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Brazil Nut (*Bertholletia excelsa*, H.B.K.) Improves Oxidative Stress and Inflammation Biomarkers in Hemodialysis Patients

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Abstract Cumulative evidence indicates that oxidative stress and inflammation frequently occurs in patients undergoing maintenance hemodialysis (HD) and as a result of overproduction of reactive oxygen species (ROS) and a decrease of antioxidant defenses such as selenium (Se). Previous studies in our laboratory showed that the supplementation of 1 unit of Brazil nut (the richest known food source of Se) a day during 3 months is effective to improve Se status and increase glutathione peroxidase (GPx) levels in HD patients. The aim of this study was to evaluate the effect of Brazil nut supplementation on oxidative stress and inflammation markers in HD patients. Forty HD patients from Rio de Janeiro, Brazil were studied. All patients received one nut per day for 3 months. The Se plasma levels and GPx, 8-isoprostane, 8-hydroxy-2-deoxyguanosine (8-OHdG), and cytokine (TNF-α and IL-6) levels and lipid profile were determined before and after 3 months of supplementation. The plasma Se and GPx activity increased, while cytokines, 8-OHdG, and 8-isoprostane plasma levels decreased significantly after 3 months supplementation. HDL-c levels increased and LDL-c levels decreased significantly. These data suggest that the consumption of only one Brazil nut per day during 3 months was effective to reduce the inflammation, oxidative stress markers, and the atherogenic risk, thereby increasing the antioxidant defenses in HD patients. Our results indicate that Brazil nut as Se source plays an important role as an anti-inflammatory and antioxidant agent in HD patients.

Keywords Selenium · Chronic kidney disease · Hemodialysis · Oxidative stress and inflammation

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Introduction

Oxidative stress in patients in hemodialysis (HD) has been reported as a result of overproduction of reactive oxygen species (ROS) due to advanced age, diabetes, chronic inflammatory state, uremic syndrome, biocompatibility of dialysis membranes, and reduced antioxidant systems including lack of vitamin C and intracellular levels of vitamin E, decreased activity of the glutathione, and lack of selenium (Se) [1].

The activation of transcription factor nuclear κB (NF-κB) by ROS intervenes in the transcription of a large number of inflammatory gene coding for cytokines and adhesion molecules

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and, furthermore, the ROS production also increased the DNA damage. All these factors in patients in HD are associated with vascular dysfunction, inflammation, and atherosclerosis [2–6].

Several authors have reported decreased levels of plasma and erythrocyte Se and glutathione peroxidase (GPx) activity (enzyme that protects membrane lipids and other cellular and extracellular components from oxidative damage) in dialysis patients, and the majority of studies recommend Se supplementation for these patients [7–13]. However, food sources are preferable to alternative supplementation practices for improving the nutritional status of a population, because they are sustainable, less expensive, and have lower risk of toxicity and in Brazil, we have a large consumption of the richest known food source of Se, the Brazil nut from the Amazon region. Thus, daily consumption of one Brazil nut could raise dietary Se intakes, which was estimated in the National Nutrition Survey to be 56 μg for men and 39 μg for women, to recommended intakes of Se [14, 15].

In fact, previous studies in our laboratory showed that the supplementation of 1 unit of Brazil nut (*Bertholletia excelsa*, family Lecythidaceae, the richest known food source of Se) a day during 3 months was effective to improve Se status and increased GPx levels in HD patients [16, 17].

However, we did not evaluate whether this nutritional compound can modulate the oxidative stress, inflammation, and lipid profile in HD patients. Instead, the aim of this study was to assess the effect of one Brazil nut daily supplementation for 3 months on oxidative stress, inflammation, and lipid profile in HD patients.

Methods and Materials

Subjects

Forty patients were studied before and after 3 months of supplementation from RenalCor Clinic in Rio de Janeiro, Brazil. Inclusion criteria were age >18 years and patients on maintenance dialysis for at least 6 months. Patients with such diseases as inflammatory, cancer, AIDS, and autoimmune and patients with phosphorus levels above 5.5 mg/dL and who use catheter access for HD and antioxidant vitamin supplements were not included. Dialysis duration was 3–4.5 h/session 3 times/week, the blood flow was greater than 250 mL/min, and the dialysate flow was 500 mL/min. The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine of the Fluminense Federal University (no 018/09), and all the patients were asked to sign the informed consent.

Experimental Protocol

The patients received one nut daily for 3 months. This time was based on European Best Practice Guidelines (EBPG) that suggests 3 to 6 months of Se supplementation [18]. The Brazil nut was offered weekly in a container with seven nuts to minimize possible problems with supplementation.

Brazil Nut

According the chemical composition, the specified supplement of one Brazil nut (*B. excelsa*, H.B.K.) (≈5 g) used for the study (given by the Agriculture Arauanã S/A) contains 0.75 g of protein, 0.45 g of carbohydrates, and 3.53 g of lipids, for a total of 36.7 kcal and 290.5 μg of Se (analyzed by atomic absorption spectrophotometry with hydride generation; HITACHI®, Z-500).

Diet Analysis

Food intake was assessed using a 2-day food record (dialysis and non-dialysis days). Subjects were instructed by a registered dietitian to keep a record of food intake, including condiments and beverages, maintaining their usual habits. An example of a detailed meal was given to the subjects. They were asked to use the usual tools to estimate their portion sizes (i.e., teaspoon/tablespoon/cup in milliliters or ounces) and, if possible, weigh their portions. Dietitians reviewed the records with the subjects so that needed information can be retrieved. The Se intake was calculated using NutWin Software (developed by the Department of Nutrition, Federal University of São Paulo–UNIFESP, São Paulo, Brazil). The Se concentrations were as determined by Ferreira et al. [19] who analyzed Se concentrations in Brazilian foods.

Analytic Procedures and Sample Processing

Blood samples were drawn from each subject in the morning, after overnight fasting, before and after 3 months of Brazil nut supplementation. Blood was drawn from the arteriovenous fistula, before the dialysis session, into a syringe containing EDTA (1.0 mg/mL) as anticoagulant. Plasma was separated (15 min, 3000× *g*, 4 °C) and stored into tubes in −80 °C until analysis. The serum levels of blood urea nitrogen, phosphorus, potassium, and calcium and body mass index (BMI) were collected from medical records of patients. The albumin and creatinine levels were determined through Bioclin® kits (K040 and K016) by automatic biochemical analyzer (Bioclin BS-120 chemistry analyzer).

To obtain the peripheral blood mononuclear cells (PBMC) 4 mL of blood with EDTA were mixed with 3 mL of ficoll, which was followed by centrifugation (15 min at 1500 rpm). The supernatant was decanted and the cloud was thoroughly resuspended in 3 mL of medium RPMI. The mixture was centrifuged at 1500 rpm/15 min. After centrifugation, the clear supernatant was discarded and the pellet was transferred

to a new sterile cryotube with DMEM (Dulbecco's modified Eagle's medium) and RPMI (Roswell Park Memorial Institute) cell culture reagent medium and stored at −80 °C.

Markers of Oxidative Stress, Inflammation, and Lipid Profile

The GPx activity was determined through Cayman's kit (Cayman Chemical, Ann Arbor, MI, USA, no 703102). Cayman's GPx assay measures GSPx activity indirectly by a coupled reaction with glutathione reductase (GR). Oxidized glutathione (GSSG), produced upon reduction of hidroperoxide by GPx, is recycled to its reduced state by GR and NADPH. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm. Under conditions in which the GPx is rate limiting, the rate of decrease in the absorbance at 340 nm is directly proportional to the GPx activity sample. The intra- and inter-assays CVs were 5.7 and 7.2 %, respectively.

The DNA-base-modified product, 8-hydroxy-2′-deoxyguanosine (8-OHdG), one of the most commonly used markers for the evaluation of oxidative DNA damage, was quantified using a competitive enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI, USA, no 589320); and the DNA purification of the PBMC was done according the kit's manual.

The plasma level of 8-isoprostane, a product of lipid peroxidation (a marker of oxidative stress), was also quantified using a competitive EIA kit (Cayman Chemical, Ann Arbor, MI, USA, no 516351).

The inflammation markers, interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α), were measured via ELISA method using R&D systems® duoset kits (DY 206 and DY 210, Minneapolis, MN, USA).

Total cholesterol (TC), HDL-cholesterol (HDL-c), and triacylglycerol were measured using Bioclin® kits (K083, K015, and K117) by automatic biochemical analyzer (Bioclin BS-120 Chemistry Analyzer). LDL-cholesterol (LDL-c) was calculated according to Friedewald's formula [20].

To assess the atherogenic risk, the Castelli I and II indices were determined. The first refers to the ratio between TC and HDL-c and the second, to the ratio between LDL-c and HDL-c [21].

Plasma Se concentrations were determined through hydride generation atomic absorption spectrometry (HG-AAS), using a HITACHI® Z-500 spectrometer. The samples were digested with nitric acid, followed by hydrochloric acid reduction. After acid digestion, the sample was added with high-purity deionized water (18.2 M Ω cm) from a Milli-Q system to make up 25 mL of sample. The blanks were carried through the procedure in the same way as the sample. All chemicals used were of analytical reagent grade. Standard solutions were prepared in the working range for adequate calibration curves. The reference material, SERONORM® Trace Elements Serum (Sero AS, Billingstad, Norway), was treated and analyzed in the same way. Our analytical results (in microgram per liter) for the determination of Se in SERONORM® (61.4 μg/L, n=3) were in good agreement with certified value (53.6–64.8 μg/L).

Statistical Analysis

The distribution of the variables was analyzed by Kolmogorov-Smirnov tests. Normally distributed variables were expressed as mean±standard deviation and non-normally distributed variables were expressed as median (interquartile range). The differences between groups were analyzed using non-parametric tests (Wilcoxon W or Mann–Whitney U) or independent samples t test or paired samples t test for parametric variables. The correlations between variables were assessed through Spearman's rho or Pearson's coefficient correlation depending on the distribution of the sample. Binary logistic regression was used to analyze if variables influenced in changes of plasma Se levels. Statistical significance was set at the level of $P<0.05$. The statistical analyses were performed through SPSS 19.0 software (Chicago, IL, USA).

Results

The mean age was 53.3±16.1 years, time on dialysis was 62.0 (8.0–207.0) months, and 15 % were diabetic. Serum levels of blood urea nitrogen (165.3±30.0 mg/dL), phosphorus (5.4±1.3 mg/dL), calcium (8.9±0.5 mg/dL), potassium (4.6±0.5 mg/dL), albumin (3.3 [3.2–3.5] g/dL), and creatinine (7.3±2.5 mg/dL) after supplementation showed no significant changes.

The mean Se intake was 31.3±15.1 μg/day and according to recommended values (45 μg/day), 97.5 % of patients presented low Se intake. After supplementation, the patients consumed on average of 321.8±15.1 μg/day; thereby, even when adding the Se intake from Brazil nut, the entire value did not exceed the Se tolerable upper intake level (UL) of 400 μg/day.

The Se plasma levels at the baseline was 17.0±11.3 μg/L, and all patients presented Se deficiency (normal values 60–120 μg/L) [22, 23]. After nut supplementation, the Se plasma values increased to 158.1±87.2 μg/L ($P<0.0001$) and only one patient showed Se levels below the normal range (Fig. 1). Additionally, the activity of GPx increased significantly as well (Fig. 2).

The 8-OHdG, 8-isoprostane (Figs. 3 and 4), TNF-α, and IL-6 levels decreased significantly after 3 months of nut supplementation (Table 1). The HDL-c levels increased and LDL-c levels decreased significantly (Table 1). No significant alterations were found in the TC and triacylglycerol levels.

After supplementation, a positive correlation between 8-OHdG and TNF-α levels was found (r=0.36, P=0.02). The

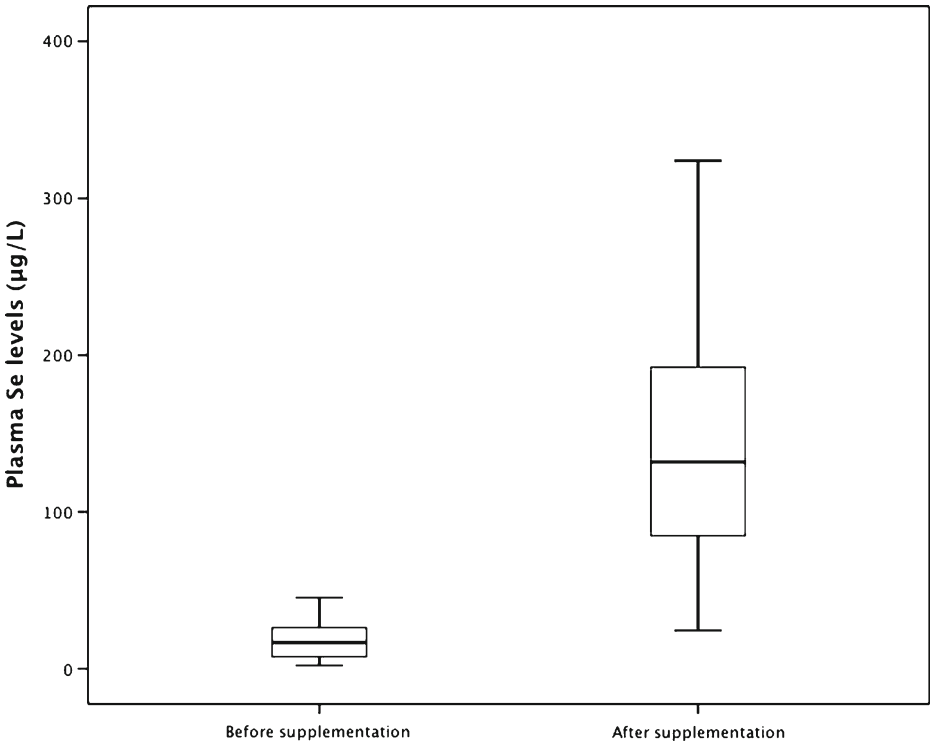

Fig. 1 Comparison of plasma Se levels before and after supplementation

statistical analysis showed, although not significant, that all patients have decreased 8-OHdG and 8-isoprostane and increased GPx activity as well as increased plasma Se.

According to the atherogenic risk measured through Castelli index, at the baseline, 48.7 % of the patients presented Castelli I index above the recommended range (<4.3 mg/dL), and in order of the Castelli II index, 61.5 % of the patients had levels above the recommended range (<2.9 mg/dL). After supplementation, Castelli I (4.2±0.2) and II (2.5±1.2) indices reduced significantly ($P<0.001$) to 3.5±1.4 and 1.8±1.0, respectively.

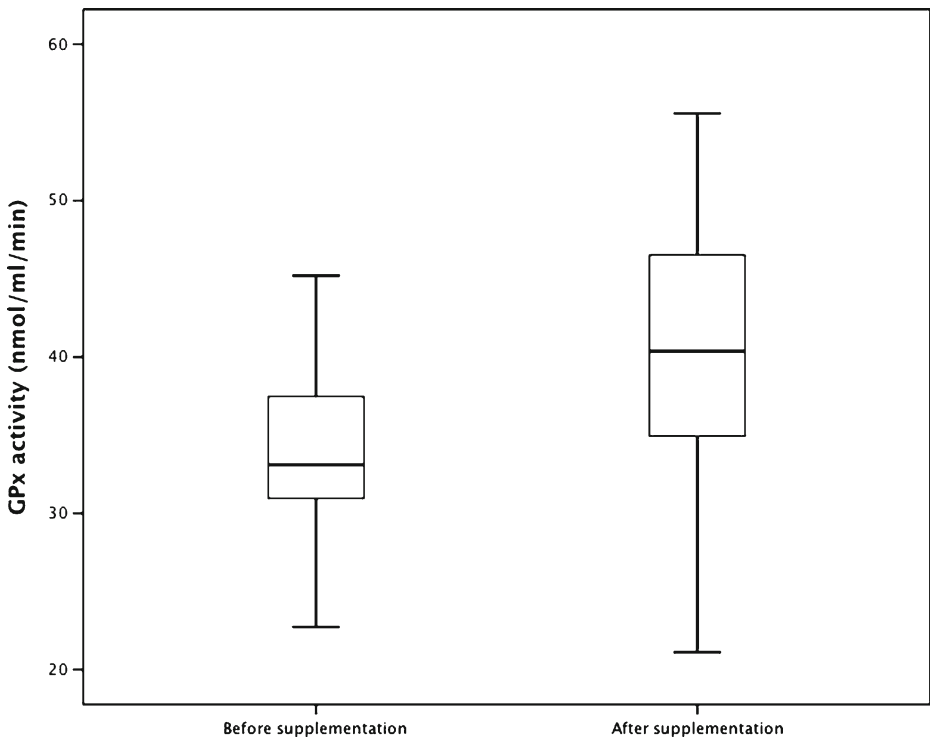

Fig. 2 Comparison of GPx activity before and after supplementation

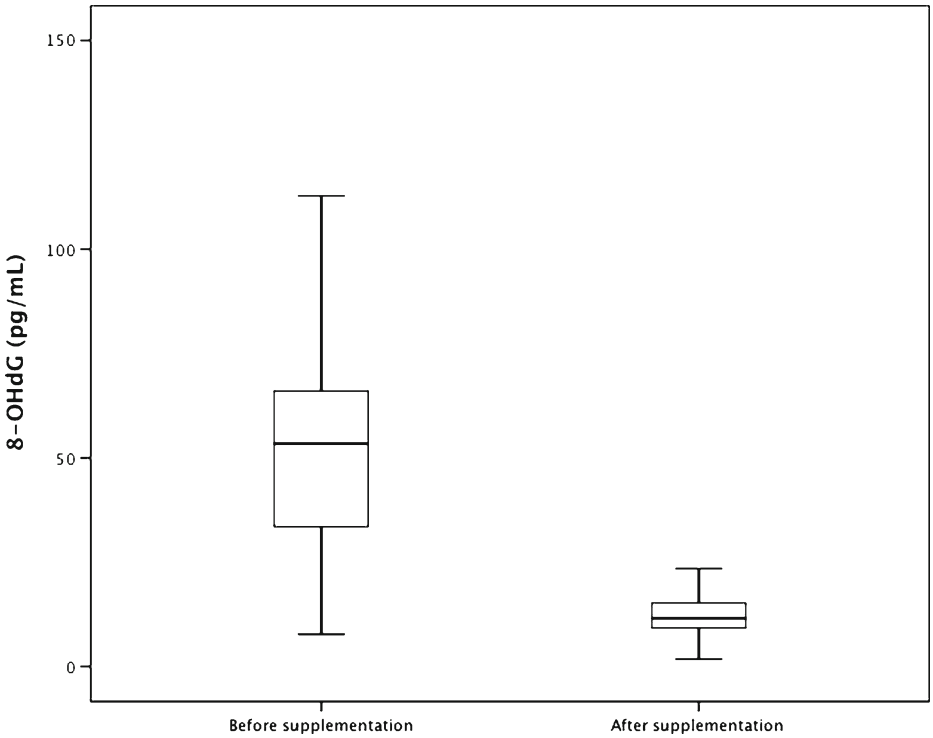

Fig. 3 Comparison of 8-OHdG levels before and after supplementation

Discussion

Our study is the first one to investigate the response of oxidative stress, inflammation, and lipid profile to Brazil nut supplementation in HD patients. The present data shows that the daily consumption of one Brazil nut was effective in decreasing the markers of oxidative stress and inflammation, increasing the antioxidant status and improving the lipid profile.

In fact, literature has reported low plasma levels of Se and GPx activity and elevated levels of oxidative stress biomarkers in HD patients and our results confirm this set in

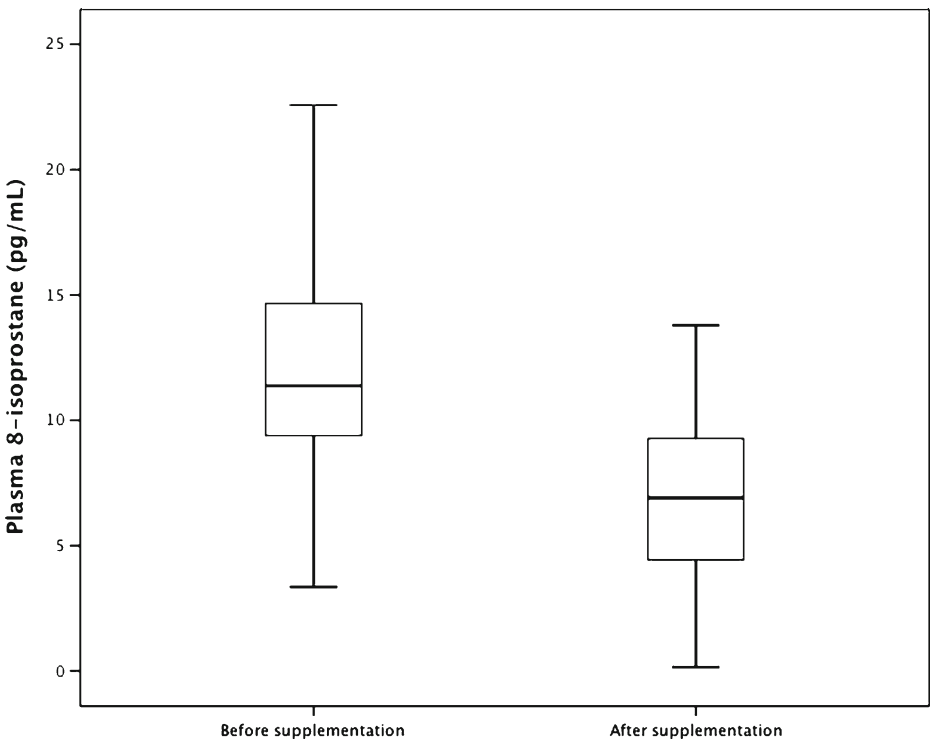

Fig. 4 Comparison of plasma 8-isoprostane levels before and after supplementation

Table 1 Oxidative markers, inflammation, and lipid profile before and after Brazil nut supplementation

	Baseline (N=40)	After supplementation (N=40)
Selenium (μg/L)	17.0±11.3	158.1±87.2*
GPx (nmol/mL/min)	33.6±5.1	40.0±8.5*
8-isoprostane (pg/mL)	12.2±4.6	6.6±4.1*
8-OHdG (pg/mL)	53.4 (31.4–66.1)	11.3 (7.8–14.4)*
IL-6 (pg/mL)	64.8±10.6	14.0±1.6*
TNF-α (pg/mL)	21.0±0.3	14.3±8.8*
TC (mg/dL)	149.5±31.5	154.0±63.1
HDL-c (mg/dL)	38.5±15.4	46.6±15.1*
LDL-c (mg/dL)	86.5±28.3	75.2±30.2*
Triacylglycerol (mg/dL)	92.0 (77.0–149.0)	113.0 (77.0–157.0)

*P<0.001

Brazilian HD patients. Several mechanisms have been reported to explain the altered Se status observed in HD patients, such as low dietary intake, increased urinary and dialytic losses, impaired intestinal absorption, abnormal binding to Se transport proteins, and drug therapy [24–27].

Overproduction of ROS can lead an oxidative damage on proteins, lipids, and DNA. Among many categories of oxidative DNA damage, 8-OHdG is one of the most abundant oxidative products of cellular DNA [28]. The most important oxygen-free radical causing damage to basic biomolecules is the hydroxyl radical (HO·), and its interaction with the nucleobases of the DNA strand, such as guanine, leads to the formation of C8 hydroxyguanine or its nucleoside form deoxyguanosine (8-hydroxy-2-deoxyguanosine) [29, 30]. Chronic kidney disease (CKD) patients present high plasma levels of this oxidative stress marker that could have a relation with endothelial dysfunction [31, 32], and recently Zachara et al. [33] in a pilot study observed that DNA damage decreased significantly from 0.9±1.07 to 0.37±0.38 (P<0.02) after Se supplementation (200 μg as Se-rich yeast). We showed that Brazil nut supplementation, as a source of Se, was also effective in decreasing the DNA damage in HD patients.

However, the effect of Se on DNA damage in patients with CKD has not been studied so far. Supplementation of the antioxidants has been suggested to scavenge free radicals and reduce the DNA damage and thus may prevent the development of cancer and atherosclerosis [34, 35]. It is thought that Se exerts its physiological effects mostly in the form of selenium-containing proteins (selenoproteins). At least 25 selenoproteins have been identified, including GPx selenoprotein P, thioredoxin reductase, iodothyronine deiodinase, and selenophosphate synthetase. Grotto et al. [36] found a negative correlation (r=−0.559; P<0.05) between GPx activity and DNA lesion in rats showing that the lower GPx activity the greater the DNA damage. In the present study, there was no correlation between GPx activity and 8-OHdG; however, the increased of GPx activity by the Brazil nut supplementation possibly reduced the oxidative DNA damage.

Isoprostanes are prostaglandin-like substances, derived from arachidonic acid peroxidation and act in all normal tissues and biological fluids; however, upon oxidative stress, the isoprostane levels are elevated, and studies suggest that the family of isoprostanes may be a useful indicator to oxidative stress in HD patients [37]. Johnson-Davis et al. [38] observed that 8-isoprostane levels were significantly elevated in CKD patients when compared to healthy subjects. Hsu et al. [39] showed that *N*-acetylcysteine (NAC), as a antioxidant, was effective in reducing plasma levels of 8-isoprostane in HD patients. In our study, we showed that Brazil nut supplementation promoted a reduction of 8-isoprostane levels, thus decreasing the oxidative stress in HD patients.

Further results of our study showed that Brazil nut consumption was also effective on reducing atherogenic risk through reducing the Castelli I and II indices. Similar improvement patterns in both Castelli indices were already detected in another study that had supplied Brazil nut in obese women [40]. According to Ingelsson et al. [41], the two indices are comparable with the ratio between apolipoproteins B and A-1 concerning the cardiovascular risk; furthermore, the cardiovascular disease is the main cause of mortality in HD patients.

Chronic inflammatory disorders are also normally associated with a decreased in Se status. Additionally, our study confirms these data and showed that Brazil nut supplementation was effective in reducing cytokines, such as TNF-α and IL-6. There are a number of ways in which Se can influence inflammatory responses, including the inhibition of the NF-κB cascade, a vital transcription factor activated by a wide range of stimuli including oxidative stress, and regulates the transcription of a broad array of genes, including those associated with inflammation as pro-inflammatory cytokines [42, 43].

A recent review published by our group showed that nutritional compounds can modulate the activation of nuclear factor-E2-related factor 2 (Nrf2), a transcription factor that activates genes that encode phase II detoxifying enzymes and antioxidant enzymes, which include GPx [43]. In fact, the preliminary data from our group show that HD patients present an increased NF-κB and reduced Nrf2 expression, which is the opposite of healthy individuals and in addition, we showed that the consumption of only one Brazilian nut per day during 3 months activated Nrf2 and reduced NF-κB expression in these patients [44].

A public health recommendation to include as few as one Brazil nut per day in the diet would avoid the need for fortification of food supplements to improve the Se status.

The EBPG Guideline on Nutrition recommends a daily intake of 55 μg of Se for chronic kidney disease patients, the same value for healthy males and females according to national research [18]. In this way, a daily consumption of just one Brazil nut could fulfill all daily required intake of Se.

Although there were some limitations, such as the small sample of patients, this was a very well-controlled protocol, which allowed us to conclude that the results are considerably relevant.

From these results, we concluded that the patients had a deficiency in relation to the nutritional Se status, and this deficiency was overcome with the supplementation of one Brazil nut a day for 3 months. Furthermore, the significant increase in the levels of GPx and the significant decrease in the levels of 8-isoprostane, 8-OHdG, and cytokines after supplementation suggest that the Brazil nut can really improve the condition of oxidative stress and inflammation in HD patients.

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Conflict of Interest The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

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