CrossMark

ORIGINAL CONTRIBUTION

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

Janaina L. S. Donadio[1] · Marcelo M. Rogero[2] · Elvira M. Guerra-Shinohara[3] ·
Charles Desmarchelier[4] · Patrick Borel[4] · Silvia M. F. Cozzolino[1]

Abstract

Purpose The consumption of Brazil nuts has been associated with benefits to lipid metabolism and reductions in total cholesterol and LDL concentrations. 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 metabolisms. 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 8 weeks, followed by 8 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 8 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 8 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.

Keywords Brazil nuts · Lipid profile · Polymorphisms · Nutrigenetics

✉ Janaina L. S. Donadio
  janainadonadio@gmail.com

1   Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil

2   Department of Nutrition, School of Public Health, University of São Paulo, São Paulo, Brazil

3   Department of Clinical and Toxicological Analyses, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil

4   NORT, Aix-Marseille Université, INRA (French National Institute for Agricultural Research), INSERM, 13005 Marseille, France

Introduction

The consumption of tree nuts (e.g. peanuts, almonds, hazelnuts, walnuts, Brazil nuts, pistachios, cashews, and 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 analyses 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, 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 concentrations [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 3–4 units per day during 16 weeks [10]. Brazil nut supplementation did not affect HDL-c and LDL-c in another study with healthy adults after ingestion of ten 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 metabolisms [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 substitutions—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 metabolisms, 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 20–60 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 8-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 8 weeks. At the end of four and 8 weeks of supplementation, another 20 mL blood sample was taken,
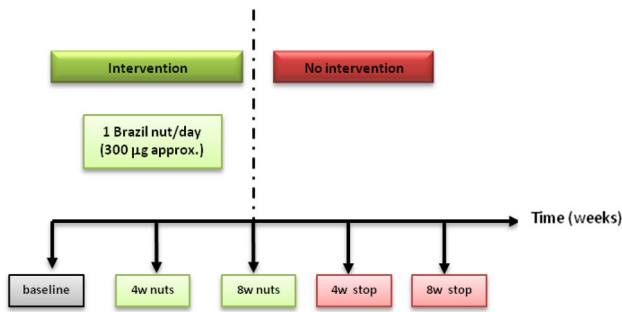
Fig. 1 Supplementation with Brazil nuts protocol. *Down arrows* indicate blood sampling collection

and then two more blood samples were taken at 4 week intervals during 8 weeks without intervention (Fig. 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 [21]). The Se content of Brazil nuts was determined using hybrid 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 3000 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 equipped with a Phenomenex (Torrance, CA, USA) reverse phase C18 column. The instrument was calibrated using 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 15 s 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 years (95% CI 26.9–30.0) and for males was 29.2 years (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 (55 µg/day).

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 4 and 8 weeks of daily consumption of

Table 1 Centesimal composition and selenium contents 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

Brazil nuts ($p < 0.001$). Total cholesterol concentrations also decreased after 8 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 8 weeks were still lower than baseline ($p < 0.001$). C-reactive protein levels at 8 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 1 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, 4 and 8 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 8 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

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

Marker	Baseline	4 weeks	8 weeks	p value
Dietary intervention with 1 Brazil nut				
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
Washout period				
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 (95% CI). 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

Significant p values are shown in bold ($p < 0.05$)

Different letters in the row mean difference among the times of intervention

na not analysed in this time point

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* (Fig. 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 4 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 8 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 8 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 concentrations. 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 US adult population with baseline plasma Se of 120 μg/L, there is an increased risk for cancer (mostly lung cancer), coronary and cardiovascular diseases after supplementation [23]. The SELECT trial, designed to investigate the role of Se and vitamin E supplementation in prostate cancer prevention, also observed

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
	Body 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
	Body 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
	Body 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

Significant p value is shown in bold ($p < 0.05$)

an increased risk for diabetes after supplementation with 200 µg of selenomethionine for 5 years. The population was 50 years old or older American males with high baseline Se status (135 µg/L) [24]. Nevertheless, in our study, the population had lower Se status at baseline (90 µg/L), and the duration of the intervention was only 2 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 considered the genetic constitution of the individuals, which the SELECT trial had not considered on its first stages. All these differences could justify the absence of potential adverse effects of the Brazil nut supplementation in our study.

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

Potential mechanisms underlying this improvement in blood lipid profile include the nutritional composition of Brazil nuts [25], the connection between Se metabolism

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

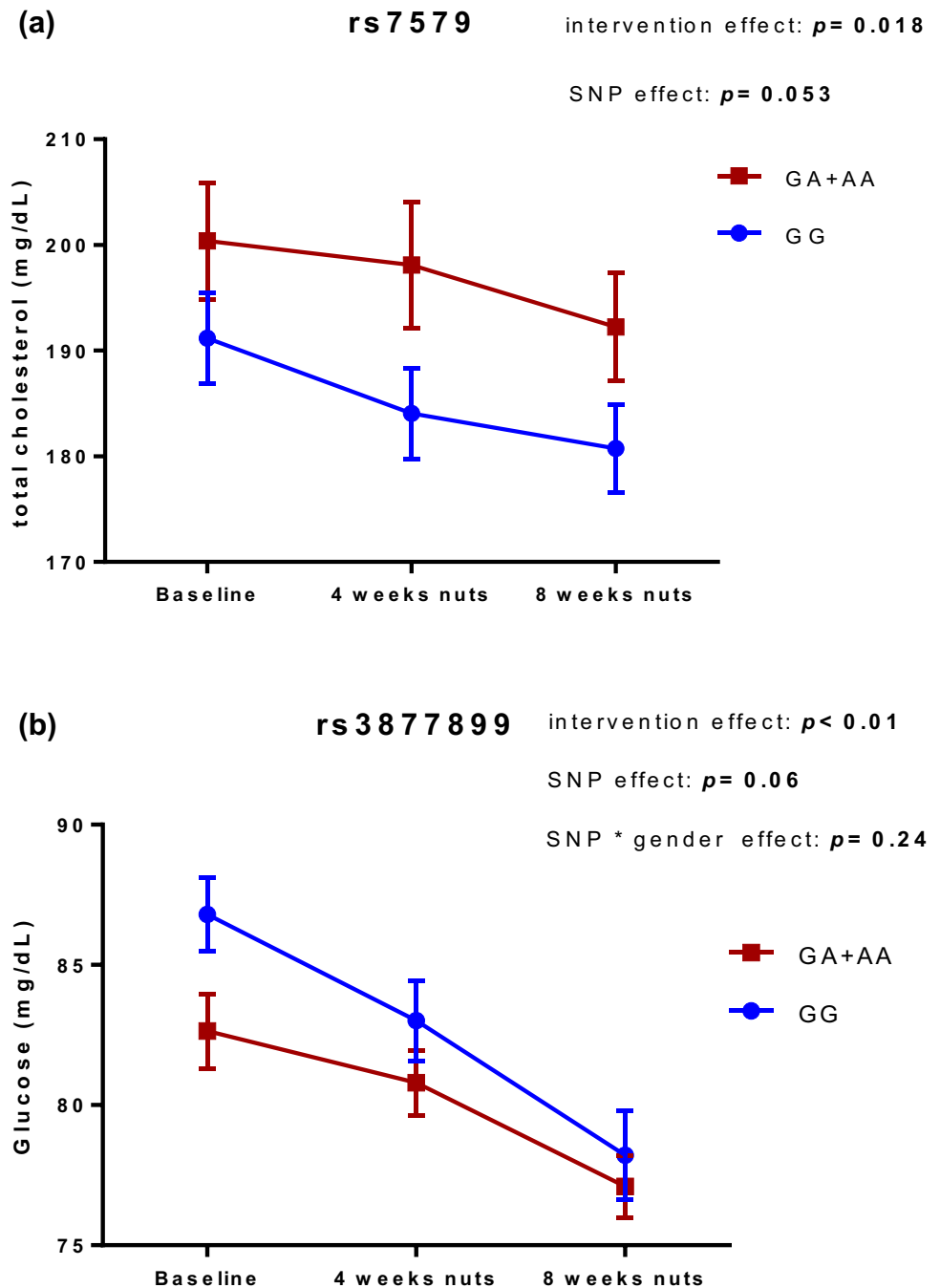| Dependent variables	Independent variables	β	St error	R^2	p value
Glucose at baseline	Gender	3.001	2.431	0.012	0.219
	Age	0.019	0.126	0.000	0.883
	Plasma Se tertile	−0.783	1.435	0.002	0.586
	Body fat percentage	0.429	0.173	0.049	**0.014**
	rs1050450	0.973	1.979	0.002	0.624
	rs713041	−1.771	1.983	0.006	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 4 weeks 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**
	Body 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
	Body 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

Significant p values are shown in bold ($p < 0.05$)

with the mevalonate pathway [26] and with cholesterol biosynthesis [27]. Although Brazil nuts have higher saturated fatty acids (SFA) concentrations compared with other nuts, the MUFA and PUFA contents are sufficient to exert their cholesterol lowering effect [4].

Another explanation for the decrease observed in total cholesterol concentration is the relation between cholesterol and selenoprotein biosynthesis. The two metabolisms are connected through the mevalonate pathway [26]. In order to be functional, selenoproteins need to have the amino acid Sec inserted in their structure during translation. This requires the synthesis and activation of the tRNA[Ser]Sec which is dependant on four bases' modifications. One of these modifications is the 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 metabolisms 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,

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

(a)   **rs 7 5 7 9**   intervention effect: ***p= 0.018***

SNP effect: ***p= 0.053***

(b)   **rs 3 8 7 7 8 9 9**   intervention effect: ***p< 0.01***

SNP effect: ***p= 0.06***

SNP * gender effect: ***p= 0.24***

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/day 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 8 weeks of supplementation with

Brazil nuts in comparison with subjects of 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 8 weeks and the maintenance of lower concentrations during the washout period could be relevant as a higher glucose lowering effect after Brazil nut intake should be expected for pre-diabetic or insulin-resistant individuals. Recently, a meta-analysis of randomized controlled trials investigating the influence of nut consumption on glycaemic control in diabetes demonstrated

that diets with tree nuts per day significantly reduced fasting glucose concentration in individuals with type 2 diabetes [32]. Furthermore, SePP has been associated with the insulin metabolism in vitro and in vivo studies. SePP is expressed in different tissues in mice [33], but it is mainly synthesised and secreted to the bloodstream by the liver [34]. The pancreas of mice also expresses SePP in the α-cells which produces glucagon and in the β-cells which produces insulin [33]. SePP expression was decreased in isolated islets after high glucose treatment [35]. Our results indicate that after supplementation with Brazil nuts the plasma SePP concentrations increased (data not shown), and the glucose levels decreased, which is in accordance with the previous findings observed in mice. The mechanism underlying this connection of SePP and glucose metabolism may be explained by the presence of a binding site for FoxO1 transcription factor in the promoter gene of *SEPP1* [35]. This transcription factor regulates the expression of gluconeogenic enzymes, such as glucose-6-phosphatase and phosphoenolpyruvate carboxykinase. These observations suggest that SePP is an important factor regulating glucose metabolism. Therefore, the presence of genetic polymorphisms in *SEPP1* gene could modify how this selenoprotein regulates the insulin metabolism.

In conclusion, this study indicates that supplementation with one Brazil nut per day for 8 weeks can reduce total plasma cholesterol concentration in healthy adults, and this response is apparently modulated by the coding SNP rs3877899 in *SEPP1*. In addition, Brazil nut supplementation can also reduce fasting plasma glucose, and this effect is possibly modulated by rs7579 in *SEPP1*. Although the observed reductions 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 out 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.

Compliance with ethical standards

References

1. Sabaté J, Ang Y (2009) Nuts and health outcomes: new epidemiologic evidence. Am J Clin Nutr 89:1643S–1648S. doi:10.3945/ajcn.2009.26736Q.Am
2. Jiang R, Jacobs DR, Mayer-Davis E et al (2006) Nut and seed consumption and inflammatory markers in the Multi-Ethnic Study of Atherosclerosis. Am J Epidemiol 163:222–231. doi:10.1093/aje/kwj033
3. O'Neil CE, Fulgoni VL, Nicklas TA (2015) Tree nut consumption is associated with better adiposity measures and cardiovascular and metabolic syndrome health risk factors in US adults: NHANES 2005–2010. Nutr J 14:64–71. doi:10.1080/07315724.2011.10719996
4. Mukuddem-Petersen J, Oosthuizen W, Jerling JC (2005) A systematic review of the effects of nuts on blood lipid profiles in humans. J Nutr 135:2082–2089
5. Welna M, Klimpel M, Zyrnicki W (2008) Investigation of major and trace elements and their distributions between lipid and non-lipid fractions in Brazil nuts by inductively coupled plasma atomic optical spectrometry. Food Chem 111:1012–1015. doi:10.1016/j.foodchem.2008.04.067
6. Segura R, Javierre C, Lizarraga MA, Ros E (2006) Other relevant components of nuts: phytosterols, folate and minerals. Br J Nutr 96(Suppl 2):S36–S44. doi:10.1017/BJN20061862
7. Bao Y, Han J, Hu FB et al (2013) Association of nut consumption with total and cause-specific mortality. N Engl J Med 369:2001–2011. doi:10.1056/NEJMoa1307352
8. Cominetti C, de Bortoli MC, Garrido AB, Cozzolino SMF (2012) Brazilian nut consumption improves selenium status and glutathione peroxidase activity and reduces atherogenic risk in obese women. Nutr Res 32:403–407. doi:10.1016/j.nutres.2012.05.005
9. Colpo E, Vilanova CDDA, Brenner Reetz LG et al (2013) A single consumption of high amounts of the Brazil nuts improves lipid profile of healthy volunteers. J Nutr Metab. doi:10.1155/2013/653185
10. Maranhão PA, Kraemer-Aguiar LG, de Oliveira CL et al (2011) Brazil nuts intake improves lipid profile, oxidative stress and microvascular function in obese adolescents: a randomized controlled trial. Nutr Metab (Lond) 8:32. doi:10.1186/1743-7075-8-32
11. Strunz CC, Oliveira TV, Vinagre JCM et al (2008) Brazil nut ingestion increased plasma selenium but had minimal effects on lipids, apolipoproteins, and high-density lipoprotein function in human subjects. Nutr Res 28:151–155. doi:10.1016/j.nutres.2008.01.004
12. Thomson CD, Chisholm A, Mclachlan SK, Campbell JM (2008) Brazil nuts: an effective way to improve selenium status. Am J Clin Nutr 87:379–384
13. Rayman MP (2012) Selenium and human health. Lancet 379:1256–1268. doi:10.1016/S0140-6736(11)61452-9
14. Hatfield DL, Gladyshev VN (2002) How selenium has altered our understanding of the genetic code. Mol Cell Biol 22:3565–3576. doi:10.1128/MCB.22.11.3565
15. Burk RF, Hill KE (2005) Selenoprotein P: an extracellular protein with unique physical characteristics and a role in selenium homeostasis. Annu Rev Nutr 25:215–235. doi:10.1146/annurev.nutr.24.012003.132120

16. Yang SJ, Hwang SY, Choi HY et al (2011) Serum selenoprotein P levels in patients with type 2 diabetes and prediabetes: implications for insulin resistance, inflammation, and atherosclerosis. J Clin Endocrinol Metab 96:1325–1329. doi:10.1210/jc.2011-0620

17. Misu H, Takamura T, Takayama H et al (2010) A liver-derived secretory protein, selenoprotein P, causes insulin resistance. Cell Metab 12:483–495. doi:10.1016/j.cmet.2010.09.015

18. Steinbrenner H (2013) Interference of selenium and selenoproteins with the insulin-regulated carbohydrate and lipid metabolism. Free Radic Biol Med 65:1538–1547. doi:10.1016/j.freeradbiomed.2013.07.016

19. Kryukov GV, Castellano S, Novoselov SV et al (2003) Characterization of mammalian selenoproteomes. Science 300:1439–1443. doi:10.1126/science.1083516

20. Méplan C, Crosley LK, Nicol F et al (2007) Genetic polymorphisms in the human selenoprotein P gene determine the response of selenoprotein markers to selenium supplementation in a gender-specific manner (the SELGEN study). FASEB J 21:3063–3074. doi:10.1096/fj.07-8166com

21. AOAC Association of Official Analytical Chemists (1990) Official methods of analysis, 15th ed. Washington

22. Carvalho RF, Huguenin GVB, Luiz RR et al (2015) Intake of partially defatted Brazil nut flour reduces serum cholesterol in hypercholesterolemic patients- a randomized controlled trial. Nutr J 14:59. doi:10.1186/s12937-015-0036-x

23. Bleys J, Navas-Acien A, Guallar E (2008) Serum selenium levels and all-cause, cancer, and cardiovascular mortality among US adults. Arch Intern Med 168:404–410. doi:10.1001/archinternmed.2007.74

24. Hatfield DL, Gladyshev VN (2009) The Outcome of Selenium and Vitamin E Cancer Prevention Trial (SELECT) reveals the need for better understanding of selenium biology. Mol Interv 9:18–21. doi:10.1124/mi.9.1.6

25. Ryan E, Galvin K, O'Connor TP et al (2006) Fatty acid profile, tocopherol, squalene and phytosterol content of brazil, pecan, pine, pistachio and cashew nuts. Int J Food Sci Nutr 57:219–228. doi:10.1080/09637480600768077

26. Moosmann B, Behl C (2004) Selenoproteins, cholesterol-lowering drugs, and the consequences: revisiting of the mevalonate pathway. Trends Cardiovasc Med 14:273–281. doi:10.1016/j.tcm.2004.08.003

27. Rayman MP, Stranges S, Griffin BA et al (2011) Effect of supplementation with high-selenium yeast on plasma lipids. Ann Intern Med 154:656–665

28. Schomburg L, Schweizer U (2009) Hierarchical regulation of selenoprotein expression and sex-specific effects of selenium. Biochim Biophys Acta 1790:1453–1462. doi:10.1016/j.bbagen.2009.03.015

29. Vunta H, Davis F, Palempalli UD et al (2007) The anti-inflammatory effects of selenium are mediated through 15-deoxy-delta 12,14-prostaglandin J2 in macrophages. J Biol Chem 282:17964–17973. doi:10.1074/jbc.M703075200

30. Klopotek A, Hirche F, Eder K (2006) PPAR gamma ligand troglitazone lowers cholesterol synthesis in HepG2 and Caco-2 cells via a reduced concentration of nuclear SREBP-2. Exp Biol Med 231:1365–1372

31. Sengupta A, Carlson BA, Hoffman VJ et al (2008) Loss of housekeeping selenoprotein expression in mouse liver modulates lipoprotein metabolism. Biochem Biophys Res Commun 365:446–452. doi:10.1038/jid.2014.371

32. Viguiliouk E, Kendall CWC, Blanco Mejia S et al (2014) Effect of tree nuts on glycemic control in diabetes: a systematic review and meta-analysis of randomized controlled dietary trials. PLoS One 9:e103376. doi:10.1371/journal.pone.0103376

33. Mao J, Teng W (2013) The relationship between selenoprotein P and glucose metabolism in experimental studies. Nutrients 5:1937–1948. doi:10.3390/nu5061937

34. Burk RF, Hill KE (2009) Selenoprotein P-expression, functions, and roles in mammals. Biochim Biophys Acta Gen Subj 1790:1441–1447. doi:10.1016/j.bbagen.2009.03.026

35. Steinbrenner H, Hotze AL, Speckmann B et al (2013) Localization and regulation of pancreatic selenoprotein P. J Mol Endocrinol 50:31–42. doi:10.1530/JME-12-0105