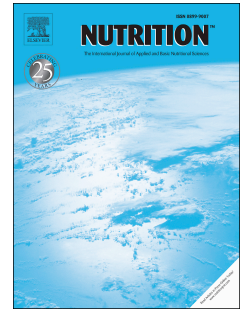


# Accepted Manuscript

Nutritional Status of Selenium in Preschool Children Receiving A Brazil Nut-Enriched Diet

Irland B.G. Martens, Barbara R. Cardoso, Dominic J. Hare, Megan M. Niedzwiecki, Franco M. Lajolo, Andreas Martens, Silvia M.F. Cozzolino



PII: S0899-9007(15)00220-8

DOI: [10.1016/j.nut.2015.05.005](https://doi.org/10.1016/j.nut.2015.05.005)

Reference: NUT 9529

To appear in: *Nutrition*

Received Date: 24 March 2015

Revised Date: 7 May 2015

Accepted Date: 10 May 2015

Please cite this article as: Martens IBG, Cardoso BR, Hare DJ, Niedzwiecki MM, Lajolo FM, Martens A, Cozzolino SMF, Nutritional Status of Selenium in Preschool Children Receiving A Brazil Nut-Enriched Diet, *Nutrition* (2015), doi: 10.1016/j.nut.2015.05.005.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

# NUTRITIONAL STATUS OF SELENIUM IN PRESCHOOL CHILDREN RECEIVING A BRAZIL NUT-ENRICHED DIET

Irland B. G. Martens<sup>1</sup>, Barbara R. Cardoso<sup>2,3,\*</sup>, Dominic J. Hare<sup>3,4,5</sup>, Megan M. Niedzwiecki<sup>5</sup>, Franco M. Lajolo<sup>2</sup>, Andreas Martens<sup>6</sup>, Silvia M. F. Cozzolino<sup>2</sup>

<sup>1</sup>*Dept. of Nutrition, Federal University of Pará, Belém, Brazil*

<sup>2</sup>*Dept. of Food and Experimental Nutrition, University of São Paulo, São Paulo, Brazil*

<sup>3</sup>*The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, Victoria, Australia*

<sup>4</sup>*Elemental Bio-imaging Facility, University of Technology Sydney, Broadway, New South Wales, Australia*

<sup>5</sup>*Exposure Biology, Lautenberg Environmental Health Sciences Laboratory, Department of Preventive Medicine, Icahn School of Medicine at Mount Sinai, New York, New York, United States of America.*

<sup>6</sup>*Institute of Inorganic and Analytical Chemistry, Technical University of Braunschweig, Braunschweig, Germany.*

**Running head:** Selenium in children fed a Brazil nut-enriched diet

**Word count:** 3,327

**Number of Tables:** 4

**Number of Figures:** 1

## **Statement of Author's Contributions to Manuscript**

IBGM, AM and SMFC: designed research; IBGM: conducted research; IBGM, AM, FMJ and SMFC: analysed data; BRC, DJH, MMN: wrote the manuscript; All authors read and approved the final manuscript.

## **\*Corresponding author:**

Bárbara R. Cardoso, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Av. Prof. Lineu Prestes 580, Bloco. 14, Butantã - 05508-000, São Paulo, SP, Brasil. Ph +55 11 30913625. Email: baritacardoso@gmail.com; barbara.rita@florey.edu.au

**Abstract****Objective:**

The Brazilian Amazon region has selenium (Se)-rich soil, which is associated with higher Se levels in populations fed locally-grown produce. Brazil nuts are a major source of dietary Se and are included with meals offered to children enrolled in public preschool in Macapá. Here, we examined Se intake and status of these children.

**Methods:**

The Macapá group consisted of 41 children from a public preschool who received 15-30 grams of Brazil nuts 3 days per week. The control group included 88 children from the nearby city of Belém, who did not receive Brazil nut-enriched meals. In both groups, school meals comprised at least 90% of the children's total food consumption. Selenium was assessed using hydride generation quartz tube atomic absorption spectroscopy in plasma, erythrocytes, nails, hair and urine. Dietary intakes (macronutrients and Se) were evaluated using the duplicate-portion method.

**Results:**

Both groups received inadequate intakes of energy and macronutrients. Selenium intake was excessive in both groups (Macapá = 155.30; Belém = 44.40 µg/day). Intake was potentially toxic in Macapá on days when Brazil nuts were added to meals. While biomarkers of Se exposure exceeded reference levels in the Macapá group, no clinical symptoms of Se overload (selenosis) were observed.

**Conclusions:**

The inclusion of Brazil nuts in school meals provided to children with already high dietary Se intakes increased Se levels and may result in an increased risk of toxicity. As selenosis is associated with some chronic diseases, we recommend continued monitoring of Se intake and status in this population.

**INTRODUCTION**

Humans require trace amounts of selenium (Se) for the synthesis of selenocysteine-containing selenoproteins, a diverse group of proteins with important roles in antioxidant defence, immune system regulation, and heavy metal detoxification [1, 2]. Dietary Se is found predominately in organic forms as selenomethionine and selenocysteine, although inorganic species are present in smaller quantities [3].

Through different pathways, both Se forms are converted to selenide ( $\text{Se}^{2-}$ ), which serves as the donor for the incorporation of Se into selenoproteins [4]. Marginal Se deficiency is associated with increased risks of immune dysfunction; cancers of the prostate, liver, lung and oesophagus; and cardiovascular, neurological and endocrine disorders [5-9]. Selenium toxicity is characterized by severe gastrointestinal distress and a strong, garlic-like breath odour, and it has suspected roles in some neurological diseases, ischaemic heart disease, renal failure and cardiomyopathy [2, 10].

The major source of Se is through diet, and the Se content of foods is largely dependent on the bioavailability of the mineral in the soil [11]. The Brazilian Amazon region is considered to have particularly Se-rich soil compared to surrounding areas [12, 13], and studies have shown that populations residing in this region have typical to very high Se nutritional status [13-15]. The Brazilian Amazon region is the leading producer of one of the richest Se food sources, the Brazil nut (*Bertholletia excelsa*, H.B.K.). Selenium in the Brazil nut is not only at a high concentration, but is also highly bioavailable [16, 17].

Since Brazil nuts are widely cultivated within the Brazilian Amazon region, they are a prominent component of the native diet and a common ingredient in local dishes. As part of public health policy, Brazil nuts are included with meals offered to children enrolled in public preschools in Macapá, the capital of Amapá, a state within the Amazon region. While Brazil nuts are often used as a strategy to improve Se status in Se-deficient populations [18-20], the effects of supplementation with this nut in populations less vulnerable to Se deficiency is not clear. Moreover, assessing Se nutritional status in children is of particular interest, as both excess and deficiency are associated with adverse health effects that may persist throughout life. Thus, we aimed to investigate Se intake and Se status of children from Macapá who receive a Brazil nut-enriched diet and to compare with children from Belém, a city in the Amazon region where Brazil nut supplementation does not occur.

## **MATERIALS AND METHODS**

### **Population study**

Forty-one preschool children from Macapá (Amapá state) and 88 preschool children from Belém (Pará state) were enrolled in this study. They were recruited from public schools where they spent 10 hours per day, 5 days per week, and received 4 meals daily: breakfast, lunch, snack and dinner. Both schools were localised in high-poverty areas of the cities, and selection criteria of participants required a monthly household income up to the Brazilian minimum wage; thus children from both groups had the same social-economic condition. To be eligible for the study, children were required to have been enrolled in school for at least seven months, with a minimum attendance rate of 75% during this period.

As part of public health policy, all children from Macapá were receiving Brazil nut-enriched meals 3 days a week at school. On average, each child received 15 to 30 g of Brazil nuts (corresponding to 3 to 6 nuts) added to recipes offered in one of the daily meals.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by Ethics Committee of the Faculty of Pharmaceutical Sciences at the University of São Paulo. Written informed consent was obtained from the children's parents.

### **Anthropometric evaluation**

The children were measured while wearing light clothing and no shoes. Body weight was measured with a Filizola scale to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm using a mounted stadiometer. Anthropometric status was classified according to World Health Organization growth standards for weight-for-age (WA), height-for-age (HA), and weight-for-height (WH) [21]. The software EPI INFO 2000 v1.1.1 (Center for Disease Control, United States) was used to determine *z* scores. Cut-off values for wasting, stunting, and thinness were 2 SD; cut-off values for overweight and obesity was 2 SD.

### **Dietary intake**

On the first day of study, parents provided a 24-hour dietary recall to verify if the children had received Brazil nuts, with no children excluded on this basis. Aside

from those provided in a controlled manner to the Macapá group, children did not consume additional Brazil nuts for the duration of the study. Results from the dietary recall estimated that, on average, meals consumed at school corresponded to at least 90% of the children's total food intake. As evaluated by leftover control, children consumed at least 90% of the school-provided meals. None of the children were receiving or had received vitamin and mineral supplementation, had consumed Brazil nuts at home, or presented with acute inflammation or gastrointestinal disturbances.

A duplicate-portion method was used to calculate dietary intake of macronutrients and Se [22]. All complete meals provided by the school were sampled daily for 7 consecutive days. Samples were collected in triplicate, weighed, sealed in demineralised polyethylene bags and stored at -20°C until analysis. Frozen meals were thawed at room temperature and mixed in a blender (WALITA Master Plus<sup>®</sup>, equipped with stainless-steel blades and cup) before freeze-drying.

Macronutrients, humidity and ash were analysed in triplicate according to Association of Official Analytical Chemists (AOAC) standards in lyophilised aliquots of the mixed samples [23]. The total carbohydrate contents were calculated by difference (100 – total grams of humidity, protein, lipids, and ash), including the fibre fraction. Selenium concentration was determined using hydride generation quartz tube atomic absorption spectroscopy (HGQTAAS) [24].

Selenium adequacy was calculated using *z* scores according to Estimated Average Requirement (EAR) and Upper Tolerable Intake Level (UL) [25] as follows:  $z = (EAR - M_i)/SD$ ;  $Z = (UL - M_i)/SD$ , where  $M_i$  is mean Se intake per day and SD is the standard deviation.

### **Biochemical assays**

Selenium status was evaluated in 41 children from Belém who were randomly assigned to sample collection and all 41 children from the Macapá group. Samples were collected at the same time as the dietary intake assessment. Fasting morning blood samples were collected by venipuncture in ethylenediaminetetraacetic acid (EDTA) evacuated tubes to determine Se concentration in plasma and erythrocytes. Plasma was separated by centrifugation at 3,000  $\times$  g for 15 min at 4 °C. The erythrocyte pellet was washed three times with 5 mL of sterile 9 g/L NaCl solution,

slowly homogenised by inversion and centrifuged at 10,000  $\times$  g for 10 minutes at 4 °C, and the supernatant was discarded. Toenail and fingernail samples were collected, cleaned with neutral detergent and deionised water, and dried at 35 °C. Selenium was measured in 50 and 100 mg sample aliquots. One hair sample was cut from the back of the head (occipital area) close to the scalp. The samples had an average mass of around 2g and were prepared for Se analysis according to the sample protocol used for nail samples. Single urine samples at 24 hours were collected in plastic demineralised bottles.

Selenium concentration was determined in plasma, erythrocyte, hair, nail and urine samples using (HGQTAAS) with HITACHI Z5000 Tandem AAS in combination with a coupled HFS-3 hydride generator [24]. Deionised water was used to prepare all solutions and to dilute the samples. Analytical accuracy and precision was assessed by analysis of the reference materials Seronorm<sup>®</sup> and NIST<sup>®</sup>1567 (wheat flour). All reagents were of analytical grade or higher purity from Merck. Nanopure water was used to prepare all solutions and to dilute samples to volume prior to analysis.

### Statistical analysis

A descriptive analysis was performed, and the results are shown as the mean  $\pm$  standard deviation (SD) for continuous variables, except for the variables of dietary intake that are presented as median. The Kolmogorov-Smirnov test was performed to verify data normality. As all variables displayed a normal distribution, a two-tailed Student's *t*-test was used to compare differences between groups. Analyses were performed using SPSS for Windows. The level of significance was established at  $p < 0.05$ .

## RESULTS

As shown in Table 1, the groups were equivalent with regard to sex, age, time of enrolment at school, weight or height.

**Table 1.** Participant details.

Parameters	Macapá (n = 41)	Belém (n = 88)
Sex (male) <sup>a</sup>	22 (53.7)	42 (47.7)
Age (y) <sup>b</sup>	4.7 $\pm$ 0.9 (3.1 – 6.3)	4.5 $\pm$ 1.2 (2.1 – 6.6)

Enrolment period at school (months) <sup>b</sup>	20.5 ± 11.6 (7.0 – 36.0)	20.8 ± 10.5 (7.0 – 36.0)
Weight (kg) <sup>b</sup>	16.8 ± 2.6 (12.4 – 25.3)	15.7 ± 2.9 (11.6 – 28.6)
Height (cm) <sup>b</sup>	103.4 ± 6.3 (94.0 – 115.0)	101.6 ± 8.7 (85.0 – 125.5)

<sup>a</sup>N (%) (all such values); <sup>b</sup>Mean ± SD (min – max) (all such values)

With regard to WA, HA, and WH parameters, most of the children from both cities were eutrophic. However, we observed that the proportion of children with stunting was significantly higher in Macapá (41%) compared to Belém (17%) ( $p < 0.01$ ).

**Table 2.** Anthropometric status of the children from Macapá and Belém according to  $z$ -score.

$z$ -score	Macapá (n = 41)			Belém (n = 88)		
	HA	WA	WH	HA	WA	WH
$z < -2$	17 (41.5)	7 (17.1)	0 (0.0)	15 (17.0)	7 (8.0)	0 (0.0)
$z -2$ to $+2$	24 (58.5)	32 (78.0)	39 (95.1)	73 (83.0)	80 (90.9)	88 (100)
$z > 2$	0 (0.0)	2 (4.9)	2 (4.9)	0 (0.0)	1 (1.1)	0 (0.0)

N (%) (all such values); HA, height-for-age; WA, weight-for-age; WH, weight-for height

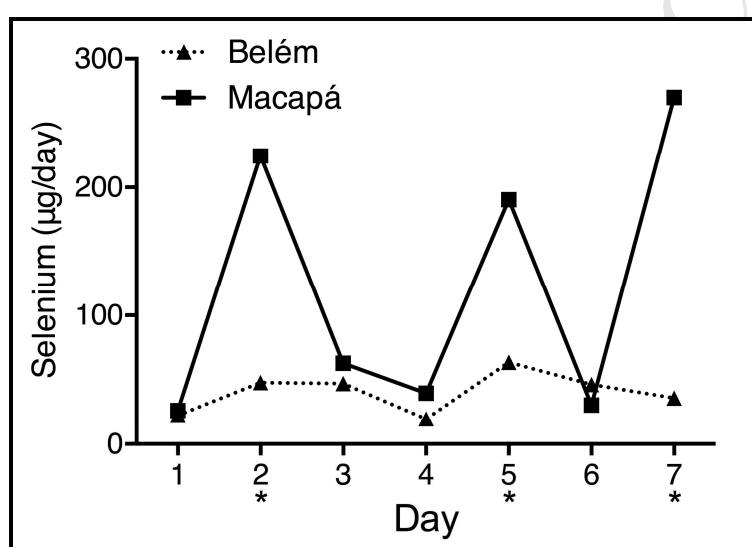
The diets from Macapá and Belém preschools presented food monotony (see Supplementary Tables for a complete list). Results from the duplicate-portion method (Table 3) showed that children from both cities had a lower caloric intake than the Recommended Dietary Allowance (RDA) of 1,300 to 1,800 kcal per day. The proportion of lipids in the diet was sufficient in both groups, and only the Macapá group achieved intake of the recommended levels of carbohydrates. Based on the recommendation of 16 to 24 g of protein daily during this life stage, both groups received excess protein intakes at least some days of the week. Children from both groups consumed excess Se compared with EAR (17  $\mu\text{g/d}$  for 1-3 years; 23  $\mu\text{g/d}$  for 4-8 years). In Macapá, this excess was more prevalent on days when Brazil nuts were added to the meals, reaching a peak of 279.3  $\mu\text{g/day}$  (Figure 1), while on the other days the median Se intake was similar to Belém children. According to  $z$  scores calculated based on EAR and UL reference values, there was no risk of inadequate Se intake in both groups, and Macapá children presented a high risk of toxicity.



**Table 3.** Macronutrients and selenium content in diets of preschool children from Macapá and Belém.

	Macapá	Belém
Energy (kcal/day)	1124.8 (994.52 – 1.265.56)	1081.5 (925.12 – 1309.52)
Protein (g/day)	31.5 (24.55 – 48.41)	42.5 (35.15 – 49.5)
Carbohydrates (%)	55.9 (50.3 – 61.2)	49.2 (41.3 – 59.2)
Lipids (%)	32.8 (27.1 – 40.0)	36.9 (24.1 – 49)
Selenium ( $\mu\text{g/day}$ )	155.30 (98.70 – 195.3)	44.40 (33.90 – 53.20)

All data are given as median (min – max)



**Figure 1.** Selenium concentration ( $\mu\text{g/day}$ ) in diets of preschool children.

\*Brazil-nut enriched meals

Table 4 shows Se concentrations in different biomarkers of children from Macapá and Belém. Selenium levels in all biomarkers were significantly higher in Macapá children, although in both groups participants showed plasma and erythrocyte values above the most accepted serum/plasma Se cutoff ( $> 84\text{--}100 \mu\text{g/L}$ ) [1].

**Table 4.** Selenium parameters of children from Macapá and Belém.

Parameters	Macapá (n = 41)	Belém (n = 41)
Plasma ( $\mu\text{g/L}$ )	$107.29 \pm 27.15$ (73.00 – 172.00)	$83.56 \pm 23.32^*$ (47.00 – 142.00)
Erythrocyte ( $\mu\text{g/L}$ )	$133.24 \pm 32.24$ (78.00 – 195.00)	$94.74 \pm 18.60^*$ (67.00 – 150.00)
Urine ( $\mu\text{g/mL}$ )	$0.27 \pm 0.12$ (0.11 – 0.47)	$0.04 \pm 0.01^*$ (0.02 – 0.10)
Hair ( $\mu\text{g/g}$ )	$0.89 \pm 0.24$ (0.44 – 1.35)	$0.31 \pm 0.10^*$ (0.12 – 0.50)

Nails ( $\mu\text{g/g}$ )	$3.43 \pm 1.81$ (0.89 – 8.43)	$1.29 \pm 0.52^*$ (0.31 – 2.16)
---------------------------	-------------------------------	---------------------------------

All data are given as mean  $\pm$  SD (min – max)

\* Significantly different from Macapá.  $p < 0.001$  (Student's t-test)

## DISCUSSION

To investigate the impact of Brazil nut-enriched diets on Se status in children residing in the Brazilian Amazon, we compared dietary Se intakes and Se status of children enrolled in public preschools in Macapá and Belém, two cities with and without school-based Brazil nut supplementation programs, respectively.

The HA index—an assessment of the delay in the child's linear growth—is one of the most important indicators used to detect child malnutrition. Although it has been reported that the prevalence of malnourishment is decreasing in Brazil [26, 27], we observed a high prevalence of stunting in our study: the prevalence observed in both cities (Macapá, 41.5%; Belém, 17.0%) is markedly above that reported by the Household Budget Survey in 2008-2009, when 6.8% of children in Brazil were reported to have growth retardation [28].

It is known that the family environment, feeding patterns, socioeconomic status and sanitation are associated with the nutritional status of children [27, 29]. Most of the children from both cities were from poor families and lived under social vulnerability, and the meals offered at school corresponded to at least 90% of their total food consumption. In some cases, children did not have access to food during weekends or on school holidays. Thus, nutritional adequacy of meals offered at school is essential to ensure appropriate nutritional intake; however, the duplicate-portion method analysis showed that the energetic content of meals was below RDA, which might contribute to the high proportion of stunting in the Macapá and Belém groups. Besides energetic deficits, daily meals offered at both schools presented inadequate macronutrient composition and food monotony.

The assessment of Se dietary intakes presents many difficulties because the Se content in primary foodstuffs varies depending on soil Se concentration. Throughout Brazil, the Se levels in soils, as well as the Se content of regional diets, are vastly different [12, 30], and the development of region-specific food composition tables is difficult. Therefore, the duplicate-portion method analysis used in this study was important to assess Se intake accurately. Previously, Rocha et al. [31] reported an association between the intake of locally-grown food and

increased Se intake and status in riverine children from Rondonia, another Brazilian state located in the Amazon basin. In both cities, we found that school meals were composed mainly of locally-grown food, which likely explains the high Se content even when Brazil nuts were not included in the meals. Daily Se intake of children living in Amazonia ranges from poor to excessive [31, 32]. On the days that Brazil nuts were not included in Macapá children's meals, Se levels were similar to Belém's, but the inclusion of the nuts made Se content peak at 279.3  $\mu\text{g/day}$ , resulting in high risk of toxicity in comparison to UL (90  $\mu\text{g/d}$  for 1-3 years; 150  $\mu\text{g/d}$  for 4-8 years).

It has been suggested that it is important to evaluate at least two biomarkers to assess nutritional Se status [1, 33]. In the present work, different biomarkers were used to cover different periods of Se exposure: plasma and urine were used as markers of current exposure; erythrocytes reflect longer-term nutritional status, due to their half-life of 120 d; and nails and hair were useful as long-term biomarkers and reflect tissue Se levels [1, 34-36]. Unfortunately, there are no specific reference values for children and thus, the results in the present study are cautiously compared with reference values for adults.

Findings from Thomson et al. [1] suggest that blood Se concentrations ranging from 84 to 100  $\mu\text{g/L}$  are necessary to maximise the activity of the selenoenzyme GPx. Based on this, children from Belém presented adequate plasma and erythrocyte levels, whereas the Macapá group had higher levels than expected—reaching potentially toxic levels when compared to the reference values established by Hays et al., who developed biomonitoring equivalents for assessing Se status according to EAR and UL values [37]. Selenium in urine reflects a higher proportion of Se dose following higher Se exposures: dietary intake is converted to selenide, and this may be metabolised to Se-containing carbohydrates, the main Se species in urine [36, 38]. Comparing the urine Se levels of both groups with those proposed by Hays et al. (EAR = 0.01  $\mu\text{g/mL}$ ; UL toxicity = 0.11  $\mu\text{g/mL}$ ) [37], we observe that the Belém group had levels considered safe, but children from Macapá had Se excretion compatible with Se intake at a toxic amount.

Hair and nails reflect tissue Se levels over a wide range of dietary intakes. While there are no recognised standard references for these biomarkers, we found that children from Macapá had higher levels compared to Belém's group, supporting the

findings from the other biomarkers. Some selenoproteins and their respective activity could be used as biomarkers of dietary Se status, in addition to total Se in biological fluids. For instance, glutathione peroxidase-3 (GPx3) is an antioxidant protein with activity directly related to dietary Se intake [39]. While we did not measure GPx3 in these children, it would be assumed that this activity would be elevated. Such a study would be of interest in the future.

Our results show that the addition of Brazil nuts to meals three times a week increased Se status of preschool children from the Amazon region. This is in agreement with other studies that reported that Brazil nuts have high content of Se, and that the intake of this nut was associated with recovery of Se deficiency and with increased antioxidant and antiinflammatory response [18-20, 40]. Studies have shown that only one nut daily is enough to recover Se status of deficient adults; thus, the ingestion of three to six nuts three times per week may result in Se toxicity to children. The main symptoms of selenosis are changes to and loss of nails and hair, skin lesions, unusual garlic odour on the breath, nervous system defects (difficulty in identifying an object by the sense of touch, tingling in hands, foot and/or mouth, tiredness in legs and/or arms, pain in legs, pain in arms, hand tremor, muscle twitches and/or cramps, joint pain) and gastrointestinal disorders (nausea, vomiting) [38, 41]. A doctor clinically evaluated the children in our study, but no signs of selenosis were observed in both groups, consistent with other studies of Amazon populations [31, 41].

The absence of symptoms of chronically-high Se intake may be due to the fact that selenomethionine is the most prevalent Se species in Brazil nuts, which comprises 75 to 90% of Se species in this food [16, 17]. Selenomethionine can be either reduced to hydrogen selenide for selenoprotein synthesis, or it can non-specifically replace methionine in proteins of plasma (mainly in albumin) and whole blood (mainly in erythrocytes); thus, this nonspecific accumulation of Se may also act as a storage pool of Se, which can be slowly released during protein turnover [25, 42]. Moreover, it has been reported that the Amazon population is highly exposed to mercury (Hg) from diet [31, 32, 43, 44], and high Se intake may counterpoise Hg-induced toxic effects because they interact to form the selenite-dimethylmercury complex, which is unstable in blood and in other tissues [45].

## CONCLUSIONS

Our data showed that Se intakes in children from two different cities localized in the Brazilian Amazon region were adequate; however, the inclusion of Brazil nuts in the school meals in Macapá resulted in excess Se dietary intakes and elevated Se levels in these children. Even though children from Macapá did not present symptoms of selenosis, based on Se levels in the assessed biomarkers, particularly in urine, we encourage the monitoring of Se levels in this population to avoid possible risks of adverse effects. Although some studies have been reported positive effects of higher Se levels on motor performance [46] and reduced risk for cataracts [47], Se toxicity may be associated with longer-term disturbances, such as diabetes and cardiovascular disease [2, 41, 48], and further study is warranted to fully establish the long-term safety of Se supplementation through diet.

## ACKNOWLEDGEMENTS

We thank Embrapa Amazonia Ocidental in Manaus, the Amapá secretaries of education and communication, and collaborators from LACEN and COMAJA. We especially want to thank all children and their families who participated in the study. Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) provided financial support.

## REFERENCES

- [1] Thomson C. Assessment of requirements for selenium and adequacy of selenium status: a review. *Eur J Nutr.* 2004;58:11.
- [2] Holben D, Smith A. The diverse role of selenium within selenoproteins: a review. *J Am Diet Assoc.* 1999;99:8.
- [3] Sun M, Liu G, Wu Q. Speciation of organic and inorganic selenium in selenium-enriched rice by graphite furnace atomic absorption spectrometry after cloud point extraction. *Food Chem.* 2013;141:66-71.
- [4] Roman M, Jitaru P, Barbante C. Selenium biochemistry and its role for human health. *Metallomics.* 2014;6:25-54.
- [5] Lin SL, Wang CW, Tan SR, Liang Y, Yao HD, Zhang ZW, et al. Selenium deficiency inhibits the conversion of thyroidal thyroxine (T4) to triiodothyronine (T3) in chicken thyroids. *Biol Trace Elem Res.* 2014;161:263-71.
- [6] Arthur JR, McKenzie RC, Beckett GJ. Selenium in the immune system. *J Nutr.* 2003;133:1457s-9s.
- [7] Cardoso BR, Ong TP, Jacob-Filho W, Jaluul O, Freitas MI, Cozzolino SM. Nutritional status of selenium in Alzheimer's disease patients. *Br J Nutr.* 2010;103:803-6.
- [8] Rita Cardoso B, Silva Bandeira V, Jacob-Filho W, Franciscato Cozzolino SM. Selenium status in elderly: relation to cognitive decline. *J Trace Elem Med Biol.* 2014;28:422-6.

- [9] Salonen JT, Alfthan G, Huttunen JK, Pikkarainen J, Puska P. Association between cardiovascular death and myocardial infarction and serum selenium in a matched-pair longitudinal study. *Lancet*. 1982;2:175-9.
- [10] Vinceti M, Bonvicini F, Rothman KJ, Vescovi L, Wang F. The relation between amyotrophic lateral sclerosis and inorganic selenium in drinking water: a population-based case-control study. *Environ Health*. 2010;9:77.
- [11] Thiry C, Ruttens A, De Temmerman L, Schneider Y-J, Pussemier L. Current knowledge in species-related bioavailability of selenium in food. *Food Chem*. 2012;130:767-84.
- [12] Favaro DI, Hui ML, Cozzolino SM, Maihara VA, Armelin MJ, Vasconcellos MB, et al. Determination of various nutrients and toxic elements in different Brazilian regional diets by neutron activation analysis. *J Trace Elem Med Biol*. 1997;11:129-36.
- [13] Lemire M, Mergler D, Fillion M, Passos CJS, Guimarães JRD, Davidson R, et al. Elevated blood selenium levels in the Brazilian Amazon. *Sci Total Environ*. 2006;366:101-11.
- [14] Pinheiro MCN, Müller RCS, Sarkis JE, Vieira JLF, Oikawa T, Gomes MSV, et al. Mercury and selenium concentrations in hair samples of women in fertile age from Amazon riverside communities. *Sci Total Environ*. 2005;349:284-8.
- [15] Lemire M, Mergler D, Huel G, Passos CJ, Fillion M, Philibert A, et al. Biomarkers of selenium status in the Amazonian context: blood, urine and sequential hair segments. *J Expo Sci Environ Epidemiol*. 2009;19:213-22.
- [16] Bodo ET, Stefanka Z, Ipolyi I, Soros C, Dernovics M, Fodor P. Preparation, homogeneity and stability studies of a candidate LRM for Se speciation. *Anal Bioanal Chem*. 2003;377:32-8.
- [17] da Silva EG, Mataveli LR, Arruda MA. Speciation analysis of selenium in plankton, Brazil nut and human urine samples by HPLC-ICP-MS. *Talanta*. 2013;110:53-7.
- [18] Rita Cardoso B, Apolinario D, da Silva Bandeira V, Busse AL, Magaldi RM, Jacob-Filho W, et al. Effects of Brazil nut consumption on selenium status and cognitive performance in older adults with mild cognitive impairment: a randomized controlled pilot trial. *Eur J Nutr*. 2015.
- [19] Stockler-Pinto MB, Mafra D, Moraes C, Lobo J, Boaventura GT, Farage NE, et al. Brazil nut (*Bertholletia excelsa*, H.B.K.) improves oxidative stress and inflammation biomarkers in hemodialysis patients. *Biol Trace Elem Res*. 2014;158:105-12.
- [20] Cominetti C, de Bortoli MC, Garrido AB, Jr., Cozzolino SM. Brazilian nut consumption improves selenium status and glutathione peroxidase activity and reduces atherogenic risk in obese women. *Nutr Res*. 2012;32:403-7.
- [21] World Health Organization. Physical status the use and interpretation of antropometry. Switzerland 1995.
- [22] L SM, Bj A, J. MV. Nutrición y salud pública métodos, bases científicas y aplicaciones. Spain: Elsevier Masson; 2012. p. 850.
- [23] Association of Official Analytical Chemists (AOAC). Official methods of analysis. Virginia, USA: Association of Official Analytical Chemists Inc.; 1990.
- [24] Gonzaga IB. Avaliação nutricional relativa ao selênio em crianças com dieta enriquecida de castanha-do-Brasil (*Bertholletia axcelsa*, L.). São Paulo - Brazil: University of São Paulo; 2002.
- [25] Institute of Medicine. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington, D.C: National Academy Press; 2000.



- [26] Restrepo-Mendez MC, Barros AJ, Black RE, Victora CG. Time trends in socio-economic inequalities in stunting prevalence: analyses of repeated national surveys. *Public Health Nutr.* 2014;1-8.
- [27] Correia LL, Silva AC, Campos JS, Andrade FM, Machado MM, Lindsay AC, et al. Prevalence and determinants of child undernutrition and stunting in semiarid region of Brazil. *Rev Saude Publica.* 2014;48:19-28.
- [28] Brasil. Instituto Brasileiro de Geografia e Estatística. Pesquisa de Orçamentos Familiares 2008-2009: antropometria e estado nutricional de crianças, adolescentes e adultos do Brasil. . Rio de Janeiro: IBGE; 2010.
- [29] de Amorim JF, Cavalcante CG, de Amorim Santos LV, Solymos GM, Toledo Florencio TM, de Paffer AT, et al. Influence of family environment on childhood stunting. *Matern Child Nutr.* 2014;10:313-4.
- [30] Shaltout AA, Castilho IN, Welz B, Carasek E, Martens IB, Martens A, et al. Method development and optimization for the determination of selenium in bean and soil samples using hydride generation electrothermal atomic absorption spectrometry. *Talanta.* 2011;85:1350-6.
- [31] Vieira Rocha A, Cardoso BR, Cominetti C, Bueno RB, de Bortoli MC, Farias LA, et al. Selenium status and hair mercury levels in riverine children from Rondonia, Amazonia. *Nutrition.* 2014;30:1318-23.
- [32] Farias LA, Favaro DIT, Maihara VA, M. B. A V, Yuyama LK, Aguiar JPL, et al. Assessment of daily dietary intake of Hg and some essential elements in diets of children from the Amazon region. *J Radioanal Nucl Chem.* 2006;270:217-23.
- [33] Slotnick MJ, Nriagu JO. Validity of human nails as a biomarker of arsenic and selenium exposure: A review. *Environ Res.* 2006;102:125-39.
- [34] Hurst R, Collings R, Harvey LJ, King M, Hooper L, Bouwman J, et al. EURRECA-Estimating selenium requirements for deriving dietary reference values. *Crit Rev Food Sci Nutr.* 2013;53:1077-96.
- [35] Satia JA, King IB, Morris JS, Stratton K, White E. Toenail and plasma levels as biomarkers of selenium exposure. *Ann Epidemiol.* 2006;16:53-8.
- [36] Terol A, Ardini F, Basso A, Grotti M. Determination of selenium urinary metabolites by high temperature liquid chromatography-inductively coupled plasma mass spectrometry. *J Chromatogr A.* 2015;1380:112-9.
- [37] Hays SM, Macey K, Nong A, Aylward LL. Biomonitoring Equivalents for selenium. *Regul Toxicol Pharmacol.* 2014;70:333-9.
- [38] Jager T, Drexler H, Goen T. Human metabolism and renal excretion of selenium compounds after oral ingestion of sodium selenate dependent on trimethylselenium ion (TMSe) status. *Arch Toxicol.* 2014.
- [39] F Combs Jr G. Biomarkers of Selenium Status. *Nutrients.* 2015;7:2209-36.
- [40] Stockler-Pinto MB, Mafra D, Farage NE, Boaventura GT, Cozzolino SM. Effect of Brazil nut supplementation on the blood levels of selenium and glutathione peroxidase in hemodialysis patients. *Nutrition.* 2010;26:1065-9.
- [41] Lemire M, Philibert A, Fillion M, Passos CJS, Guimarães JRD, Barbosa Jr F, et al. No evidence of selenosis from a selenium-rich diet in the Brazilian Amazon. *Environ Int.* 2012;40:128-36.
- [42] Navarro-Alarcon M C-VC. Selenium in food and the human body: A review. *Sci Total Environ.* 2008;400:26.
- [43] Barbosa AC, de Souza J, Dorea JG, Jardim WF, Fadini PS. Mercury biomagnification in a tropical black water, Rio Negro, Brazil. *Arch Environ Contam Toxicol.* 2003;45:235-46.

- [44] Faial K, Deus R, Deus S, Neves R, Jesus I, Santos E, et al. Mercury levels assessment in hair of riverside inhabitants of the Tapajos River, Para State, Amazon, Brazil: Fish consumption as a possible route of exposure. *J Trace Elem Med Biol.* 2015;30:66-76.
- [45] Naganuma A, Imura N. Mode of in vitro interaction of mercuric mercury with selenite to form high-molecular weight substance in rabbit blood. *Chem Biol Interact.* 1983;43:271-82.
- [46] Lemire M, Fillion M, Frenette B, Passos CJS, Guimarães JRD, Barbosa Jr F, et al. Selenium from dietary sources and motor functions in the Brazilian Amazon. *NeuroToxicology.* 2011;32:944-53.
- [47] Lemire M, Fillion M, Frenette B, Mayer A, Philibert A, Passos CJ, et al. Selenium and mercury in the Brazilian Amazon: opposing influences on age-related cataracts. *Environ Health Perspect.* 2010;118:1584-9.
- [48] Bleys J, Navas-Acien A, Guallar E. Serum selenium and diabetes in U.S. adults. *Diabetes Care.* 2007;30:829-34.



## NUTRITIONAL STATUS OF SELENIUM IN PRESCHOOL CHILDREN RECEIVING A BRAZIL NUT-ENRICHED DIET

### **Highlights:**

- Brazil nuts can be used as a dietary selenium supplement.
- Children from an Amazonian school fed a Brazil nut enriched diet had high levels of selenium.
- These children were asymptomatic, but at risk of toxicity.
- Children not receiving a supplemented diet had normal levels of selenium.
- Selenium supplementation should be preceded by assessment of selenium levels in the recipients.

**Table 1S.** Variety and frequency of foods provided on the school meals in Macapá.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Frequency (n)	Frequency (%)
<b>Breakfast</b>									
Orange juice	1					1		2	7.69
Sugar	1	1	1	1	1	1	1	7	26.92
Salty biscuit	1				1			2	7.69
Coffee		1						1	3.85
Bread		1				1		2	7.69
Milk		1	1	1	1		1	5	19.23
Margarine		1				1		2	7.69
Oat			1				1	2	7.69
Chocolate powder				1				1	3.85
Tapioca flour				1				1	3.85
Rice cereal					1			1	3.85
Total	3	5	3	4	4	4	3	26	100.00
<b>Lunch</b>									
Chicken	1	1				1	1	4	6.45
Potato	1	1		1				3	4.84
Coriander	1						1	2	3.23
Carrot	1	1	1	1				4	6.45
Pasta	1	1		1				3	4.84
Óil	1	1	1	1	1	1	1	7	11.29
Paprika	1	1	1	1	1	1	1	7	11.29
Manioc flour	1	1	1	1	1	1	1	7	11.29
Pinapple	1							1	1.61
Salt	1	1	1	1	1	1	1	7	11.29
Beans			1					1	1.61
Cow tripe			1					1	1.61
Cole			1					1	1.61
Rice			1		1	1	1	4	6.45
Egg			1					1	1.61
Onions			1		1		1	3	4.84

Cow meat				1				1	1.61
Cow liver					1			1	1.61
Wheat flour							1	1	1.61
Dendê oil							1	1	1.61
Coconut milk							1	1	1.61
Brazil nuts							1	1	1.61
Total	10	8	11	8	7	6	12	62	100.00

**Snack**

Watermelon	1			1				2	18.18
Sugar		1						1	9.09
Milk		1						1	9.09
Rice cereal		1						1	9.09
Papaya		1				1		2	18.18
Cereal		1						1	9.09
Banana			1				1	2	18.18
Pineapple					1			1	9.09
Total	1	5	1	1	1	1	1	11	100.00

**Dinner**

Potato							1	1	2.27
Coriander				1			1	2	4.55
Oil			1	1			1	3	6.82
Paprika			1	1			1	3	6.82
Manioc flour			1				1	2	4.55
Banana						1		1	2.27
Salt			1	1			1	3	6.82
Acerola juice	1			1	1			3	6.82
Sugar	1	1	1		1	1		5	11.36
Onion				1				1	2.27
Cow meat				1			1	2	4.55
Manioc	1				1			2	4.55
Margarine	1				1			2	4.55
Fennel	1				1			2	4.55
Coconut milk	1				1			2	4.55

Brazil nuts		1			1			2	4.55
Milk		1				1		2	4.55
Açaí			1					1	2.27
Fish			1					1	2.27
Wheat flour			1					1	2.27
Bread				1				1	2.27
Black pepper				1			1	2	4.55
Total	6	3	8	9	7	3	8	44	100.00

**Table 2S.** Variety and frequency of foods provided on the school meals in Belém.

[illegible]

Salt	1	1	1	1	1	1	1	7	8.33
Beans			1	1	1	1		4	4.76
Cole	1		1		1			3	3.57
Rice	1	1	1	1	1	1	1	7	8.33
Egg				1				1	1.19
Onion				1		1	1	3	3.57
Cow meat		1	1			1		3	3.57
Chayote	1	1						2	2.38
Parsley	1							1	1.19
Garlic	1					1	1	3	3.57
Watermelon	1					1		2	2.38
Orange		1			1			2	2.38
Beef cherkyy			1	1	1	1		4	4.76
Tomato				1				1	1.19
Cabbage				1		1		2	2.38
Banana				1				1	1.19
Fish					1			1	1.19
Green beans						1		1	1.19
Milk							1	1	1.19
Guava jam							1	1	1.19
Total	14	10	9	12	11	15	13	84	100.00

**Snack**

Cereal		1						1	4.17
Sugar	1	1	1	0	1	1	1	6	25.00
Salty biscuit				1				1	4.17
Milk	1	1	1			1	1	5	20.83
Apple							1	1	4.17
Sweet cookies	1				1	1		2	12.50
Avocado		1					1	2	8.33
Papaya			1				1	2	8.33
Passiofruit juice					1			1	4.17
Sandwich cookies			1					1	4.17
Grapefruit juice				1				1	4.17

Total	3	4	4	2	3	3	5	24	100.00
<b>Dinner</b>									
Chicken				1			1	2	3.28
Potato		1		1			1	3	4.92
Coriander				1	1			2	3.28
Carrot	1	1		1		1	1	5	8.20
Pasta		1			1			2	3.28
Oil	1	1	1	1	1	1	1	7	11.48
Paprika		1		1	1	1	1	5	8.20
Manioc flour	1		1	1		1	1	5	8.20
Salt	1	1	1	1	1	1	1	7	11.48
Cole	1					1		2	3.28
Rice		1		1			1	3	4.92
Egg		1			1			2	3.28
Onion			1			1	1	3	4.92
Cow meat	1	1	1			1		4	6.56
Tomato		1						1	1.64
Cabbage	1					1		2	3.28
Green beans			1					1	1.64
Soup	1		1			1		3	4.92
Pumpkin	1		1					2	3.28
Total	9	10	8	9	6	10	9	61	100.00