterile, demineralized tubes until the analyses were performed.

**Biochemical parameters**

Concentrations of glucose, C-reactive protein and lipid profile (total cholesterol (TC), HDL-c, LDL-c and triglycerides) in fasting serum were measured spectrometrically using commercial kits (Labtest, Minas Gerais, Brazil) adapted to a biochemical analyser (LabMax 240, Labtest, Minas Gerais, Brazil). Malondialdehyde (MDA) was measured in plasma by high performance liquid chromatography (HPLC) on a Shimadzu (Kyoto, Japan) instrument with a Phenomenex (Torrance, CA, USA) reverse phase C18 column. The instrument was calibrated with a MDA standard stock solution in the following concentrations: 0.0, 0.25, 0.5, 1.0, 2.0, 4.0, 6.0 and 12.0 μM.

**Genotyping**

Total DNA was extracted from 200 μL of whole blood using the Purelink Genomic DNA Minikit (Invitrogen, Life Technologies, California, USA). The final concentration was measured by using a NanoDrop ND 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and adjusted for further analysis. SNPs in selenoprotein genes were determined by real-time PCR using Taqman SNP Genotyping Assays (Thermo Fisher Scientific, Wilmington, DE, USA). Samples were assayed along with no-template and internal controls for each genotype and run in the

StepOne Plus Real Time PCR system under the following conditions: enzyme activation at 95 °C for 10 min, followed by 40 cycles at 92 °C for 15s and 60 °C for 1 min for annealing and extension. The allelic discrimination was obtained by performing an endpoint read. The SNPs selected were in *GPX1* gene (rs1050450), *GPX4* gene (rs713041), *SEPP1* gene (rs3877899 and rs7579), *SELS* gene (rs34713741) and *SEP15* gene *(*rs5845).

**Statistical Analysis**

Continuous variables were tested for normality using the Kolmogorov-Smirnov test. The data were presented as geometric means (CI 95%). Concentrations of blood lipids, glucose and C-reactive protein were compared in the different time points using ANOVA repeated measures or Friedman's test. For MDA, paired t Student test was used. A genetic dominant model was used to assess differences in the presence of the rare allele. In this model, individuals with the rare allele were combined in one category, leaving the common genotype in another category. Multivariate linear regression models were created using total cholesterol and glucose at each intervention as dependent variables. Age, body fat composition, gender, tertiles of plasma Se, and six SNPs were included as independent variables. Repeated measures analysis of covariance (ANCOVA) was performed to investigate the effect of the genotypes for SNPs appointed in the multivariate linear regression models. Covariates included for outcome total cholesterol was age and for outcome glucose were gender, tertiles of plasma Se and body fat composition. The Chi-square test with continuity correction was used to determine whether genotype frequencies followed the Hardy-Weinberg Equilibrium. The haplotype distribution and linkage disequilibrium were done in the software Haploview 4.2. SNPs were considered in linkage disequilibrium when D' was > 0.5. Differences were considered significant at $p < 0.05$. The analyses were performed

using the Statistical Package for the Social Sciences software version 17.0 for Windows (SPSS, Chicago, IL, USA) and GraphPad Prism (GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, CA, USA).

## Results

### Demographic and anthropometric characteristics of the participants

A total of 130 healthy volunteers completed the entire study protocol. Females constituted 75% of the group and 72% of the group self-reported as being Caucasian. Mean age for females was 28.4 y (95% CI: 26.9 – 30.0) and for males was 29.2 y (95% CI: 26.4 – 32.3). Family history of chronic diseases, such as cancer, diabetes mellitus and cardiovascular disease, was reported by 87% of the volunteers. At baseline, there was a difference between females and males for BMI (22.5 (95% CI: 21.8 – 23.1) vs. 24.7 (95% CI: 23.5 – 26.0), respectively ($p < 0.001$) and body fat percentage (26.8 (95% CI: 25.6 – 28.0) vs. 21.4 (95% CI: 19.0 – 24.1), respectively ($p < 0.001$).

### Centesimal composition and Se content of Brazil nuts

The Se content and centesimal composition of Brazil nuts are shown in Table 1. Four different batches were used during the supplementation. The mean ± standard deviation for Se content of these four batches was 100.4 ± 5.3 μg/g. The average weight of the nuts was from 3 to 4 g, therefore each nut provided approximately 300 μg of Se, which is approximately five times higher than the RDA for adults (55μg/d).

### Lipid profile alterations after supplementation with Brazil nuts

The lipid profile and MDA levels are reported in Table 2. During the intervention, fasting glucose concentrations decreased after four and eight weeks of

daily consumption of Brazil nuts ($p < 0.001$). Total cholesterol concentrations also decreased after eight weeks of supplementation. No significant differences were observed for triglycerides, HDL-c, LDL-c and MDA levels. After interruption of Brazil nut intake, fasting glucose concentrations at four and eight weeks were still lower than baseline ($p < 0.001$). C-reactive protein levels at eight weeks after interruption were higher than baseline ($p = 0.023$). No significant differences were observed for total cholesterol, triglycerides, HDL-c, LDL-c and MDA levels.

**Influence of age on total cholesterol concentrations**

Multivariate linear regression models for total cholesterol were created in order to explain the variations observed during Brazil nut supplementation (Table 3). Age was the only variable associated with total cholesterol at 4 weeks of intervention, in which the increase of one year of age was related to the increase of 1.123 mg/dL of total cholesterol.

**Body fat percentage, gender, plasma Se and rs3877899 on *SEPP1* gene influence on glucose concentrations**

The multivariate linear regression models created for glucose can be seen in Table 4. The body fat percentage was associated with glucose concentrations at three time points. An increase of one unit of body fat percentage increased 0.429, 0.495 and 0.583 mg/dL of glucose at baseline, four and eight weeks of intervention, respectively. The presence of the SNP rs3877899 in *SEPP1* gene was associated with glucose concentrations at baseline, in which the presence of the variant allele A was related to a reduction of 4.520 mg/dL of glucose ($p = 0.025$). The increase of plasma Se concentrations was related to an increase in glucose concentrations at 4 weeks of

intervention ($p$ = 0.046). Gender was associated with glucose concentrations at eight weeks of intervention: being male was related to an increase of 8.145 mg/dL in glucose concentrations ($p < 0.001$). The frequency of the genotypes and alleles for rs3877899 in *SEPP1* gene were GG 54% (n= 70), GA 36% (n= 47) and AA 10% (n= 13) and for rs7579 were GG 38% (n= 50), GA 42% (n= 55) and AA 19% (n= 25). Genotype distribution did not significantly deviate from Hardy-Weinberg equilibrium for any of the SNPs. Haplotype analysis showed evidence of linkage disequilibrium for the two SNPs in the *SEPP1* gene (rs7579, rs3877899) (D'= 1.0 and $r^2$ = 0.15) with three haplotypes observed: haplotype a (common) GG (44%), haplotype b AG (28%) and haplotype GA (28%).

**Influence of SNPs in *SEPP1* on serum cholesterol and glucose concentrations**

Total cholesterol and glucose were stratified by SNPs in *SEPP1* (Figure 2). The SNP rs7579 modulated cholesterol response to supplementation. During the intervention, total cholesterol decreased in both groups, but carriers of the rare allele A had higher cholesterol concentrations during the supplementation, almost reaching statistical significance after four weeks of intervention ($p$ = 0.054). Glucose concentrations were modulated by the coding SNP rs3877899. During intervention, glucose concentrations decreased significantly in both groups, however carriers of the rare allele A had lower values at baseline ($p$ = 0.004) and after eight weeks of intervention ($p$ = 0.013).

**Discussion**

Previous studies have demonstrated that the intake of Brazil nuts improves blood lipid profile in adults [8–10, 22]. The present results support and extend these observations by showing that supplementation with one Brazil nut per day for eight weeks decreases total serum cholesterol and glucose concentrations in healthy adults. In addition, genetic variations in *SEPP1* (rs3877899 and rs7579) modulated the effect of Brazil nut supplementation on plasma glucose and total cholesterol levels. This study is the first to observe an association between plasma cholesterol and glucose levels and the polymorphisms in *SEPP1* after Brazil nut supplementation.

The supplementation with one Brazil nut provided an average of 300 μg of Se for each nut. This is a high amount of Se and after a long term supplementation ( e.g., > 1 year) could potentially increase the risk of metabolic diseases. Some epidemiological studies have demonstrated that high plasma Se levels (above 150 μg/L) increase the risk of all-cause mortality and cardiovascular disease. In the U.S. adult population with baseline plasma Se of 120 μg/L, there is an increased risk of mortality for cancer – mostly lung cancer – coronary and cardiovascular diseases [23]. The SELECT trial, designed to investigate the role of Se and vitamin E supplementation in prostate cancer prevention, also observed an increased risk for diabetes after supplementation with 200μg of selenomethionine for 5 years. The population was fifty years old or older American males with high baseline Se status (135μg/L)[24] . Nevertheless, in our study the population had lower Se status at baseline (90μg/L) and the duration of the intervention was only two months, which is much shorter than the duration of the SELECT trial. Moreover, our intervention was with one Brazil nut and not an isolated compound as the trial used and we are considering the genetic constitution of the individuals, which the SELECT trial did not considered. All these differences could

295 justify the absence of potential adverse effects of the Brazil nut supplementation in our
296 study.

297 Recent reports have demonstrated that the intake of Brazil nuts could increase
298 HDL-c concentrations in obese females [8] and healthy adults [9], decrease total
299 cholesterol and LDL-c in obese female adolescents [10] and decrease total cholesterol in
300 dyslipidaemic adults [22]. In the present work, we found that the daily intake of one
301 Brazil nut for eight weeks decreased total cholesterol levels. Although the decrease in
302 total cholesterol concentration can be considered of limited clinical application because
303 these volunteers were not dyslipidaemic, this reduction after eight weeks of ingestion
304 and the following increase after interruption of the intervention suggest that Brazil nuts
305 may interfere with cholesterol metabolism and this might be relevant for populations at
306 risk for metabolic syndrome or dyslipidaemia.

307 Potential mechanisms underlying this improvement in blood lipid profile include
308 the nutritional composition of Brazil nuts [25], the connection between Se metabolism
309 with the mevalonate pathway [26] and with cholesterol biosynthesis [27]. Although
310 Brazil nuts have higher saturated fatty acids (SFA) concentrations compared to other
311 nuts, the MUFA and PUFA contents are sufficient to exert their cholesterol lowering
312 effect [4].

313 Another explanation for the decrease observed in total cholesterol concentration
314 is the relation between cholesterol and selenoprotein biosynthesis. The two metabolisms
315 are connected through the mevalonate pathway [26]. In order to be functional,
316 selenoproteins need to have the amino acid Sec inserted in their structure during
317 translation. This requires the synthesis and activation of the $tRNA^{[Ser]Sec}$ which is
318 dependant on four bases modifications. One of these modifications is the
319 isopentenylation of adenosine 37, which requires isopentenyl pyrophosphate (IPP), a

direct metabolite of mevalonate during cholesterol biosynthesis [26]. Our hypothesis is that during the supplementation with a high Se content nut, the synthesis of selenoproteins might be stimulated, which could be directing the IPP for the isopentenylation of adenosine 37 of the $tRNA^{[Ser]Sec}$, to the detriment of cholesterol synthesis. Selenoproteins that are ranked high in the hierarchy of selenoprotein expression, such as GPx4, could be preferentially synthesized under such conditions [28]. The metabolism of Se and cholesterol are also connected via sterol response element binding protein 2, SREBP2. Previously it was observed that Se supplementation increased expression of 15-deoxy-12,14-prostaglandin J2 [29], a ligand of the peroxisome proliferator-activated receptor-γ, PPAR-γ, which reduces SREBP2 decreasing cholesterol biosynthesis [30]. Moreover, studies in mice deleted for the *Trsp* gene that encodes the specific transfer RNA for Sec, the $tRNA^{[Ser]Sec}$, prevented selenoproteins expression and increased plasma cholesterol [31]. All these results indicate that one or more selenoproteins may be involved in the regulation of cholesterol metabolism.

Experimental evidence has demonstrated that SNP rs3877899 in *SEPP1* influences plasma Se concentration after supplementation with 200 μg/d of sodium selenite for 4 weeks [20]. Our results extend these earlier observations, indicating that individuals with the rare allele A (GA+AA) had lower concentrations of glucose at baseline and after eight weeks of supplementation with Brazil nuts in comparison to subjects homozygous GG. As observed for total cholesterol, values were within the range expected for healthy adults, limiting the clinical application of this result. However, the reduction after just eight weeks and the maintenance of lower concentrations after interruption of the intervention could be relevant as a higher glucose lowering effect after Brazil nut intake should be expected for pre-diabetic or

345 insulin-resistant individuals. Recently, a meta-analysis of randomized controlled trials
346 investigating the influence of nut consumption on glycaemic control in diabetes
347 demonstrated that diets with tree nuts per day significantly reduced fasting glucose
348 concentration in individuals with type 2 diabetes [32]. Furthermore, SePP has been
349 associated with the insulin metabolism *in vitro* and *in vivo* studies. SePP is expressed in
350 different tissues in mice [33] but it is mainly synthesised and secreted to the
351 bloodstream by the liver [34]. The pancreas of mice also expresses SePP in the α-cells
352 which produces glucagon and in the β-cells which produces insulin [33]. SePP
353 expression was decreased in isolated islets after high glucose treatment [35]. Our
354 results indicate that after supplementation with Se the plasma SePP concentrations
355 increased (data not shown) and the glucose levels decreased, which is in accordance
356 with the previous findings observed in mice. The mechanism underlying this connection
357 of and glucose metabolism may be explained by the presence of a binding site for
358 FoxO1 transcription factor in the promoter gene of *SEPP1* [35]. This transcription
359 factor regulates the expression of gluconeogenic enzymes, such as glucose-6-
360 phosphatase and phosphoenolpyruvate carboxykinase. These observations suggest that
361 SePP is an important factor regulating glucose metabolism. Therefore, the presence of
362 genetic polymorphisms in *SEPP1* gene could modify how this selenoprotein regulates
363 the insulin metabolism.

364 In conclusion, this study indicates that supplementation with one Brazil nut per
365 day for eight weeks can reduce total plasma cholesterol concentration in healthy adults
366 and this response is apparently modulated by the coding SNP rs3877899 in *SEPP1*. In
367 addition, Brazil nut supplementation can also reduce fasting plasma glucose and this
368 effect is possibly modulated by rs7579 in *SEPP1*. Although the observed reductions
369 were still within the normal range for clinical practice, these results may be important in

future nutritional interventions with the goal of investigating the effect of supplementation with Brazil nut on cholesterol and glucose metabolisms. Any future Brazil nut supplementation study conducted for long term periods should be carried so as to produce a more modest increase in plasma Se levels in order to achieve concentrations not higher than 200 μg/L, with the aim of avoiding adverse effects observed in previous studies. These future studies should consider the baseline nutritional status, the gender and the genetic background of the participants.

## Conflict of interest

There are no actual or potential conflicts of interest that might influence judgment on the part of any author.

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**Table 1** Centesimal composition and selenium content in Brazil nuts used during the protocol

| Nutrient | Mean ± sd |
|---|---|
| Energy (kcal) | 732.9 ± 2.8 |
| Carbohydrates (g) | 15.0 ± 0.6 |
| Proteins (g) | 13.1 ± 0.2 |
| Lipids (g) | 69.0 ± 0.6 |
| Ash (%) | 3.1 ± 0.9 |
| Humidity (%) | 4.7 ± 0.7 |
| Selenium (μg/g) | 100.4 ± 5.3 |

Values are mean ± standard deviation for the four batches used.

**Table 2** Biochemical parameters during and after diary intervention with one unit of Brazil nut for 8 weeks in healthy subjects.

| Marker | Diary intervention with 1 Brazil nut | | | |
|---|---|---|---|---|
| | baseline | 4 weeks | 8 weeks | *P* value |
| Glucose (mg/dL) | 83.6 (81.7-85.6)[a] | 81.0 (79.2-82.9)[b] | 76.8 (75.0-78.7)[c] | **<0.001** |
| Total cholesterol (mg/dL) | 191.3 (184.5-198.4)[a] | 185.9 (178.9-193.3)[a,b] | 182.2 (175.7-188.9)[b] | **0.009** |
| Triglycerides (mg/dL) | 87.2 (80.2-94.7) | 88.7 (81.7-96.3) | 88.7 (81.9-96.1) | 0.431 |
| HDL-c (mg/dL) | 55.5 (52.9-58.2) | 55.6 (53.2-58.1) | 55.2 (52.6-58.0) | 0.756 |
| LDL-c (mg/dL) | 104.5 (99.7-109.4) | 105.4 (99.5-111.6) | 106.7 (101.3-112.5) | 0.398 |
| C-Reactive Protein (mg/dL) | 1.04 (0.85-1.28) | 1.10 (0.89-1.37) | 1.22 (0.99-1.51) | 0.641 |
| MDA (mg/dL) | 0.36 (0.31-0.42) | na | 0.46 (0.41-0.51) | 0.071 |
| Marker | After interruption of intervention | | | |
| | baseline | 4 weeks | 8 weeks | *P* value |
| Glucose (mg/dL) | 83.6 (81.7-85.6)[a] | 79.7 (77.9-81.6)[b] | 79.2 (77.2-81.2)[b] | **<0.001** |
| Total cholesterol (mg/dL) | 191.3 (184.5-198.4) | 186.5 (179.1-194.3) | 190.4 (182.9-198.3) | 0.729 |
| Triglycerides (mg/dL) | 87.2 (80.2-94.7) | 84.0 (77.6-91.0) | 89.7 (82.4-97.6) | 0.838 |
| HDL-c (mg/dL) | 55.5 (52.9-58.2) | 57.0 (54.3-59.7) | 56.5 (53.8-59.4) | 0.699 |
| LDL-c (mg/dL) | 104.5 (99.7-109.4) | 104.7 (99.3-110.5) | 105.3 (100.4-110.4) | 0.581 |
| C-reactive Protein (mg/dL) | 1.04 (0.85-1.28)[a] | 1.07 (0.86-1.34)[a,b] | 1.32 (1.08-1.61)[b] | **0.023** |

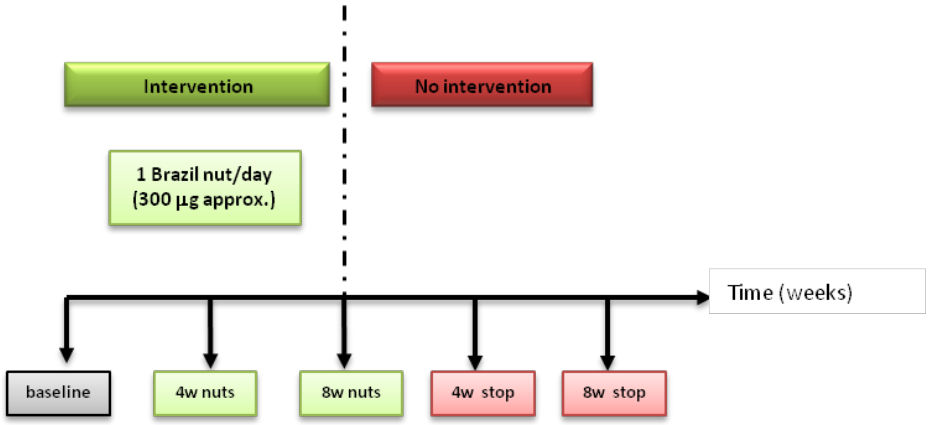
Values are geometric mean (CI 95%). ANOVA repeated measures with *post hoc* Tukey for glucose and total cholesterol, Friedman test with post hoc Dunn for triglycerides, HDL-c, LDL-c, and C-reactive protein, Wilcoxon test for MDA.
Different letters in the row mean difference among the times of intervention.
na: not analyzed in this time point

**Table 3** Multivariate linear regression models for dependent variable total cholesterol during intervention with one unit of Brazil nut in healthy subjects

| Dependent variables | Independent variables | β | St error | $R^2$ | P value |
|---|---|---|---|---|---|
| total cholesterol at baseline | gender | -10.847 | 8.815 | 0.012 | 0.221 |
| | age | 0.831 | 0.456 | 0.027 | 0.071 |
| | Plasma Se tertile | 1.636 | 5.204 | 0.000 | 0.754 |
| | fat percentage | 0.127 | 0.626 | 0.000 | 0.840 |
| | rs1050450 | 0.935 | 7.177 | 0.000 | 0.897 |
| | rs713041 | -4.854 | 7.190 | 0.003 | 0.501 |
| | rs3877899 | 1.768 | 7.206 | 0.000 | 0.807 |
| | rs7579 | 5.998 | 7.432 | 0.005 | 0.421 |
| | rs34713741 | -1.925 | 7.323 | 0.000 | 0.793 |
| | rs5845 | 0.511 | 7.187 | 0.000 | 0.943 |
| total cholesterol at 4 weeks of intervention | gender | -13.266 | 9.392 | 0.016 | 0.160 |
| | **age** | 1.123 | 0.446 | 0.050 | **0.013** |
| | Plasma Se tertile | 3.864 | 6.178 | 0.003 | 0.532 |
| | fat percentage | -0.232 | 0.649 | 0.001 | 0.721 |
| | rs1050450 | -10.227 | 7.143 | 0.016 | 0.155 |
| | rs713041 | -8.043 | 7.286 | 0.010 | 0.272 |
| | rs3877899 | 3.385 | 7.259 | 0.001 | 0.642 |
| | rs7579 | 11.394 | 7.586 | 0.018 | 0.136 |
| | rs34713741 | 2.664 | 7.442 | 0.001 | 0.721 |
| | rs5845 | 5.126 | 7.221 | 0.004 | 0.479 |
| total cholesterol at 8 weeks of intervention | gender | -5.118 | 9.407 | 0.003 | 0.587 |
| | age | 0.149 | 0.438 | 0.001 | 0.734 |
| | Plasma Se tertile | -1.616 | 5.113 | 0.001 | 0.753 |
| | fat percentage | -0.032 | 0.675 | 0.000 | 0.963 |
| | rs1050450 | -3.884 | 6.897 | 0.003 | 0.574 |
| | rs713041 | 0.019 | 6.989 | 0.000 | 0.998 |
| | rs3877899 | -2.998 | 6.995 | 0.001 | 0.669 |
| | rs7579 | 12.384 | 7.203 | 0.024 | 0.088 |
| | rs34713741 | 8.449 | 7.189 | 0.010 | 0.242 |
| | rs5845 | 5.183 | 6.936 | 0.004 | 0.456 |

**Table 4** Multivariate linear regression models for dependent variable glucose during intervention with one unit of Brazil nut in healthy subjects

| Dependent variables | Independent variables | β | St error | $R^2$ | P value |
|---|---|---|---|---|---|
| Glucose at baseline | gender | 3.001 | 2.431 | 0.012 | 0.219 |
| | age | 0.019 | 0.126 | 0.000 | 0.883 |
| | plasma Se tertile | -0.783 | 1.435 | 0.002 | 0.586 |
| | **fat percentage** | 0.429 | 0.173 | 0.049 | **0.014** |
| | rs1050450 | 0.973 | 1.979 | 0.002 | 0.624 |
| | rs713041 | -1.771 | 1.983 | 0.006 | 0.374 |
| | **rs3877899** | -4.520 | 1.987 | 0.041 | **0.025** |
| | rs7579 | 0.556 | 2.050 | 0.000 | 0.787 |
| | rs34713741 | -0.895 | 2.020 | 0.001 | 0.658 |
| | rs5845 | -0.580 | 1.982 | 0.000 | 0.770 |
| Glucose at 4weeks of intervention | gender | 1.381 | 2.362 | 0.002 | 0.560 |
| | age | -0.127 | 0.112 | 0.010 | 0.259 |
| | **plasma Se tertile** | 3.128 | 1.551 | 0.033 | **0.046** |
| | **fat percentage** | 0.495 | 0.163 | 0.071 | **0.003** |
| | rs1050450 | -1.153 | 1.796 | 0.003 | 0.522 |
| | rs713041 | -2.271 | 1.833 | 0.012 | 0.218 |
| | rs3877899 | -2.684 | 1.826 | 0.017 | 0.144 |
| | rs7579 | -1.446 | 1.908 | 0.004 | 0.450 |
| | rs34713741 | 1.124 | 1.872 | 0.003 | 0.549 |
| | rs5845 | -2.142 | 1.816 | 0.011 | 0.241 |
| Glucose at 8 weeks of intervention | **gender** | 8.145 | 2.475 | 0.083 | **0.001** |
| | age | -0.031 | 0.115 | 0.000 | 0.790 |
| | plasma Se tertile | 1.499 | 1.345 | 0.010 | 0.267 |
| | **fat percentage** | 0.583 | 0.178 | 0.082 | **0.001** |
| | rs1050450 | -0.505 | 1.814 | 0.000 | 0.781 |
| | rs713041 | -0.600 | 1.839 | 0.001 | 0.745 |
| | rs3877899 | -1.348 | 1.840 | 0.004 | 0.465 |
| | rs7579 | -0.970 | 1.895 | 0.002 | 0.610 |
| | rs34713741 | -0.905 | 1.891 | 0.002 | 0.633 |
| | rs5845 | -2.230 | 1.825 | 0.012 | 0.224 |

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533 **Fig. 1** Supplementation with Brazil nuts protocol.

534 Down arrows indicate blood sampling collection.

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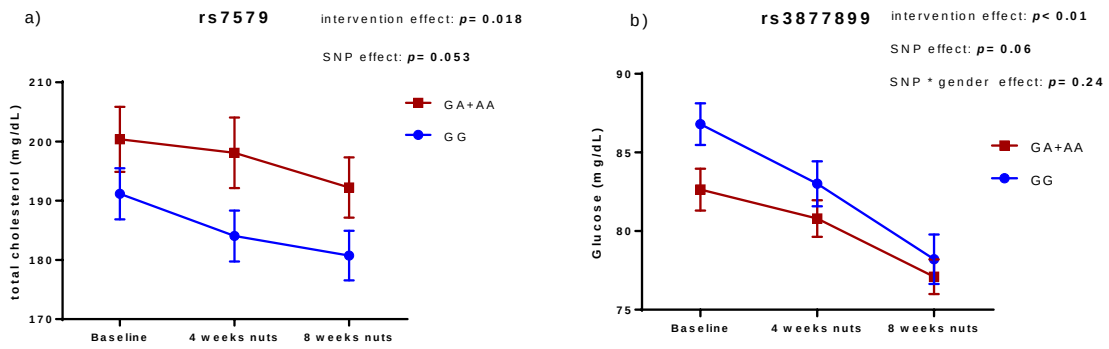


**Fig. 2** Total cholesterol and glucose stratified by polymorphisms in *SEPP1* gene

Values are means ± st error. Two way ANCOVA repeated measures adjusted for multiple comparisons by Bonferroni test for total cholesterol (a) and glucose (b) levels. Covariates used: age for cholesterol and body fat composition, gender and plasma Se tertiles for glucose levels.

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