Clinical Nutrition xxx (xxxx) xxx

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Association between creatine kinase activity, oxidative stress and selenoproteins mRNA expression changes after Brazil nut consumption of patients using statins

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ARTICLE INFO

Article history:
Received 13 December 2019
Accepted 10 February 2020

Keywords:
Statin
Selenium
Selenoproteins
Creatine kinase activity
Oxidative stress

SUMMARY

Background & aims: Although the mechanisms by which statins promote muscle disorders remain unclear, supplementation with dietary antioxidants may mitigate statins' side effects. This study aimed to investigate whether the consumption of Brazil nuts modulates serum creatine kinase (CK) activity in patients regularly using statins.

Methods: The study was performed in the Ribeirão Preto Medical School University Hospital. Thirty-two patients in regular use of statins were divided according to CK activity levels (G1: increased or G2: normal) and received one unit of Brazil nut daily for 3 months. Body composition, blood selenium (Se) concentrations, erythrocyte glutathione peroxidase (GPX) activity, oxidative stress parameters, and CK activity were evaluated before and after supplementation.

Results: In both groups, supplementation with one Brazil nut daily for 3 months contributed to achieve decreased levels of CK activity in serum, with positive changes in plasma and erythrocyte Se concentrations ($p < 0.0001$), and increased levels of GPX activity. Among the parameters related to curbing of oxidative stress, we observed reduced levels of malondialdehyde (MDA) and superoxide dismutase (SOD) in both groups after supplementation. We also found a moderately negative association between CK and GPX activity ($r = -41$; $p < 0.02$). Expression of selenoproteins *GPX1*, *SELENOP*, and *SELENON* after Brazil nut supplementation was unchanged.

Conclusion: Brazil nut consumption enhanced the control of CK activity by improving oxidative stress biomarkers in patients using statins but did not modulate mRNA expression of selenoproteins.

1. Introduction

Statins are the most prescribed and effective pharmacological therapy for the treatment of hypercholesterolemia and the prevention of cardiovascular events [1]. They are specific and potent inhibitors of cholesterol biosynthesis in the mevalonate pathway [2–4]. This pathway is also important for the maturation of selenocysteine tRNA (Sec tRNA), which is responsible for the expression of selenoproteins [5,6]. When this pathway is inhibited, diminished availability of selenoproteins can occur [4,7]. Selenoprotein deficits have been associated with side effects of statins, such as myopathies and increased oxidative stress [4,7]. An appealing candidate for curtailed expression in statin-induced myopathy is selenoprotein N (*SELENON*) [8,9]. Other candidates are selenoprotein P

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https://doi.org/10.1016/j.clnu.2020.02.012
0261-5614/

(*SELENOP*), important to transport selenium from the liver to target tissues [10], and glutathione peroxidase (*GPX*), which could also present reduced expression, and is involved in hydrogen peroxide and hydroperoxide detoxification, protecting against oxidative injury [10].

Creatine kinase (CK) is an enzyme found in both cytosol and mitochondria of tissues where energy demands are high, most notably skeletal muscle. Elevated serum CK activity can indicate tissue damage and are observed in several pathological conditions, including statin-induced myopathy [3,11,12]. According to Consensus Statements and Clinical Practice Guidelines established by International Health Societies, statin-related myopathy characterization is complex and has been defined as myalgia or muscle weakness associated with serum CK levels ranging between 5 and 10-fold the upper limit of normal [3,13–15]. However, some studies have described the presence of statin-associated muscle disorders in patients even with normal or slightly elevated levels of serum CK [3,14,16]. Symptoms of statin-related myopathy usually occur soon after initiation of statin therapy, but sometimes may appear even after years of treatment [17].

Dietary Selenium (Se) supplementation was shown to mitigate the statins' side effects [4,7,18]. However, the mechanisms underlying the beneficial effects have not yet been fully elucidated. One dietary supplement that has become popular among recent studies is Brazil nut (*Bertholletia excelsa*, family Lecythidaceae), especially due to its great amount of Se in the main form of Selenomethionine (SeMet), which has a high bioavailability and low toxicity [19,20]. Supplementation with Brazil nuts has been shown to improve Se status in different conditions and populations [19,21–26].

In the present study, we sought to investigate whether the consumption of Brazil nuts assists in the control of serum CK activity of patients in regular use of statins. We hypothesized that the daily consumption of Brazil nuts would have benefits on Se status, increase antioxidant enzyme activity, and modulate mRNA expression of selenoproteins involved in the antioxidant process, muscle homeostasis and Se transportation.

2. Methods

2.1. Ethic statement

All procedures followed in this study have been performed in accordance with the ethical standards as laid down in the Declaration of Helsink. The study protocol was approved by the Ethics Committee of the Ribeirão Preto Medical School at the University of São Paulo, Brazil (protocol number CAAE: 56221916.5.0000.5440). Informed consent was obtained from all individual participants. The trial was registered at Brazilian Clinical Trial Registry under identification number (RBR-7rwgzt).

2.2. Study population

This was an open, non-randomized, controlled, single-center study conducted at the Ribeirão Preto Medical School University Hospital, University of São Paulo, Brazil, from January 2017 to July 2017. Inclusion criteria were being between 18–60 years of age, both sexes, and in regular use of statin. Out of the initial 55 eligible participants identified, seven participants were excluded because they were not using the statin continuously or had the type or dosage of medication changed by the doctor in recent months ($n = 7$) or refused to participate ($n = 4$). Thus, according with measurements of CK activity previously obtained from medical records, 44 participants were allocated into Group 1: ($n = 22$) patients with increased CK activity (>189 U/L) or Group 2: ($n = 22$) patients with normal CK activity (<189 U/L), either to receive Brazil nut supplementation (Fig. 1). Patients with nuts allergy, taking multivitamins and mineral supplements, in use of antibiotics or other medications that are also metabolized by cytochrome P450 3A4 (CYP3A4), in current tobacco and alcohol consumption, athletes or individuals practicing intense physical activity, with serious cardiac complications, thyroid disorders, liver disease, kidney failure and, neoplasia were not included in the study.

2.3. Study procedures

Clinical evaluation and interview were conducted at the beginning of the study to obtain general information for anamnesis. During the first visit, height and weight were measured for each participant and these values were used to calculate body mass index (BMI). We also collected venous blood samples for biochemical evaluations. Volunteers received, at no cost, three vacuum-sealed bags containing Brazil nuts enough for all intervention period. They were oriented to consume one Brazil nut daily for 3 months. Periodically, researchers contacted the participants to monitor the compliance of Brazil nuts consumption. All subjects were instructed to maintain their normal diet and to avoid additional nuts during the study. Once the 3-month study period was completed, we again assessed anthropometric data and collected a second blood sample to perform post supplementation biochemical measurements.

2.4. Centesimal composition of Brazil nuts

Brazil nuts were originally from the Brazilian state of Amazon and were acquired in partnership with the Excelsa Institute (Itacoatiara, AM, Brazil). A random sample of Brazil nuts used in the study was analyzed in triplicate according to AOAC [27] for determining humidity, ash, protein, and lipids. The total carbohydrates were calculated by difference (100—total grams of humidity, protein, lipids, and ash), including fiber fraction.

2.5. Sample collection

The collection of blood samples occurred before and after 3 months supplementation with Brazil nuts and was performed in the University Hospital Clinical Research Unit (UPC) after 8 h of fasting and the separation of whole blood to obtain plasma and erythrocytes occurred immediately after collection. An aliquot of 500 μl of whole blood was stored into 1.5 mL sterile plastic tubes used for RNA extraction and subsequent gene expression. The samples were stored at −80° until the time of analysis.

2.6. Biochemical evaluation

Commercially available kits by Labtest (Minas Gerais, Brazil) were performed following the manufacturer protocol to measure serum CK activity (Cat. No. 117) by UV kinetic increasing reaction according to the International Federation of Clinical Chemistry and Laboratory Medicine and quantitative end point colorimetric assay of total cholesterol in serum (Cat. No. 76).

2.7. Selenoprotein gene expression

Total RNA was isolated from whole blood using TRIzol reagent (Invitrogen Life Technologies) and final concentration was measured in a NanoDrop ND 1000 spectrophotometer (NanoDrop ND 1000, Thermo Scientific, Wilmington, DE, USA). RNA integrity was considered acceptable when the absorbance ratios in 260 and 280 nm wavelengths were between 1.8 and 2. cDNA was synthesized by reverse transcription polymerase chain reaction

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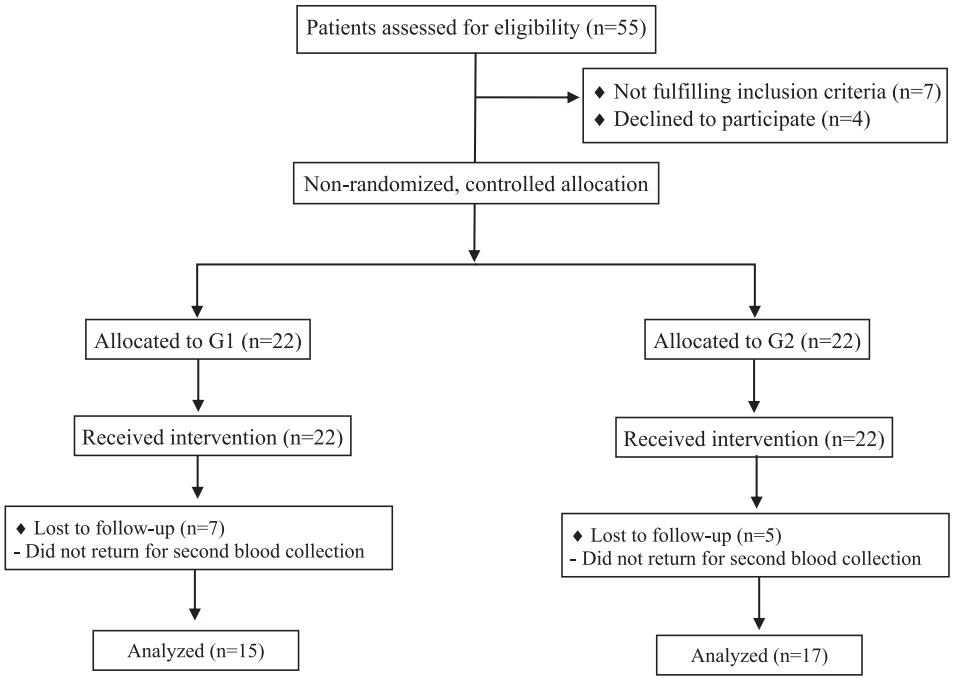

Fig. 1. Flowchart of inclusion and follow-up.

(PCR) using the High Capacity Reverse Transcriptase kit (Applied Biosystems, Thermo Scientific, Foster City, CA, USA). Analysis of gene expression was performed by real-time quantitative PCR using Taqman Gene expression Assays for *GPX1* (Hs00829989_gH), *SELENOP* (Hs01032845_m1) and, *SELENON* (Hs00898723_ml). *β-actin* (4352935E) mRNA expression was used as a reference gene. The relative expressions were calculated using the $2^{-\Delta\Delta Ct}$ method.

2.8. Evaluation of oxidative damage: malondialdehyde (MDA)

For dosage of malondialdehyde 100 μl plasma sample was used. Three hundred microliters of 10 mM solution of 1-methyl-phenyl-indole was added in acetonitrile and methanol (2:1, v/v) and 75 μl of hydrochloric acid (HCl) pure (37%). The tubes were vortexed and incubated in water bath at 45 °C for 40 min. After the bath, samples were cooled in ice and then centrifuged at 4000 rpm for 10 min. Supernatant was read for absorbance at a wavelength of 586 nm. Malondialdehyde concentration was calculated by comparing it to a curve 1,1,3,3 - tetramethoxypropane (TMP) hydrolyzate.

2.9. Glutathione peroxidase activity (GPX)

GPX activity was measured in erythrocytes according to Paglia and Valentine (1976) [28]. The method is based on the reaction in which GPX catalyzes the oxidation of reduced GSH by a hydroperoxide. In the presence of GPX nicotinamide adenine dinucleotide phosphate (NADPH), oxidized GSH is converted to the reduced form with a concomitant oxidation of NADPH NADPH +. The decrease in absorbance at 340 nm was then measured.

2.10. Superoxide dismutase (SOD)

Plasma SOD levels was evaluated using a commercially available kit (19160, Sigma–Aldrich, St. Louis, MO, USA) according to the manufacturer's instruction. The assay utilizes a tetrazolium salt WST-1 that produces a water- soluble formazan dye upon reduction with superoxide anion. The rate of WST-1 reduction is linearly related to the inhibition activity of xanthine oxidase (XO) by SOD. SOD catalyzes the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen resulting in decrease of WST-1 reduction. This inhibition activity of SOD was measured by colorimetric method at OD 450 nm.

2.11. Selenium

The determination of the total concentration of selenium in plasma and erythrocytes was performed by an Inductively Coupled Plasma Mass Spectrometry (ICP-MS), fitted with a dynamic reaction cell (DRC) (Perkin Elmer Sciex Norwalk, CT USA). Samples were diluted in the ratio 1:50 with a solution containing Triton X-100 0.01% (v/v), HNO_3 0.05% (v/v) and 10mg/L-1 rhodium (Rh) as an internal standard. The concentration of the analytical calibration standards ranged from 0 to 50 μg/L [29].

2.12. Sample size calculation

The sample size calculation was based on CK activity of patients using cholesterol-lowering medications. We conducted a pilot study with 104 patients selected at University Hospital and from who we evaluated serum CK activity. Patients were classified according to the assay range (Labtest, Minas Gerais, Brazil) as normal: when CK activity were up to 189 U/L or out of range: when CK activity were greater than 189 U/L. Considering mean CK activity in normal group (66.7U/L) and out of range group (229.3 U/L), standard deviation (SD) 114 U/L, alpha 5%, and 99% power the sample size was calculated as 12 for each group.

2.13. Statistical analysis

Continuous variables were tested for normality using the Shapiro–Wilk test and nonparametric tests were used when appropriate. The data were presented as mean ± SD with its respective p-values. Baseline characteristics and baseline measured

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outcomes were compared with post treatment using paired t-test or Wilcoxon test. The Pearson's correlation coefficient or the Spearman's correlation was calculated according to the presence or absence of a normal distribution. To compare averages of gene expression results Wilcoxon test was applied. Values are expressed as median, minimum and maximum, and interquartile range. Data were plotted in Statistical Package for the Social Sciences software version 14.0 (SPSS, Chicago, IL, USA) and GraphPad Prism software (GraphPad Software Inc., San Diego, CA). Differences were considered significant if $p < 0.05$.

3. Results

The Se content and centesimal composition of Brazil nuts are shown in Table 1. The average weight of Brazil nuts was 5 g, therefore each nut provided approximately 290 μg of Se, which is higher than the RDA (Recommended Dietary Allowances) for adults (55 μg/d) but less than upper limit (400 μg/d) established by the Institute of Medicine, USA (IOM), 2000 [30].

A total of 32 patients completed the entire study protocol. Compliance of Brazil nuts consumption was reported by patients at the end of the study. Moreover, increased Se levels in plasma and erythrocyte post treatment (Table 2) confirmed the adherence to the supplementation. According to the analysis of non-consecutive dietary food records, at baseline, the energy intake of participants in G1 (2050 ± 180.2 kcal) and G2 (2130 ± 237.3 kcal) was comparable ($p = 0.9591$) and, all participants maintained their normal diet during the period of supplementation (data not shown). At the beginning of the study, participants were asked about the practice of physical activity. Most of the participants in G1 and G2 reported not practicing regular physical activity and, they did not change this habit during the study. Regarding the type of cholesterol-lowering medication used by study participants, 71.9% was using Simvastatin (40 mg) and 28.1% Atorvastatin (40 mg). When stratifying the type of statin according to the study group, in G1, 80% was using Simvastatin and 20% Atorvastatin, and in G2, 64.7% was using Simvastatin and 35.3% Atorvastatin. We did not find differences in the type of statin between groups ($p = 0.3369$). It is relevant to consider that during the 3-month period of supplementation there were no changes in medical prescription of statin dosage or type. Females constituted 40.6% of the group and mean age was 50.1 ± 7.6 years. The study groups were not different in relation to sex ($p = 0.07$) or age ($p = 0.78$). The body composition, CK activity, total cholesterol, Se status, and oxidative stress parameters of the volunteers pre and post supplementation are summarized in Table 2. According to BMI, patients in G1 were classified as with obesity while in G2 patients were classified as overweight. After supplementation with Brazil nuts, individuals in both groups lost a significantly amount of weight with consequent reduction in BMI. In both G1 and G2 groups, Brazil nut supplementation contributed to decreased levels of CK activity in serum. The levels of total cholesterol were adequate at the baseline and remained unchanged in both groups after supplementation.

3.1. Biomarkers of selenium status and oxidative stress

Biomarkers of Se status and oxidative stress measured at baseline and post treatment are shown in Table 2. At baseline, the majority of patients showed adequate Se levels in plasma (>80–95 μg/L), with only 3 individuals being slightly deficient [6]. Changes in plasma and erythrocyte Se concentrations were significantly positive in both groups after supplementation ($p < 0.0001$) and enough to restore selenium deficiency in the few Se-deficient individuals. The levels of GPX activity also increased in both groups post treatment (G1 $p < 0.01$, G2 $p < 0.005$). Among the oxidative stress parameters, we observed reduced levels of MDA and SOD in both groups after Brazil nut supplementation. Changes in Se status after supplementation were associated with changes in antioxidant parameters in different ways (Table 3). While increased levels of erythrocyte selenium were positively associated with erythrocyte GPX activity ($r = 0.54$; $p < 0.002$) higher levels of plasma selenium were negatively associated with SOD ($r = -44$; $p < 0.01$). No correlations were observed between changes in Se status and changes in CK activity but, interestingly, we observed a moderately negative association between CK activity and GPX activity ($r = -41$; $p < 0.02$) which in turn, was associated with increased selenium levels (Table 3).

Table 1
Centesimal composition and selenium contents in Brazil nuts used during the protocol.

Nutrient	Mean ± SD
Energy (kcal)	696.0 ± 4.3
Carbohydrates (g)	12.9 ± 0.4
Proteins (g)	10.9 ± 0.51
Lipids (g)	71.6 ± 0.09
Ash (%)	2.91 ± 0.23
Humidity (%)	1.75 ± 0.33
Selenium (μg/g)	58.1 ± 2.1

Values are mean ± standard deviation (SD). Except for selenium, all nutrients were calculated considering 100g of Brazil nut.

3.2. Selenoproteins gene expression

Gene expression of three selenoproteins (*GPX1, SELENOP*, and *SELENON*) was analyzed before and after 3 months of Brazil nut supplementation. The results are shown in Table 4. Surprisingly, we did not detect changes in the expression of selenoproteins *GPX1, SELENOP*, and *SELENON* after Brazil nut supplementation in both groups.

4. Discussion

Previous studies in other populations have demonstrated that the supplementation with one unit of Brazil nuts significantly improved Se status [8–10]. Supporting these studies, we found that one Brazil nut daily for 3 months outstandingly enhanced Se intake, did not exceed the tolerable upper intake level (400 μg/day) [6] and, was sufficient to increase plasma and erythrocyte Se for all participants.

Our patients were in regular and chronic use of statin, and most of them were using simvastatin. Simvastatin is the most common and cost-effective medication prescribed in the Brazilian Unified Health System (SUS) [31]. However, statin-associated muscle symptoms are more common with simvastatin than other available statins [3]. It occurs due to its high lipophilicity that displays greater levels of passive diffusion across cellular membranes, increasing the distribution of the medication [1].

Although we observed a significant decrease in body weight after Brazil nut consumption, we did not find an association between Se status or oxidative stress parameters and BMI (data not shown). This observation concurs with previous studies. For exemple, in the SELGEN study, participants received 100 μg sodium selenite/day for 6 weeks, and the association between Se status and BMI was canceled by Se supplementation, indicating that the effect of BMI only occurs in suboptimal Se intake [32].

In our study, after supplementation, the levels of CK activity decreased in both groups indicating that the consumption of one Brazil nut could benefit muscle homesotasis of patients with

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Table 2
Body composition, CK, lipid profile, Se status, and oxidative stress parameters according to group after 3 months of Brazil nut supplementation.

Parameters	Group 1 (n = 15)			Group 2 (n = 17)		
	Pre Mean ± SD	Post Mean ± SD	*P-value*	*Pre* Mean ± SD	Post Mean ± SD	*P-value*
Weight (kg)	95.1 ± 21.6	92.2 ± 20.3	0.04	81.1 ± 20.4	78.1 ± 20.9	0.0001
BMI (kg/m^2)	33.1 ± 5.9	31.9 ± 5.2	0.04	29.4 ± 7.4	28.3 ± 7.4	0.0002
CK (U/L)†	347.9 ± 133.3	228.2 ± 122.5	0.005	102.9 ± 45.7	78.3 ± 34.2	0.005
Cholesterol (mg/dL)	177.0 ± 39.3	176.5 ± 45.4	0.97	181.2 ± 44.3	152.8 ± 25.3	0.056
Plasma Se (μg/L)	81.5 ± 3.7	258.9 ± 73.8	<0.0001*	88.5 ± 6.9	286.5 ± 146.5	<0.0001*
Erythrocyte Se (μg/L)	82.33 ± 11.5	298.3 ± 84.5	<0.0001	91.3 ± 18.2	388.1 ± 208.4	<0.0001
MDA (nmol/mL)	3.3 ± 1.1	2.1 ± 1.1	0.003	2.8 ± 0.6	1.7 ± 0.9	<0.0001
GPX activity (U P/g)	24.7.5 ± 3.0	29.8 ± 6.0	0.01*	23.5 ± 4.4	35.4 ± 29.5	0.005*
SOD (U/L)	1.4 ± 0.4	0.3 ± 0.1	<0.0001	1.6 ± 0.8	0.4 ± 0.3	0.0001

Note: Paired t-test; Wilcoxon *; t test for independent samples †. Values are expressed as mean ± standard deviation. Pre: pre supplementation; Post: post supplementation; SD: standard deviation. BMI: body mass index; CK: creatine kinase activity; Se: selenium; MDA: malondialdehyde; GPX: glutathione peroxidase; SOD: superoxide dismutase.

Table 3
Correlation analysis between changes in the oxidative stress parameters and changes in Se status.

Correlation (r)						
Parameters	Erythrocyte Se	Serum Se	CK	GPX activity	MDA	SOD
Erythrocyte Se[b]		0.78***	0.05	0.55**	0.15	−0.49**
Serum Se[b]	0.78***		−0.03	0.46*	0.16	−0.44*
CK[b]	0.05	−0.03		−0.41*	0.18	−0.17
GPX[b]	0.55**	0.46*	−0.41*		0.07	−0.40*
MDA[a]	0.15	0.16	0.18	0.07		−0.07
SOD[b]	−0.49*	−0.44*	−0.17	−0.40	−0.07	

***p < 0.0001; **p < 0.005; *p < 0.05.
[a] Pearson's correlation coefficient.
[b] Spearman's correlation. CK: creatine kinase activity; Se: selenium; MDA: malondialdehyde; GPX: glutathione peroxidase; SOD: superoxide dismutase.

variable levels of CK activity. In contrast with our results, Bosgrud et al. (2012) found a significant elevation of CK levels after supplementation with 200 μg of Se and 400 mg of coenzyme Q10 [33]. Neverthless, the authors concluded that increases in CK levels probably reflect the effect of multiple comparison rather than a true effect of supplementation [33]. We did not find an association between higher levels of Se and CK activity, but interestingly, a moderately negative association between CK activity and GPX activity was observed. Thus, attenuation of statin-related muscle damage measured by the decrease of CK activity may be directly associated with the improvement in curbing oxidative stress. Indeed, a study has demonstrated that statin may increase the free radical load in the skeletal muscles causing oxidative damage and myopathy in these tissues [34]. Therefore, the administration of statins with dietary antioxidants might be beneficial reducing the muscular side effects [34].

Table 4
Gene expression of selenoproteins according to group assessed by quantitative polymerase chain reaction (qPCR) after three months of Brazil nut supplementation.

Gene	Pre		Post		p-value
	Median (min − max)	IQR	Median (min − max)	IQR	
Group 1					
GPX	0.67 (0.30−1.3)	0.41	0.61 (0.14−2.3)	0.78	0.30
SELENOP	0.05 (0.03−0.14)	0.08	0.11 (0.02−0.83)	0.36	0.07
SELENON	0.035 (0.001−0.08)	0.07	0.03 (0.001−0.12)	0.06	0.90
Group 2					
GPX	0.23 (0.05−0.59)	0.31	0.28 (0.15−0.74)	0.21	0.36
SELENOP	0.03 (0.01−0.07)	0.04	0.03 (0.01−0.31)	0.19	0.44
SELENON	0.03 (0.01−0.06)	0.03	0.04 (0.01−0.08)	0.03	0.34

Note: Values are expressed as median (minimum − maximum) and interquartile range (IQR) and were normalized to *β-actin* mRNA levels. Wilcoxon test was used to compare averages. Pre: pre supplementation; Post: post supplementation. GPX: glutathione peroxidase; *SELENOP*: selenoprotein P; *SELENON*: selenoprotein N.

In the present study, the enhancement of Se status after Brazil nut supplementation reduced oxidative stress. We observed that one Brazil nut supplementation daily was effective in decreasing lipid peroxidation by reducing MDA levels, one of the most commonly and widely used oxidative stress biomarkers [35]. Elevated GPX activity after supplementation was also associated with higher levels of erythrocyte Se. Considering that lipid peroxidation is closely related to oxidative stress [21] these results may indicate a protective effect of Brazil nut consumption, connecting the GPX activity and MDA levels. Elevated GPX activity leads to decreases in hydrogen peroxide, lipid and phospholipid hydroperoxides [36], reducing the stimulus of lipid peroxidation and consequently decreasing MDA synthesis [43]. Although our results demonstrated the reduction of MDA levels after supplementation, studies with different populations have shown controversial results about the effectiveness of Brazil nuts regarding MDA levels [21,23,25,37]. In these studies, the general characteristics of patients, such as advanced age and a pre-existing health condition, could contribute to the distinct oxidative stress response after Se supplementation. Moreover, according to Cardoso et al. (2015), in a context of low levels of free radicals, the consumption of antioxidants may be protective at low levels, and an additional intake may not be relevant [21]. On the other hand, increased GPX activity after Brazil nut supplementation is observed in most studies, corroborating with our findings [21,23,44]. Dietary components may interact to reflect different responses to the intervention regarding the modulation of the antioxidant response. In the present study, levels of SOD decreased after supplementation and were negatively associated with higher levels of Se. A possible explanation would be that by increasing Se intake, we also increased metabolites of selenium and selenium-containing organic molecules that are generally more potent antioxidants and would also contribute to antioxidant mechanisms decreasing the synthesis of endogenous antioxidants such as SOD [20,38]. Also, Brazil nuts have other

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exogenous antioxidant agents such as bioactive compounds and other minerals that could contribute to SOD decreased levels [22].

Statin-induced myopathy may be associated with a reduction in the expression and activity of selenoproteins [2,3,7,17,18]. This reduction occurs due to the inhibition of the mevalonate pathway, which also inhibits a whole series of endogenous metabolites, among them isopentenyl pyrophosphate, a metabolite responsible for isopentenylation of adenosine at position 37 of tRNA[Ser]Sec. Lack of isopentenylation may result in lower rate of synthesis of selenoproteins [4,7,18,39,40]. Moosmann and Behl (2004) hypothesized that dietary Se supplementation could decrease the statin-induced side-effects in patients since higher levels of Se could increase the catalytic efficacy and turnover of the remaining, mature SectRNA molecules [18]. Furthermore, Se supplementation, by organic and inorganic selenium compounds, has been shown to increase selenoprotein expression and activity [20]. In the present study, after Brazil nut supplementation, we confirmed increases in GPX activity but we did not find differences in mRNA expression of *GPX1, SELENOP,* and *SELENON* post-treatment. A possible explanation could be a saturation effect of selenoproteins gene expression at optimum Se levels. Under this condition, any excess supply of Se results in increased metabolism, but marginal or no further increases in selenoprotein biosynthesis [41]. A review of Reska et al. (2012), indicated that mRNA expression of different selenoproteins in leukocytes after Se supplementation does not result in changes in selenoprotein mRNA levels, corroborating with our findings, and indicating that protein synthesis may already be saturated at sufficient Se concentration [42].

The absence of a control group is one limitation of our study. However, since the main goal of this study was to investigate the effect of the dietary intervention in CK activity, oxidative stress, and gene expression, we decided that each person before treatment would be a more appropriate control. The other limitation was the non-performance of biopsy in the skeletal muscle that could enhance our understanding about the specific mechanisms involving Se metabolism and statin side effects on muscle.

In conclusion, the present study showed that the supplementation with one unit of Brazil nut was sufficient to increase the Se status and contribute to the improvement of oxidative stress parameters, whereas the latter was directly associated with decreased serum CK activity that could benefit the muscle homeostasis of patients using statins. However, the improvement of Se status post-treatment did not contribute to the enhancement of selenoproteins gene expression indicating a possible saturation effect at optimum Se levels. Thus, more studies are required to further address the beneficial effects of Se supplementation to muscle homeostasis of patients using statins, mainly including the molecular and genetic analysis of muscle cells.

Funding sources

This work was supported by Brazilian grants from Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) to LMW under grant 2016/05677-7 and to FBJ and AMN under grant 2018/24069-3. Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) also supported LMW under grant 1591486. Funding sources had no involvement in study design, collection, analysis and interpretation of data from the present study.

6. Statement of authorship

LMW, FBJ and AMN made the conception and design of the study. LMW and LFL did the collection and carried out the sample analyses. RFB, DT, ACB, MCFF, and TMBC participated in the sample analyses and revision of the manuscript. LMW did all the data analysis and interpretation with help of ACB. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare they have no financial conflict of interest.

Acknowledgments

We are grateful for the help of laboratory technicians from University of São Paulo (USP): Paula Payão Ovídio, MSc (Ribeirão Preto Medical School) and Vanessa Cristina de Oliveira Souza, Ph.D (Ribeirão Preto School of Pharmaceutical Sciences).

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Please cite this article as: Watanabe LM et al., Association between creatine kinase activity, oxidative stress and selenoproteins mRNA expression changes after Brazil nut consumption of patients using statins, Clinical Nutrition, https://doi.org/10.1016/j.clnu.2020.02.012