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Genetically determined variations of *selenoprotein P* are associated with antioxidant, muscular, and lipid biomarkers in response to Brazil nut consumption by patients using statins

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Abstract

Several single nucleotide polymorphisms (SNPs) could indirectly, as well directly, influence metabolic parameters related to health effects in response to selenium (Se) supplementation. This study aimed to investigate whether the selenoprotein SNPs were associated with the response of Se status biomarkers to the Brazil nut consumption in patients using statins and if the variation in Se homoeostasis could affect antioxidant protection, lipid profile, muscle homoeostasis and selenoproteins mRNA. The study was performed in the Ribeirão Preto Medical School University Hospital. Thirty-two patients using statins received one unit of Brazil nut daily for 3 months. Body composition, blood Se concentrations, erythrocyte glutathione peroxidase (GPX) activity, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triacylglycerol (TAG), creatine kinase (CK) activity and gene expression of *GPX1* and selenoprotein P (*SELENOP)* were evaluated before and after Brazil nut consumption. The volunteers were genotyped for SNP in *GPX1* (rs1050450) and *SELENOP* (rs3877899 and rs7579). SNPs in selenoproteins were not associated with plasma and erythrocyte Se, but SNPs in *SELENOP* influenced the response of erythrocyte GPX activity and CK activity, TAG and LDL after Brazil nut consumption. Also, Brazil nut consumption increased *GPX1* mRNA expression only in subjects with rs1050450 CC genotype. *SELENOP* mRNA expression was significantly lower in subjects with rs7579 GG genotype before and after the intervention. Thus, SNP in *SELENOP* could be associated with interindividual differences in Se homeostasis after Brazil nut consumption, emphasising the involvement of genetic variability in response to Se consumption towards health maintenance and disease prevention.

Key words: **Se: Selenoproteins: SNP: Oxidative stress: Statin**

3-Hydroxy-3-methylglutaryl-CoA reductase inhibitors, known as statins, are the most prescribed and effective pharmacological therapy for treating hypercholesterolemia and prevent cardiovascular events[1–3]. Despite their favourable overall safety profile, significant adverse effects of statin treatment have been reported, especially statin-associated muscle symptoms, defined as myalgia or muscle weakness with or without an elevation in serum creatine kinase (CK) levels[1,2,4–7]. It has been suggested that the statin-associated muscle symptoms are associated with oxidative stress, in which the imbalance between antioxidants and the over-generation of free radicals, including reactive oxygen species, could induce apoptosis in the skeletal muscles[4]. The inclusion of foods with antioxidant characteristics may be a worthwhile strategy to improve antioxidant capacity[8] and reduce statin-associated muscle symptoms risk in patients using statins. The consumption of Brazil nuts (*Bertholletia excelsa*, family Lecythidaceae) has become popular among recent studies due to its significant amount of Se in the main form of

Abbreviations: CK, creatine kinase; GPX, glutathione peroxidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Se, selenium; SELENOP, selenoprotein P; TAG, triacylgrycerol.

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selenomethionine, being a viable strategy to increase dietary intake of Se in different conditions and populations[9,10]. Moreover, the increase of Se intake due to the regular consumption of Brazil nuts has been associated with antioxidant and anti-inflammatory effects, modulation of blood lipids, decreased atherogenic risk[10] and considered as coadjutant therapy in the prevention/treatment of CVD[11].

In the human population, the individual variation in response to Se consumption, irrespective of baseline Se status, may indicate gene variants' influence[12]. Genetic polymorphisms have been recognised as an important source of interindividual variation in response to nutritional supplementation. Several SNP in selenoprotein genes could give rise to interindividual variations in Se metabolism and response to Se supplementation[9,13,14]. In particular, SNP in glutathione peroxidase *(GPX1)* (encoding GPX1) and selenoprotein P (*SELENOP*) (encoding SELENOP) have been shown to affect blood Se or selenoprotein levels after supplementation. The *GPX1 rs1050450* polymorphism is a proline to leucine substitution at codon 197, resulting in reduced enzyme activity and higher DNA damage levels[15]. The impact of *SELENOP* variation in codon 234, associated with alanine to threonine change (*rs3877899*) and guanine to adenine transition within 3' untranslated region (UTR) of *SELENOP* mRNA (*rs7579*), may result in changes in Se metabolism, but the involved mechanisms are not entirely elucidated[14]. Moreover, polymorphisms or mutations in selenoproteins' genes and synthesis cofactors may negatively influence the natural protection against oxidative stress and could be potential determinants of blood lipid levels, increasing the risk for many diseases[8,16,17].

In a previous dietary intervention with patients using statins regularly[18], we found that one Brazil nut intake for 3 months modulated the oxidative stress by increasing erythrocyte GPX activity and blood Se levels while influencing the decreased levels of malondialdehyde. We also found that Se status post-Brazil nut consumption did not enhance selenoproteins gene expression[18]. A hypothesis that may enrich the discussion presented in our previous study is that the response to Brazil nut consumption is influenced by specific polymorphisms, especially those involved in Se transportation and distribution, affecting biomarkers of metabolic pathways Se acts. Thus, in the present study, we expanded our work on the same thirty-two patients from the previous study to investigate whether SNP in *GPX1* (*rs1050450)* and *SELENOP* (*rs3877899* and *rs7579*) modify the effect of increased dietary Se intake after Brazil nut consumption on biomarkers of Se status, antioxidant protection, muscle homoeostasis, lipid profile and selenoproteins mRNA expression in the same study population.

Subjects and methods

Study design

This was an open, non-randomised, single-centre study performed at the Ribeirão Preto Medical School University Hospital, University of São Paulo, Brazil, from January 2017 to September 2018. Thirty-two patients fulfilled the inclusion criteria: age between 18 and 60 years, both sexes and using statin continuously. We did not include patients under at least one of the following conditions: nuts allergy, taking multivitamins or mineral supplements, using antibiotics or other medications that are also metabolised by cytochrome P450 3A4 (CYP3A4), in current tobacco or alcohol consumption, engaging in intense physical activity, severe cardiac complications, thyroid disorders, liver disease, kidney failure or neoplasia.

In order to investigate the influence of SNP in response to daily Brazil nut consumption, the volunteers were genotyped for SNP in *GPX1* (*rs1050450*) and *SELENOP* (*rs3877899* and *rs7579*) and distributed as homozygous wild type, heterozygous and homozygous variant.

All participants received one unit of Brazil nut daily for 3 months. The composition of Brazil nuts was described previously[18]. The Se content in Brazil nuts was 58·1 (SEM 2·1) μg/g, and the average weight was 5 g; therefore, each nut provided approximately 290 μg of Se[18]. Clinical evaluation and interviews were conducted at the beginning of the study to obtain general information (age, sex) and personal habits (co-morbidities, presence of allergies, use of medications, vitamins and mineral supplements consumption, and physical activity). Additionally, we collected venous blood samples for biochemical assays. The blood sample collection occurred before (pre-intervention period) and after (post-intervention period) 12 weeks of Brazil nuts consumption. The blood collection was performed in the University Hospital Clinical Research Unit (UPC) after 8 h of fasting. For blood plasma collection, blue-capped trace metals-free tubes containing K2EDTA anticoagulant (BD Vacutainer®) were used. Separation of whole blood to obtain plasma and erythrocytes occurred immediately after collection. An aliquot of 500 μl of whole blood was stored in 1·5 ml sterile plastic tubes for RNA extraction and subsequent gene expression. Samples were stored at –80° until the time of analysis. Data of oxidative stress, CK activity and selenoproteins mRNA expression were previously reported in[18] but were re-examined under a different stratification in the present study, according to the genotype for SNP in *GPX1* (*rs1050450*) and *SELENOP* (*rs3877899* and *rs7579*).

The Ethics Committee of Ribeirão Preto Medical School at the University of São Paulo, Brazil (protocol number CAAE: 56221916.5.0000.5440) approved the study protocol. Informed consent was obtained from all individual participants. The trial was registered at the Brazilian Clinical Trial Registry under identification number (RBR-7rwgzt).

Anthropometric evaluation

Weight was measured with an electronic platform (Filizola) scale with a precision of 0·1 kg and a maximum capacity of 300 kg. Height was measured with a vertical shaft with 0·5 cm graduation. BMI was calculated by dividing the body mass by the square of the body height, universally expressed in units of kg/m^2.

Evaluation of dietary intake

Dietary intake was assessed by food record over three non-consecutive days. Patients were oriented to self-record the type and amount of food and beverages consumed. The information was processed in the nutritional analysis programme Dietpro®, 5i

version. Brazilian databases were used to determine the food chemical composition: the food chemical composition table developed by Sonia Tucunduva Philippi[19] and the Brazilian food chemical composition table, TACO[20].

Biochemical assays

The Clinical Analysis Laboratories of HCFMRP-USP take part in rigorous external quality control programmes nationally and internationally, among them: Laboratory Testing Proficiency, Brazilian Society of Clinical Pathology/Laboratory Medicine (SBPC/ML), College of American Pathologists (CAP) and Oneworld Accuracy.

Commercially available kits by Labtest (Minas Gerais) were used following the manufacturer's protocol. Serum CK activity was measured by UV kinetic increasing reaction (Cat. No. 117). Total cholesterol in serum was measured by quantitative endpoint colorimetric assay (Cat. No. 76). LDL (Cat. No. 111) and HDL (Cat. No. 145) were measured by selective surfactant. TAG (Cat. No. 87) were measured by the Enzyme-Trinder method. The intra-assay CV was less than 10 % for all of the serum markers. As each biomarker was made within the same assay, we did not consider the inter-assay variability. GPX activity was measured in erythrocytes according to the method of Paglia and Valentine[21], as described previously[18]. The determination of total Se concentration in plasma and erythrocytes was performed according to Batista *et al.*[22] by an Inductively Coupled Plasma MS (NexIon 2000B, Perkin Elmer). Samples were diluted in the ratio 1:50 with a solution containing Triton X-100 0·01 % (v/v), HNO3 0·05 % (v/v) and 10 mg/l-1 rhodium (Rh) as an internal standard. The concentration of the analytical calibration standards ranged from 0 to 50 μg/l[22]. To verify the accuracy and precision of data, the analysis of reference materials – QMEQAS07B06 and QMEQAS07B03 human blood from the L'Institut National de Santé Publique du Quebec (Canada) – was performed at the beginning of the assay and after running ten samples. Originated values were constantly in agreement with target values (recoveries > 90 % and *t* test, 99 %). Repeatability (intra-assay precision) was < 8 %. The method detection limit is 0·2 μg/l.

Genotyping of the rs1050450, rs7579 and rs3877899 SNP

Isolation of DNA from whole blood was carried out using a PureLink Genomic DNA kit (Invitrogen, Life Technologies Inc.), and the concentration was measured using a NanoDrop ND 1000 spectrophotometer (Thermo Fisher Scientific). Polymorphisms in *GPX1* (rs1050450) and *SELENOP* (rs7579 and rs3877899) genes were determined by real-time quantitative PCR with TaqMan SNP Genotyping assays (Life Technologies). The assays were obtained as pre-designed from Applied Biosystems for rs7579 and rs3877899 (ID Assays C___8806056_10 and C___2841533_10, respectively) and custom-made through Custom TaqMan® Genomic Assays service for rs1050450 (*GPX1*/rs1050450: primers: F: 5′-TGT GCC CCC TAC GCA GGT ACA-3′, R: 5′-CCC CCG AGA CAG CAG CA-3′, T-allele: 5′-FAM-CTG TCT CAA GGG CTC AGC TGT-MGB-3′, C-allele: 5′-VIC-CTG TCT CAA GGG CCC AGC TGT-MGB-3′). The quantitative PCR reaction contained 3·125 μl of 1Å~ GoTaq® Master Mix (Promega), 0·156 μl of the 20Å~ SNP Genotyping Assay (Life Technologies) and 20 ng of genomic DNA in a 6·741 μl total reaction volume. Samples were run on Applied Biosystems 7500 real-time PCR system (Applied Biosciences/Thermo Fisher Scientific) under standard conditions. The validity of *GPX1* rs1050450 quantitative PCR results was verified by direct sequencing 5 % of the samples using the BigDye FN Sequencing Kit (PE Applied Biosystems) and the following primers: primer forward 5′-CATCGAAGCCCTGCTGTCT-3′; primer reverse 5′-CACTGCAACTGCCAAGCA-3′. The agreement of the genotypes determined for the blinded quality control samples was 100 %

GPX1 *and* SELENOP *gene expression*

We previously described the procedures of RNA extraction and cDNA synthesis[18]. The analysis of gene expression was performed by quantitative PCR using TaqMan Gene expression Assays for *GPX1* (Hs00829989_gH) and *SELENOP* (Hs01032845_m1). *β*-actin (4352935E) mRNA expression was used as a reference gene. The relative expressions were calculated using the $^{2-\Delta\Delta Ct}$ method[23].

Statistical analysis

For all statistical analyses, homozygous and heterozygous individuals for the variant alleles were combined in one category, leaving the homozygous wild type in another category to increase the statistical power. The χ^2 test was used to determine whether genotype frequencies followed the Hardy–Weinberg equilibrium. A repeated-measures ANOVA adjusted for multiple comparisons with the Bonferroni test was used to determine the intergroup effect between genotypes. According to genotype, the post-intervention data (after Brazil nut consumption) were compared with pre-intervention data (before Brazil nut consumption) and were assessed by the paired Student's *t* test or Wilcoxon test. Pearson's test was applied to determine the correlations between genotype, GPX enzyme activity and erythrocyte Se during the intervention period. Data were plotted in Statistical Package for the Social Sciences software version 14·0 (SPSS) and GraphPad Prism software (GraphPad Software Inc.). Differences were considered significant if $P < 0{\cdot}05$.

Results

Thirty-two participants were included and completed the study protocol. Among them, 59·4 % were men, and 40·6 % were women. Participants' mean age was 50·1 (SEM 7·6) years. According to BMI, patients were classified as obese or overweight (31·1 (SEM 3·8) kg/m^2). According to the analysis of non-consecutive dietary food records, at baseline, the participants' energy intake was 8487·9 (SEM 1266·5) kJ/d and, all participants maintained their regular diet during the intervention (data not shown). At the beginning of the study, as part of the questionnaire of personal habits, participants were asked about the type, intensity and frequency of physical activity. Most of the participants were not practicing regular physical activity (84·4 %), and they did not change this habit during the study. Regarding the type of cholesterol-lowering medication, 71·9 % were using

British Journal of Nutrition

simvastatin (40 mg) and 28·1 % atorvastatin (40 mg). The pre-supplementation variables of the study population were not different among genotype groups (data not shown).

Genotype distribution and variant allele frequencies for the *GPX1 rs1050450, SELENOP rs7579* and *SELENOP rs3877899* polymorphisms are shown in Table 1. One participant has excluded from *SELENOP rs7579* evaluation due to the inconclusive genotype. The genotype distribution of the studied polymorphisms was in Hardy–Weinberg equilibrium (data not shown).

The pre-and post-Brazil nut consumption and genotype effect on erythrocyte GPX activity, plasma and erythrocyte Se, and CK activity for each SNP are shown in Table 2. In the present study, the significant increase in plasma and erythrocyte Se after Brazil nut consumption occurred regardless of the genotypes. Also, the increase in both erythrocyte GPX activity and CK activity was not significant post-intervention in the presence of variant alleles in *SELENOP*.

Total cholesterol, TAG, LDL and HDL levels were stratified according to *SELENOP rs3877899* genotypes (Table 3). After the intervention, TAG and LDL levels decreased significantly in wild-type individuals. However, in allele T carriers' presence, we did not find significant differences between pre- and post-intervention for the same parameters, and the intergroup comparison indicated higher levels of TAG and LDL in these individuals than the wild-type carriers. No differences were observed in the lipid profile in the presence of rs7579 and rs1050450 polymorphisms (data not shown).

We also investigated the effects of genotype and Brazil nut consumption in selenoprotein genes' mRNA expression (Fig. 1). After Brazil nut consumption, *GPX1* expression's up-regulation occurred only in individuals with CC genotype for rs1050450 ($P = 0{\cdot}0361$) but not in CT + TT ($P = 0{\cdot}4375$). (Fig. 1(a)). In the presence of the variant allele A for rs7579, we observed an up-regulation of *SELENOP* mRNA expression when compared with the wild type despite the intervention ($P_{\text{Genotype}} = 0{\cdot}05$) (Fig. 1(c)). *SELENOP* mRNA expression was not influenced by rs3877899 polymorphism in response to Brazil nut consumption (Fig. 1(b)).

Discussion

Individual variations in Se supplementation response may partly be reflected by the selenoprotein gene variant[12,15,24]. Recent studies have demonstrated that selenoprotein SNP, including *GPX1* rs1050450, *SELENOP* rs3877899 and *SELENOP* rs7579, may interfere with Se utilisation and effectiveness[12,24]. The study of Méplan *et al.* provided 100 μg of sodium selenite/d and observed that SNP in selenoproteins might predict the behaviour of biomarkers of Se status and response to supplementation, influencing interindividual differences in Se metabolism[14]. In the present study, participants received about three times more Se, approximately 290 μg of Se/d, which is higher than the RDA for adults (55 mg/d) but less than the upper limit (400 mg/d) established by the Institute of Medicine (IOM), 2000[18]. Nevertheless, we did not find significant differences in blood Se levels between selenoprotein SNP genotypes, pre- and post-intervention. Thus, the current analysis indicated that the variations in Se biomarkers assessed were strongly dependent on Se status, and at saturated Se condition, some functional differences of gene polymorphisms could be masked.

Considering the role of GPX in the antioxidant defense system, it appears that subjects with the variant allele in *SELENOP* rs7579 and rs3877899 had lower enzyme activity, indicating that these SNP could affect the antioxidant protection. Studies have demonstrated that the presence of *SELENOP* variant alleles could modify the efficiency of selenocysteine incorporation into SELENOP, the stability of SELENOP and cellular Se uptake, affecting the Se metabolism, blood concentrations and the synthesis of other selenoproteins, including antioxidant enzymes[8,9,14,15,25]. Previous studies have also correlated the rs7579 polymorphism with several clinical conditions characterised by oxidative stress, such as diabetes and cancer[26,27]. Moreover, Gharipour *et al.* observed that the existence of variant alleles in rs3877899 decreased the antioxidant activity in Iranian patients with CVD, confirming the importance of SELENOP in antioxidant defense[27].

The muscular side effects associated with statin treatment could resemble the symptoms of Se deficiency[28]. Thus, Se supplementation could mitigate statin's side effects, mainly due to its antioxidant defense role[18]. Nonetheless, in the presence of *SELENOP* rs7579 and rs3877899 variant alleles, the CK activity, a biomarker to muscle damage, did not change after Brazil nut consumption, indicating a possible impairment in the Se carrying capacity to extra-hepatic tissues via SELENOP[27].

Previous studies have demonstrated that the intake of Brazil nuts improves blood lipid profile in adults[29,30], which could be an auxiliary strategy in treating dyslipidaemia, resulting in a reduction in the statin dosage and avoiding muscular side effects dose dependent. However, a genetic variation in *SELENOP rs3877899* altered the response to Brazil nut consumption regarding LDL and TAG levels, suggesting that this polymorphism influenced Se's ability to improve biomarkers of lipid profile.

Alterations in selenoprotein activity and concentration during Se depletion and repletion are accompanied by changes in the mRNA levels[12]. In the present study, Brazil nut consumption effectively increased *GPX1* mRNA expression in circulating blood leukocytes only in individuals of the CC genotype at rs1050450, corroborating with Cardoso *et al.* and Donadio *et al.*[8,9,13]. Nevertheless, other studies did not find an association between Se supplementation and selenoprotein transcript levels in circulating leukocytes and whole blood[12,31,32]. A possible explanation for these conflicting findings could be the excess levels of circulating Se after Brazil nut consumption affecting selenoproteins' expression and activity[18,33,34]. Jablonska *et al.*[35] also found a functional significance of rs1050450 polymorphism resulting in a different response of GPX1 activity to Se supplementation. They pointed to the importance of the genetic background in assessing the Se status, especially in the supplementation trials[35]. Another possibility could be Se's chemical form since the Se compounds with singular chemical characteristics are metabolised by distinct pathways, varying in their abilities to produce distinct Se metabolites[36,37]. Interestingly, Se in Brazil nuts is found in the main form of

Table 1. Genotype and allele frequencies for polymorphisms in *GPX* and *SELENOP* genes of participants (*n* 32)
(Numbers and percentages)

	Wild type		Heterozygous + variant		MAF	
Polymorphism	*n*	%	*n*	%	*n*	%
GPX						
rs1050450 *n* 32	CC		CT + TT		T	
	20	62·5	12	37·5	8	25
SELENOP						
rs3877899 *n* 32	CC		CT + TT		T	
	7	21·9	25	78·1	14	43·8
rs7579 *n* 31	GG		GA + AA		A	
	17	54·8	14	45·2	8	25·8

C, cytosine; T, thymine; A, adenine; G, guanine; GPX, glutathione peroxidase; SELENOP, selenoprotein P; MAF, minor allele frequency.

Table 2. Pre- and post-Brazil nut consumption and genotype effect of erythrocyte GPX activity, plasma Se, erythrocyte Se and CK activity according to the studied genotypes†
(Mean values with their standard errors of mean; numbers; 95 % confidence intervals)

	Pre		Post		Change		
Polymorphism	Mean	SEM	Mean	SEM	*n*	95 % CI	*P*
Erythrocyte GPX activity (U/g Hb)							
rs1050450							
CC	24·6	4·3	29·1	4·5	4·5	0·9, 8·1	0·01
CT + TT	22·8	2·7	28·4	6·8	5·6	0·9, 10·3	0·02
rs3877899							
CC	24·1	3·9	29·2	5·5	5·1	1·9, 8·2*	0·01
CT + TT	23·3	3·8	27·5	4·7	4·2	–2·1, 10·6*	0·25
rs7579							
GG	23·7	4·6	29·9	5·5	6·2	2·2, 10·2*	0·002
GA + AA	24·2	2·8	27·8	5·2	3·6	–0·3, 7·5*	0·08
Plasma Se (ng/ml)							
rs1050450							
CC	82·9	12·4	294·4	128·6	211·5	149, 274	< 0·0001
CT + TT	88·9	16·0	252·6	80·2	163·7	79, 248	0·0002
rs3877899							
CC	86·1	14·3	291·1	119·1	205	147, 263	< 0·0001
CT + TT	82·9	12·7	240·0	91·0	157	50, 264	0·003
rs7579							
GG	84·6	12·7	286·3	70·6	201·8	130, 273	< 0·0001
GA + AA	85·5	15·4	272·3	149·8	186·8	113, 261	< 0·0001
Erythrocyte Se (ng/ml)							
rs1050450							
CC	86·4	18·5	399·0	173·0	312·6	228, 397	< 0·0001
CT + TT	85·9	11·1	302·3	103·3	216·5	105, 328	0·0002
rs3877899							
CC	87·5	16·7	358·4	177·0	270·9	190, 322	< 0·0001
CT + TT	85·5	13·4	301·7	121·7	216·1	62, 370	0·005
rs7579							
GG	88·1	16·3	367·1	143·5	279	179, 379	< 0·0001
GA + AA	84·9	15·7	340·9	184·1	256	153, 359	< 0·0001
Creatine kinase activity (nmol/ml)							
rs1050450							
CC	228·5	35	174·6	30·4	–59·7	0·6, 119·5	0·001
CT + TT	206·0	49	109·3	13·47	–96·7	18·2, 175	0·02
rs3877899							
CC	220·8	35	152·5	25·3	–68·3	14, 122	0·01
CT + TT	218·2	25	163·3	20·2	–74·8	–33, 183	0·23
rs7579							
GG	192·6	33	116·8	16·5	–75·9	7·8, 143·7	0·03
GA + AA	198·3	44	136·7	29·3	–61·6	–13, 137	0·13

C, cytosine; T, thymine; A, adenine; G, guanine; GPX, glutathione peroxidase.
† Repeated-measures two-way ANOVA adjusted for multiple comparison with Bonferroni test. Differences in genotype effect are represented as *$P < 0·05$.

Table 3 Lipid profile pre- and post-Brazil nut consumption with Se according to *SELENOP rs3877899* genotypes† (Mean values with their standard errors of mean)

	Pre		Post		
Lipid profile	Mean	SEM	Mean	SEM	*P*
Total cholesterol					
CC	184·8	43·4	163·2	39·2	0·07
CT + TT	164·7	24·9	158·0	31·7	0·69
TAG					
CC	141·9	46·8	112·5	44·5*	0·03
CT + TT	158·3	66·9	142·2	52·5*	0·56
LDL					
CC	113·3	38·2	89·4	25·5*	0·008
CT + TT	102·7	44·5	106·6	48·9*	0·47
HDL					
CC	42·9·6	9·5	41·1	9·9	0·83
CT + TT	38·5	44·2	40·4	7·5	0·44

C, cytosine; T, thymine; A, adenine; G, guanine.
† Wilcoxon matched-pairs signed-rank test. Differences in genotype effect are represented as $^*P < 0{\cdot}05$.

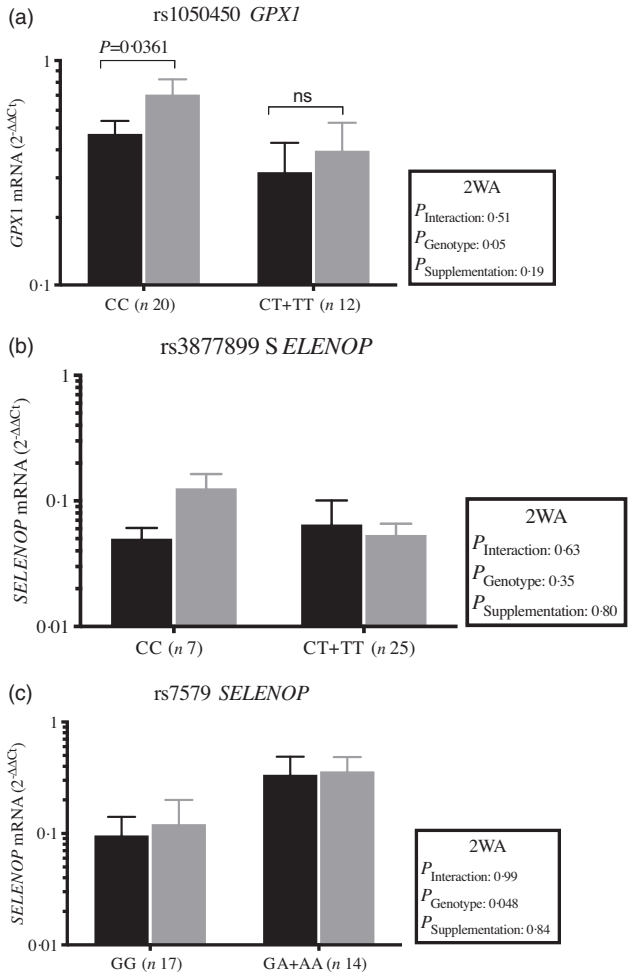

Fig. 1. Effects of dietary consumption of Brazil nuts on selenoprotein expression in previously genotyped volunteers. (a) GPX1 mRNA expression as a function of genotype for rs1050450; (b) SELENOP mRNA expression as a function of genotype for rs3877899 and (c) for rs7579. Values were normalised to β-actin and are represented as median with interquartile range, plotted as individual values (log10). Two-way ANOVA (2WA) repeated measures adjusted for multiple comparisons with Bonferroni. $^*P < 0{\cdot}05$, Wilcoxon test. A, adenine; C, cytosine; G, guanine; T, thymine; GPX, glutathione peroxidase; SELENOP, selenoprotein P. ■, before; ■, after.

selenomethionine (concentration ranges from 75 to 90 %), which has a high bioavailability and low toxicity[18,38], being a valuable alternative for Se supplementation[10].

The variant allele for rs7579 was associated with increased *SELENOP* expression pre- and post-Brazil nut supplementation. One possibility is the influence of *SELENOP* polymorphisms in the ratio of SELENOP isoforms in plasma, affecting the *SELENOP* mRNA expression, especially the 60 kDa isoform, found in higher amounts in the presence of the variant allele A for rs7579[8,9,12,25].

In this study, due to the small size sample, it was not possible to detect the effect of sex on biochemical parameters. The other limitation was the absence of a control group. However, since this study's primary purpose was to investigate the effect of the genotypes on the dietary intervention response, we decided that the wild-type genotype for each SNP would be the best control.

In conclusion, our results indicated that SNP in *SELENOP* were associated with interindividual differences in Se utilisation after Brazil nut consumption in patients using statins, indicating that the antioxidant protection, muscle homoeostasis and lipid profile are depending upon the presence of genetic polymorphisms. We also demonstrated the influence of selenoprotein polymorphisms rs1050450 and rs7579 on the mRNA expression of the selenoproteins GPX1 and SELENOP. However, more studies are required to understand the specific molecular mechanisms by which the genetic components interact with other interindividual variations, affecting the response of Se supplementation and, consequently, the Se metabolism. This knowledge could assist in a more individual approach to Se supplementation in clinical practice.

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The authors declare that they have no financial conflict of interest.

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