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

3

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22 J.L.S.D, C. D. and E. M. G. S. performed the statistical analysis. J.L.S.D. wrote the
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24 the final version of the manuscript for submission.

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29 **Abstract**

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

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

54 **Introduction**

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

67 There are some mechanisms that could explain the positive effect of tree nuts on
68 cardiovascular health. The most accepted is the lipid-lowering effect, since tree nuts
69 have a high content of PUFA that could explain the reduction of total cholesterol and
70 LDL-c levels, observed for almonds, peanuts, walnuts and pecan nuts [4]. The lipid-
71 lowering effect of Brazil nuts has been investigated in several studies. The
72 supplementation of one Brazil nut per day during 18 weeks in obese females reduced
73 cardiovascular risk by increasing HDL-c levels [8]. The increase in HDL-c and the
74 decrease in LDL-c were observed in healthy adults after an ingestion of 4 units of Brazil
75 nuts for 4 weeks [9] and a decrease in total cholesterol and LDL-c was observed in
76 obese female adolescents after the ingestion of 3 to 4 units per day during 16 weeks
77 [10]. Brazil nut supplementation did not affect HDL-c and LDL-c levels in another
78 study with healthy adults after ingestion of 10 units of nuts per day during 2 weeks,

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

83 Brazil nuts are the richest source of selenium (Se) known in nature [12]. As the
84 amino acid selenocysteine (Sec), Se is inserted into selenoproteins, which have
85 important functions in the antioxidant system, lipid peroxidation, immune function,
86 brain function, diabetes risk, among others [13]. The insertion of Sec into selenoproteins
87 occurs during translation and it requires the presence of a specific structure in the
88 3'untranslated region (3'UTR) of the mRNA, a specific RNA for Sec (tRNA^{[Ser]Sec}), and
89 other structures [14].

90 In contrast to other selenoproteins, Selenoprotein P (SePP) has ten Sec residues,
91 which are expressed primarily in liver but also in other tissues such as brain, gut, heart,
92 and kidneys [15]. In Se-deficient conditions, SePP is preferentially directed to the brain
93 and testis, which implies the presence of SePP receptors APOER2 in these two tissues
94 [15]. Plasma Se and SePP concentrations are the most used biomarkers of Se status.
95 SePP concentration and mRNA expression have been associated with insulin resistance.
96 A higher concentration of plasma SePP was found in people with type 2 diabetes and in
97 rodent models of type 2 diabetes, hepatic mRNA of *SEPP1* was elevated [16]. In
98 addition, treatment of mice with two intraperitoneal injections of 1 mg/kg body weight
99 of purified human SePP induced glucose intolerance and insulin resistance [17]. Se and
100 selenoprotein levels have been related to lipid and carbohydrate metabolism [18] and
101 also to diabetes risk, where patients with type 2 diabetes have higher plasma
102 concentrations of SePP [16]. Moreover, plasma SePP was positively associated with
103 carotid intima media thickness [16]. In animal studies, Se supplementation either with

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

108 SePP is encoded by the gene *SEPP1* located in chromosome 5p12. It has two
109 functional single nucleotide polymorphisms (SNPs) [19, 20]. Both are a G to A
110 substitution, the first one is located in the coding region of the gene changing the amino
111 acid Alanine to Threonine at position 234 of the protein (rs3877899). This
112 polymorphism modulates plasma Se levels in healthy adults in response to
113 supplementation with sodium selenite [20]. The second one (rs7579) is located in the
114 3'UTR, important for Sec insertion, and it modulates plasma SePP concentrations [20].
115 Genetic variants in *SEPP1* have not been previously associated with lipid profile or
116 glucose levels in response to supplementation with Brazil nuts. Therefore, due to the
117 evidence associating SePP with glucose and lipid metabolism, the hypothesis of this
118 study was that functional polymorphisms in selenoprotein genes would influence the
119 response of serum lipids and glucose after supplementation with a high-Se and high-
120 lipid nut such as the Brazil nut.

121 **Methods**

122 **Study population and supplementation protocol**

123 One hundred and thirty unrelated healthy adults (males and females) aged 20 to
124 60 years old were selected at University of São Paulo, Brazil. Volunteers taking
125 multivitamins and mineral supplements, anti-inflammatory drugs, with excessive
126 alcohol consumption, athletes, obese (BMI > 30) and with chronic diseases such as
127 cancer, diabetes and cardiovascular disease were not included in the study. The

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

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

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

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

149 **Blood sampling**

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

152 and subsequent genotyping. Another 5 mL of blood were collected in a tube without
153 anticoagulant to obtain serum. Plasma was separated by centrifugation at 3,000 *rpm* for
154 15 min at 4 °C. The erythrocyte pellet was washed three times with 5 mL sterile 9 g/L
155 NaCl solution, slowly mixed by inversion, and centrifuged at 10,000 *rpm* for 10 min
156 (Eppendorf, C5408) at 4 °C, and the supernatant fluid was discarded. Aliquots of whole
157 blood, serum, plasma and erythrocytes were frozen at -80 °C in sterile, demineralized
158 tubes until the analyses were performed.

159 **Biochemical parameters**

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

168 **Genotyping**

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

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

182 **Statistical Analysis**

183 Continuous variables were tested for normality using the Kolmogorov-Smirnov
184 test. The data were presented as geometric means (CI 95%). Concentrations of blood
185 lipids, glucose and C-reactive protein were compared in the different time points using
186 ANOVA repeated measures or Friedman's test. For MDA, paired t Student test was
187 used. A genetic dominant model was used to assess differences in the presence of the
188 rare allele. In this model, individuals with the rare allele were combined in one
189 category, leaving the common genotype in another category. Multivariate linear
190 regression models were created using total cholesterol and glucose at each intervention
191 as dependent variables. Age, body fat composition, gender, tertiles of plasma Se, and
192 six SNPs were included as independent variables. Repeated measures analysis of
193 covariance (ANCOVA) was performed to investigate the effect of the genotypes for
194 SNPs appointed in the multivariate linear regression models. Covariates included for
195 outcome total cholesterol was age and for outcome glucose were gender, tertiles of
196 plasma Se and body fat composition. The Chi-square test with continuity correction was
197 used to determine whether genotype frequencies followed the Hardy-Weinberg
198 Equilibrium. The haplotype distribution and linkage disequilibrium were done in the
199 software Haploview 4.2. SNPs were considered in linkage disequilibrium when D' was
200 > 0.5 . Differences were considered significant at $p < 0.05$. The analyses were performed

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

204

205 **Results**

206 **Demographic and anthropometric characteristics of the participants**

207 A total of 130 healthy volunteers completed the entire study protocol. Females
208 constituted 75% of the group and 72% of the group self-reported as being Caucasian.
209 Mean age for females was 28.4 y (95% CI: 26.9 – 30.0) and for males was 29.2 y (95%
210 CI: 26.4 – 32.3). Family history of chronic diseases, such as cancer, diabetes mellitus
211 and cardiovascular disease, was reported by 87% of the volunteers. At baseline, there
212 was a difference between females and males for BMI (22.5 (95% CI: 21.8 – 23.1) vs.
213 24.7 (95% CI: 23.5 – 26.0), respectively ($p < 0.001$) and body fat percentage (26.8 (95%
214 CI: 25.6 – 28.0) vs. 21.4 (95% CI: 19.0 – 24.1), respectively ($p < 0.001$).

215 **Centesimal composition and Se content of Brazil nuts**

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

221 **Lipid profile alterations after supplementation with Brazil nuts**

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

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

231 **Influence of age on total cholesterol concentrations**

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

237

238 **Body fat percentage, gender, plasma Se and rs3877899 on *SEPP1* gene influence on** 239 **glucose concentrations**

240 The multivariate linear regression models created for glucose can be seen in
241 Table 4. The body fat percentage was associated with glucose concentrations at three
242 time points. An increase of one unit of body fat percentage increased 0.429, 0.495 and
243 0.583 mg/dL of glucose at baseline, four and eight weeks of intervention, respectively.
244 The presence of the SNP rs3877899 in *SEPP1* gene was associated with glucose
245 concentrations at baseline, in which the presence of the variant allele A was related to a
246 reduction of 4.520 mg/dL of glucose ($p = 0.025$). The increase of plasma Se
247 concentrations was related to an increase in glucose concentrations at 4 weeks of

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

258

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

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

269

270 **Discussion**

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

279 The supplementation with one Brazil nut provided an average of 300 µg of Se
280 for each nut. This is a high amount of Se and after a long term supplementation (e.g., >
281 1 year) could potentially increase the risk of metabolic diseases. Some epidemiological
282 studies have demonstrated that high plasma Se levels (above 150 µg/L) increase the risk
283 of all-cause mortality and cardiovascular disease. In the U.S. adult population with
284 baseline plasma Se of 120 µg/L, there is an increased risk of mortality for cancer –
285 mostly lung cancer – coronary and cardiovascular diseases [23]. The SELECT trial,
286 designed to investigate the role of Se and vitamin E supplementation in prostate cancer
287 prevention, also observed an increased risk for diabetes after supplementation with
288 200µg of selenomethionine for 5 years. The population was fifty years old or older
289 American males with high baseline Se status (135µg/L)[24] . Nevertheless, in our study
290 the population had lower Se status at baseline (90µg/L) and the duration of the
291 intervention was only two months, which is much shorter than the duration of the
292 SELECT trial. Moreover, our intervention was with one Brazil nut and not an isolated
293 compound as the trial used and we are considering the genetic constitution of the
294 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 isopentenylolation of adenosine 37, which requires isopentenyl pyrophosphate (IPP), a

320 direct metabolite of mevalonate during cholesterol biosynthesis [26]. Our hypothesis is
321 that during the supplementation with a high Se content nut, the synthesis of
322 selenoproteins might be stimulated, which could be directing the IPP for the
323 isopentenylation of adenosine 37 of the tRNA^{[Ser]Sec}, to the detriment of cholesterol
324 synthesis. Selenoproteins that are ranked high in the hierarchy of selenoprotein
325 expression, such as GPx4, could be preferentially synthesized under such conditions
326 [28]. The metabolism of Se and cholesterol are also connected via sterol response
327 element binding protein 2, SREBP2. Previously it was observed that Se
328 supplementation increased expression of 15-deoxy-12,14-prostaglandin J2 [29], a ligand
329 of the peroxisome proliferator-activated receptor- γ , PPAR- γ , which reduces SREBP2
330 decreasing cholesterol biosynthesis [30]. Moreover, studies in mice deleted for the *Trsp*
331 gene that encodes the specific transfer RNA for Sec, the tRNA^{[Ser]Sec}, prevented
332 selenoproteins expression and increased plasma cholesterol [31]. All these results
333 indicate that one or more selenoproteins may be involved in the regulation of
334 cholesterol metabolism.

335 Experimental evidence has demonstrated that SNP rs3877899 in *SEPP1*
336 influences plasma Se concentration after supplementation with 200 μ g/d of sodium
337 selenite for 4 weeks [20]. Our results extend these earlier observations, indicating that
338 individuals with the rare allele A (GA+AA) had lower concentrations of glucose at
339 baseline and after eight weeks of supplementation with Brazil nuts in comparison to
340 subjects homozygous GG. As observed for total cholesterol, values were within the
341 range expected for healthy adults, limiting the clinical application of this result.
342 However, the reduction after just eight weeks and the maintenance of lower
343 concentrations after interruption of the intervention could be relevant as a higher
344 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

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

377

378 **Conflict of interest**

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

381

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

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

493 Values are mean \pm standard deviation for the four batches used.

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

Marker	Diary intervention with 1 Brazil nut			P value
	baseline	4 weeks	8 weeks	
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			P value
	baseline	4 weeks	8 weeks	
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

498 Values are geometric mean (CI 95%). ANOVA repeated measures with *post hoc* Tukey for glucose and total cholesterol, Friedman
499 test with *post hoc* Dunn for triglycerides, HDL-c, LDL-c, and C-reactive protein, Wilcoxon test for MDA.

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

501 na: not analyzed in this time point

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

Dependent variables	Independent variables	β	St error	R²	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

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

Dependent variables	Independent variables	β	St error	R²	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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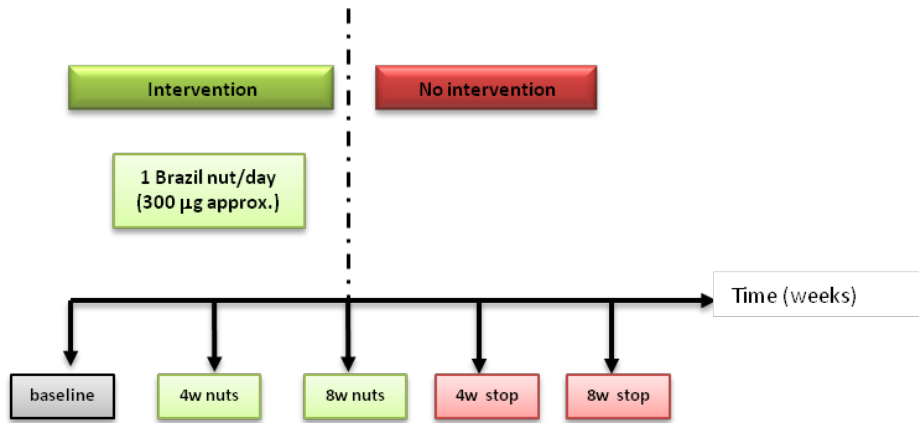
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533 **Fig. 1** Supplementation with Brazil nuts protocol.

534 Down arrows indicate blood sampling collection.

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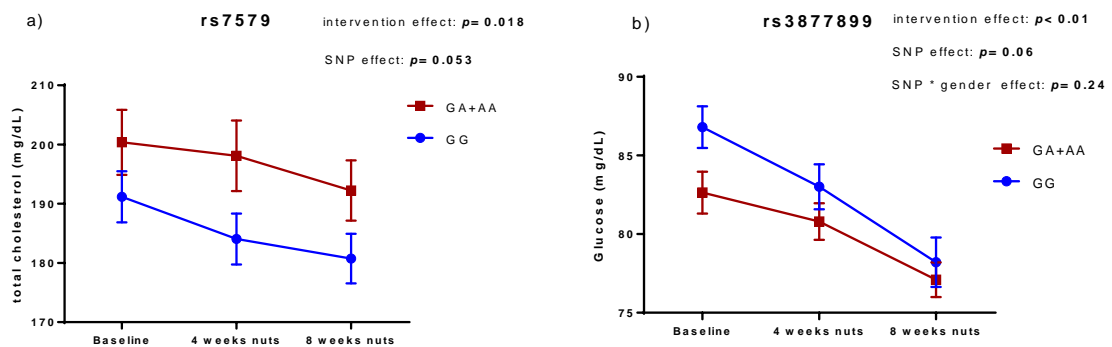


Fig. 2 Total cholesterol and glucose stratified by polymorphisms in *SEPP1* gene

Values are means \pm 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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