



Original/Otros

## Effect of selenium supplementation via Brazil nut (*Bertholletia excelsa*, HBK) on thyroid hormones levels in hemodialysis patients: a pilot study

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### Abstract

**Background:** thyroid function depends on trace mineral selenium (Se), being at the active center of the iodothyronine deiodinase that catalyzes the conversion of the thyroxine (T4) to the active form of thyroid hormone, triiodothyronine (T3). Hemodialysis (HD) patients have reduced T3 levels partly due to impaired hormonal conversion that can be related to Se deficiency, a common feature in these patients. This study evaluated the effect of Brazil nuts (richest Se source) on thyroid hormone levels in HD patients.

**Methods:** we performed an uncontrolled intervention with 40 HD patients (53.3 ± 16.1 yrs, dialysis vintage 62.0 (8.0 - 207.0) months) that received one nut (≈5g, average 58.1 µg Se/g) per day for three months. Se plasma levels were determined by atomic absorption spectrophotometry with hydride generation and, serum T3, free T4 (FT4), TSH as well as glutathione peroxidase (GPx) activity were measured by ELISA.

**Results:** all patients were Se deficient and presented low T3 levels at baseline. After intervention, Se plasma levels (from 17.6 ± 11.6 to 153.4 ± 86.1 µg/L), GPx activity (from 33.7 ± 5.9 to 41.4 ± 11.2 nmol/min/mL), T3 (from 27.3 ± 8.8 to 50.2 ± 4.8 ng/dL) and FT4 levels (0.87 ± 0.2 to 0.98 ± 0.4 ng/dL) were significantly increased (p < 0.05), while TSH levels were reduced (from 2.17 ± 1.3 to 1.96 ± 1.1 uIU/mL), but not significantly.

**Conclusion:** in conclusion, increasing Se levels via Brazil nut supplementation was associated with improvement in thyroid hormone levels in HD patients, although the amount of Se given was not able to restore T3 to normal levels.

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Key words: Hemodialysis. Thyroid hormones. Selenium. Brazil nut.

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### EFFECTO DE LA SUPLEMENTACIÓN DE SELENIO A TRAVÉS DE LA NUEZ DE BRASIL (*BERTHOLLETIA EXCELSA*, HBK) EN LOS NIVELES DE HORMONAS TIROIDEAS EN PACIENTES DE HEMODIÁLISIS: UN ESTUDIO PILOTO

#### Resumen

**Introducción:** la función tiroidea depende de minerales traza de selenio (Se), que está en el centro activo de la deiodinasa yodotironina, que cataliza la conversión de la tiroxina (T4) a la forma activa de la hormona tiroidea, triyodotironina (T3). Hemodiálisis (HD) de los pacientes ha reducido los niveles de T3 de los pacientes, debido en parte a la conversión hormonal alterada que puede estar relacionada con la deficiencia de Se, una característica común en estos pacientes. Este estudio evaluó el efecto de las nueces de Brasil (la más rica fuente de Se) en los niveles de hormonas tiroideas en pacientes en HD.

**Métodos:** se realizó una intervención no controlada con 40 pacientes en HD (53,3 ± 16,1 años, diálisis vendimia 62,0 (8,0 - 207,0 meses)), que recibieron una nuez (≈ 5, promedio 58,1 mg Se/g) por día durante tres meses. Determinaron los niveles plasmáticos de Se por espectrofotometría de absorción atómica con generación de hidruros y los niveles de T3, T4 libre (FT4), TSH en suero, así como la actividad de la glutatión peroxidasa (GPx) por ELISA.

**Resultados:** todos los pacientes tenían niveles bajos de Se y T3 al inicio del estudio. Después de la intervención, los niveles plasmáticos de Se (de 17,6 ± 11,6 a 153,4 ± 86,1 mg/L), actividad GPx (de 33,7 ± 5,9 a 41,4 ± 11,2 nmol/min/ml), T3 (de 27,3 ± 8,8 a 50,2 ± 4,8 ng/dL) y T4L (0,87 ± 0,2 a 0,98 ± 0,4 ng/dL) se incrementaron significativamente (p < 0,05), mientras que los niveles de TSH se redujeron (de 2,17 ± 1,3 a 1,96 ± 1,1 IUU/ml), pero no de forma significativa.

**Conclusión:** en conclusión, el aumento de los niveles de Se vía suplementación con nuez brasileña se asocia con una mejoría en los niveles de hormonas tiroideas en pacientes en HD, aunque la cantidad de Se dada no fue capaz de restablecer la T3 a los niveles normales.

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Palabras clave: Hemodiálisis. Hormonas tiroideas. Selenio. Nuez de Brasil.

## Introduction

Up to 80% of chronic kidney disease (CKD) patients undergoing dialysis present low levels of total and free triiodothyronine (T3), an entity known as the low-T3 syndrome or non-thyroidal illness<sup>1,2</sup>. Traditionally, these low-T3 levels have been considered a physiological adaptation to an energy shortage by reduction of the metabolic rate. Intriguingly, recent epidemiological observations link low T3 levels with endothelial dysfunction, arterial stiffness, systemic inflammation and increased cardiovascular mortality risk in this patient population<sup>3-7</sup>. While it has been postulated that these associations may be causally linked, there is currently insufficient interventional evidence on the possible benefits of T3 restoration in HD patients<sup>8</sup>.

The underlying pathophysiology of these derangements is likely multifactorial, involving aging, iodine retention, altered serum protein binding capacity, systemic inflammation, malnutrition, metabolic acidosis and peripheral deiodinase activity<sup>9-11</sup>. Adequate supplies of both iodine and the essential trace element selenium (Se) are required for optimal thyroid function. Selenium is at the active center of a number of selenoenzymes required for thyroid function: The selenoenzymes, glutathione peroxidase and thioredoxin reductase are crucial to the protection of the thyroid from oxygen species and hydrogen peroxide produced by the thyroid which is essential to oxidize iodide in the thyroid hormone biosynthesis. In addition, the iodothyronine deiodinases constitute a family of selenoenzymes largely expressed in the thyroid that catalyze the removal of iodine from the outer ring or inner ring of the thyroid hormones. Deiodinases type I (D1) and type II (D2) are required for the interconversion of thyroxine (T4) to the active form T3<sup>12</sup>.

Studies have repeatedly demonstrated that dialysis patients commonly present selenium deficiency and its supplementation improves nutritional status, antioxidant defenses and reduce inflammation<sup>13-17</sup>. To the best of our knowledge, only one study in 1996 has addressed the hypothesis that restoration of selenium deficiency may raise T3 levels in HD patients<sup>18</sup>. This may open an interesting and safe perspective for intervention in this population. Our laboratory has shown the effectiveness of Brazil nuts supplementation (the richest known food source of selenium) in increasing selenium status in HD patients<sup>17,19,20</sup> and the aim of this study was to evaluate the effect of Brazil nut supplementation on thyroid hormones levels in HD patients.

## Subjects and methods

### Subjects

Forty HD patients from RenalCor Clinic in Rio de Janeiro, Brazil, were studied before and after 3 months

of Brazil nut supplementation. Inclusion criteria were age > 18 yrs and patients on maintenance dialysis for at least 6 months. Patients with inflammatory disease, cancer, AIDS, autoimmune disease, thyroid nodules, phosphorus levels above 5.5 mg/dL, use of catheter access for HD and use of antioxidant vitamin supplements were not included. Dialysis duration was 3-4.5 h/session three times/week, the blood flow greater than 250 ml/min and the dialysate flow was of 500 ml/min. The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine of the Fluminense Federal University (n°018/09) and all the patients were asked to sign the informed consent.

## Methods

### Nutritional Assessment

The following anthropometric parameters were assessed: body weight, height and waist circumference (WC). Body mass index (BMI) was calculated from the equation  $BMI = \text{weight (kg)} / \text{height}^2 \text{ (m)}^2$ . Measurements were made after the dialysis session by a trained staff member.

### Experimental protocol

The patients received one nut daily for three months. This time period was based on European Best Practice Guidelines (EBPG) that suggests 3 to 6 months of selenium supplementation<sup>22</sup>. The Brazil nut was offered weekly in container containing seven nuts to minimize possible problems with supplementation.

### Brazil nut

According the chemical composition, one Brazil nut (*Bertholletia excelsa*, H.B.K.) ( $\approx 5\text{g}$ ) (given up by the Agriculture Arauanã S/A) contains 0.75g of protein, 0.45g of carbohydrates and 3.53g of lipids, for a total of 36.7 kcal and 290.5  $\mu\text{g}$  of selenium (analyzed by atomic absorption spectrophotometry with hydride generation- HITACHI®, Z-500).

### 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 (1.0mg/mL) as anticoagulant. Plasma was separated (15 min, 3000 x g, 4°C), and stored into tubes in -80°C until analysis. Serum levels of blood urea nitrogen, phosphorus,

potassium, calcium were collected from medical records of patients. Albumin and creatinine levels were determined through Bioclin® kits (K040 and K016) by automatic biochemical analyzer (Bioclin BS-120 Chemistry Analyzer).

Plasma T3 (normal values: 40-180ng/dL), free T4 (FT4) (normal values: 0.7-1.8 ng/dL) and TSH levels (normal values: 0.5-5.0 uIU/mL) were measured by immunoenzymatic assay using Bioclin commercial kits (K101 and K098 Bioclin, Quibasa Química Ltda, Brazil) and the ratio T4/T3 were calculated to verify the hormone levels changes after supplementation.

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 340 nm is directly proportional to the GPx activity sample. The intra- and inter-assays CVs were 5.7 and 7.2%, respectively.

Plasma selenium concentrations were determined through hydride generation atomic-absorption spectrometry (HG-AAS), using a HITACHI® Z-500 spectrometer. Samples were digested with nitric acid, followed by a hydrochloric acid reduction. After acid digestion, the sample was made up to 25mL with high purity deionised water (18.2 M Ωcm) from a Milli-Q system. 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. Reference material, SERONORM® Trace Elements Serum (Sero AS, Billingstad, Norway), was treated and analysed in the same way. Our analytical results (in µg/L) for the determination of selenium 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 the Kolmogorov-Smirnov tests. Normally distributed variables were expressed as mean ± standard deviation and non-normally distributed variables were expressed as median (interquartil range). The differences between groups were analyzed using nonparametric 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 Rho or Pearson's coefficient correlation depending on the distribution of the sample. 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

General characteristics of patients are depicted in Table I. The etiology of renal failure in these patients was hypertension (75%), diabetes (15%), and others (10%). The mean of BMI was  $23.0 \pm 5.1$  kg/m<sup>2</sup>, 2 (5%) patients presented BMI values below 18.5 kg/m<sup>2</sup> and, 14 (35%) presented values above 25 kg/m<sup>2</sup>. The waist circumference was above normal values in 20% of patients and the mean was  $88.4 \pm 15.4$  cm for men and  $85.5 \pm 17.7$  cm for women.

All patients presented selenium deficiency (normal values: 60–120 µg/L)<sup>23,24</sup> and after supplementation, the selenium plasma levels increased significantly in all patients. The GPx activity also increased significantly. At inclusion, all patients presented T3 levels below normal values and, these levels were increased significantly after supplementation (Table II). The TSH levels were reduced, after supplementation, but not significantly. The Se levels before supplementation were correlated with FT4 levels ( $r = 0.5$ ,  $p = 0.04$ ). No side or adverse effects attributable to the intervention were reported by patients.

## Discussion

We showed in this study that dietary selenium supplementation through one Brazil nut daily for three months was effective in raising selenium plasma levels, and we also observed improved GPx activity and thyroid hormone profile after intervention. This is consistent with the role of selenium in the activity of a number of selenoenzymes required for thyroid function.

The thyroid is a gland with high content of selenium because it expresses several specific selenoproteins

**Table I**  
General Characteristics of the HD patients

Parameters	Patients
Men/Women	17/23
Age (yrs)	$53.3 \pm 16.1$
HD time (mo)	62.0 (8.0 - 207.0)
Urea nitrogen (mg/dL)	$165.3 \pm 30.0$
Phosphorus (mg/dL)	$5.4 \pm 1.3$
Calcium (mg/dL)	$8.9 \pm 0.5$
Potassium (mg/dL)	$4.6 \pm 0.5$
Albumin (g/dL)	3.3 (3.2 - 3.5)
Creatinine (mg/dL)	$7.3 \pm 2.5$

**Table II**  
Assessment biochemical parameters before and after Brazil nut supplementation (N=40)

Parameters	Before Supplementation	After Supplementation
Plasma Se ( $\mu\text{g/L}$ )	17.6 $\pm$ 11.6	153.4 $\pm$ 86.1*
GPx (nmol/min/mL)	33.7 $\pm$ 5.9	41.4 $\pm$ 11.2*
T3 (ng/dL)	27.3 $\pm$ 8.8 (80-180)	50.2 $\pm$ 4.8*
FT4 (ng/dL)	0.87 $\pm$ 0.2 (0,70 a 1,80)	0.98 $\pm$ 0.4*
TSH (uUI/mL)	2.17 $\pm$ 1.3 (0,5 a 5,0)	1.96 $\pm$ 1.1
FT4/T3 ratio	0.7 $\pm$ 0.1	0.2 $\pm$ 0.06*

\* $P < 0.05$ .

implicated in thyroid hormone metabolism<sup>25</sup>. Then, selenium deficiency can lead to changes in thyroid hormones levels. The only one study published about the impact of selenium supplementation on thyroid hormone levels in HD patients showed that this supplementation might be helpful in partially improving thyroid function in dialysis patients<sup>18</sup>. In different populations from CKD patients, the results are contradictories. Rayman *et al.*, 2008<sup>26</sup> did not show any effect of selenium intervention on thyroid hormones in elderlies. Thomson *et al.*, 2009<sup>27</sup> showed that selenium supplementation to older New Zealand population was effective to increase the plasma selenium and GPx activity; however, no significant changes were found in T3 and T4 levels. In contrast, Combs *et al.*, 2009<sup>28</sup> showed that selenium supplementation increased T3 concentration in men healthy adults, but not in women.

According to Thomson *et al.*, 2009<sup>27</sup> during selenium deficiency, the mineral is well maintained in the thyroid gland and the deiodinases are high on the hierarchy of selenoproteins, such that selenium status must be insufficient to modify the activity of these enzymes and hormones. This may be particularly pertinent in typically selenium-deficient chronic diseases such as CKD, and may explain our observation that selenium restoration impacts on thyroid hormones putatively via improved deiodinases activity in the thyroid gland.

The selenium species in the Brazil nut in other studies indicates that selenomethionine is the main specie. Studies in rats showed that the bioavailability of the Brazil nut is equal to that of sodium selenite<sup>29</sup>. The greater increase in whole blood selenium after Brazil nut consumption suggests that selenium from this nut may be more bioavailable than others forms of selenium supplementation.

A simple dietary modification such as to include as little as one Brazil nut/day in the diet of dialysis patients would avoid the need for fortification of foods supplements. Natural food sources are preferable to alternative supplementation practices, because they are sustainable, less expensive, and have lower risk of toxicity. Besides selenium, Brazil nuts also provide other vitamins and minerals as well as n-6 polyunsaturated fat that may positively impact on health status<sup>30</sup>.

Our study has a number of limitations to be considered, starting by the lack of controlled group and few patients. Besides that, this study did not analyse the deiodinases activity. Nevertheless, the evaluation of deiodinase activity needs much effort because it can only be measured in the tissues where enzymes are expressed such as thyroid, lung and kidney. Therefore, our study can only be interpreted as hypothesis generating, providing evidence on the possible link between selenium restoration and thyroid hormones levels. Further controlled studies are necessary to confirm or refute these findings.

Despite the increased T3 levels, TSH was within normal range in HD patients before selenium supplementation as expected as FT4 was also in the normal range. Although after selenium supplementation FT4 and T3 increased significantly, while the TSH levels slightly decreased. This data suggest that selenium supplementation, at least in the doses used in this work might be able to increase the deiodinases (D1 and D2) activities, as T3 levels were also increased. A higher dose of selenium might restore T3 levels to normal range.

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### Disclosure

Authors have nothing to disclosure.

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