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Selenium status in preschool children receiving a Brazil nut–enriched diet

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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. The aim of this study was to examine Se intake and status of these children.
Methods: The Macapá group consisted of 41 children from a public preschool who received 15 to 30 g of Brazil nuts 3 d/wk. 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 ≥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 (155.30 and 44.40 μg/d, in Macapá and Belém, respectively). Intake was potentially toxic in Macapá on days when Brazil nuts were added to meals. Although 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 for 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 defense, 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 (Se^{2-}), which serves as

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the donor for the incorporation of Se into selenoproteins [4]. Marginal Se deficiency is associated with increased risk for immune dysfunction; cancers of the prostate, liver, lung and esophagus; and cardiovascular, neurologic, and endocrine disorders [5–9]. Selenium toxicity is characterized by severe gastrointestinal distress and a strong garlic-like breath odor, and it has suspected roles in some neurologic diseases, ischemic 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 with 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].

Because 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. Although 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 are 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. The children were recruited from public schools where they spent 10 h/d, 5 d/wk, and received four meals daily: breakfast, lunch, snack, and dinner. Both schools were localized 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 socioeconomic condition. To be eligible for the study, children were required to have been enrolled in school for at least 7 mo, 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 d/wk at school. On average, each child received 15 to 30 g of Brazil nuts (corresponding to three to six 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 participants/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 (Centers for Disease Control and Prevention, Washington DC, USA) was used to determine *z* scores. Cutoff values for wasting, stunting, and thinness were 2 SD; cutoff values for overweight and obesity was 2 SD.

Dietary intake

On the first day of study, parents provided a 24-h dietary recall to verify that the children had received Brazil nuts; no children were excluded on this basis. In addition to 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 ≥90% of the children's total food intake. As evaluated by leftover control, children consumed ≥90% of the school-provided meals. None of the children were receiving or had received vitamin and mineral supplementation, consumed Brazil nuts at home, or presented 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 demineralized polyethylene bags, and stored at −20°C until analysis. Frozen meals were thawed at room temperature and mixed in a blender (WALITA Master Plus®, equipped with stainless-steel blades and cup) before freeze-drying.

Macronutrients, humidity, and ash were analyzed in triplicate according to Association of Official Analytical Chemists (AOAC) standards in lyophilized aliquots of the mixed samples [23]. The total carbohydrate contents were calculated by difference (100 – total g of humidity, protein, lipids, and ash), including the fiber 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: $Z = (\text{EAR}–\text{Mi})/\text{SD}$; $Z = (\text{UL}–\text{Mi})/\text{SD}$, where Mi 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 3000g for 15 min at 4°C. The erythrocyte pellet was washed three times with 5 mL of sterile 9 g/L NaCl solution, slowly homogenized by inversion, and centrifuged at 10 000g for 10 min at 4°C, and the supernatant was discarded. Toe- and fingernail samples were collected, cleaned with neutral detergent and deionized 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 ~2 g and were prepared for Se analysis according to the sample protocol used for nail samples. Single urine samples at 24 h were collected in plastic demineralized 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]. Deionized 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 and NIST1567 (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 before analysis.

Statistical analysis

A descriptive analysis was performed, and the results are shown as the mean ± 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, length of enrollment at school, weight, and height. 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%) than in Belém (17%; $P < 0.01$) Table 2.

Table 1
Participant characteristics

Parameters	Macapá (n = 41)	Belém (n = 88)
Sex*	22 (53.7)	42 (47.7)
Age (y)†	4.7 ± 0.9 (3.1–6.3)	4.5 ± 1.2 (2.1–6.6)
Enrollment period at school (mo)†	20.5 ± 11.6 (7–36)	20.8 ± 10.5 (7–36)
Weight (kg)†	16.8 ± 2.6 (12.4–25.3)	15.7 ± 2.9 (11.6–28.6)
Height (cm)†	103.4 ± 6.3 (94.–115)	101.6 ± 8.7 (85–125.5)

* N (%) (male).
† Mean ± SD (min–max) (all such values).

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

Nutrients	Nutrients Macapá	Belém
Energy (kcal/d)	1124.8 (994.52–1.265.56)	1081.5 (925.12–1309.52)
Protein (g/d)	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)	36.9 (24.1–49)
Selenium (μg/d)	155.30 (98.70–195.3)	44.40 (33.90–53.20)

* All data are given as median (min–max).

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 lower caloric intake than the recommended dietary allowance of 1300 to 1800 kcal/d. 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/d of protein 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 μg/d for 1–3 y; 23 μg/d for 4–8 y). In Macapá, this excess was more prevalent on days when Brazil nuts were added to the meals, reaching a peak of 279.3 μg/d (Fig. 1), whereas 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 for inadequate Se intake in either group, and Macapá children presented a high risk for toxicity.

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 participants from both groups showed plasma and erythrocyte values above the most accepted serum/plasma Se cutoff (>84–100 μg/L) [1].

Discussion

To investigate the effect of Brazil nut–enriched diets on Se status in children residing in the Brazilian Amazon, we compared dietary Se intake 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 malnutrition 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%) is markedly above that reported by the Household Budget Survey in 2008 to 2009, when 6.8% of children in Brazil were reported to have growth retardation [28].

Table 2
Anthropometric status of 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)	15 (17)	7 (8)	0 (0)
z –2 to +2	24 (58.5)	32 (78)	39 (95.1)	73 (83)	80 (90.9)	88 (100)
z >2	0 (0.0)	2 (4.9)	2 (4.9)	0 (0)	1 (1.1)	0 (0)

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

It is known that the family environment, feeding patterns, socioeconomic status, and sanitization 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 ≥90% of their total food consumption. In some cases, children did not have access to food on weekends or during 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 recommended dietary allowance, which might contribute to the high proportion of stunting in both 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. 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, was previously reported [31]. 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 the meals given to Macapá children, Se levels were

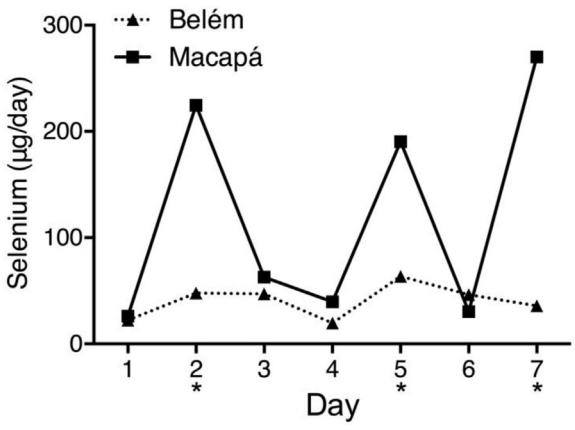

Fig. 1. Selenium concentration (μg/d) in diets of preschool children. *Brazil nut–enriched meals.

Table 4
Selenium parameters of children from Macapá and Belém*

Parameters	Macapá (n = 41)	Belém (n = 41)
Plasma (μg/L)	107.29 ± 27.15 (73.00–172.00)	83.56 ± 23.32† (47.00–142.00)
Erythrocyte (μg/L)	133.24 ± 32.24 (78.00–195.00)	94.74 ± 18.60† (67.00–150.00)
Urine (μg/mL)	0.27 ± 0.12 (0.11–0.47)	0.04 ± 0.01† (0.02–0.10)
Hair (μg/g)	0.89 ± 0.24 (0.44–1.35)	0.31 ± 0.10† (0.12–0.50)
Nails (μg/g)	3.43 ± 1.81 (0.89–8.43)	1.29 ± 0.52† (0.31–2.16)

* All data are given as mean ± SD (min–max).
† Significantly different from Macapá. $P < 0.001$ (Student's *t* test).

similar to children from Belém, but the inclusion of the nuts made Se content peak at 279.3 μg/d, resulting in a high risk for toxicity in comparison to UL (90 μg/d for 1–3 y; 150 μg/d for 4–8 y).

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.

Previous findings suggest that blood Se concentration (singular, not plural) ranging from 84 to 100 μg/L is necessary to maximize the activity of the selenoenzyme glutathione peroxidase (GPx) [1]. 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 with 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 metabolized to Se-containing carbohydrates, the main Se species in urine [36,38]. Comparing the urine Se levels of both groups with those previously proposed (EAR = 0.01 μg/mL; UL toxicity = 0.11 μg/mL) [37], we observed 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. Although there are no recognized standard references for these biomarkers, we found that children from Macapá had higher levels than the Belém 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, GPx-3 is an antioxidant protein with activity directly related to dietary Se intake [39]. Although we did not measure GPx3 in these children, it can be assumed that this activity would be elevated. Such a study would be of interest in the future.

Our results demonstrated 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 increased antioxidant and anti-inflammatory 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 in children. The main symptoms of selenosis are changes to and loss of nails and hair, skin lesions, unusual garlic odor on the breath, nervous system defects (difficulty in identifying an object by the sense of touch; tingling in hands, feet, and/or mouth; tiredness in legs and/or arms; pain in legs and/or 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 either group, 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 non-specific 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 intake of children from two different cities localized in the Brazilian Amazon region was adequate; however, the inclusion of Brazil nuts in the school meals in Macapá resulted in excess Se dietary intake and elevated Se levels in these children. Although 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 for adverse effects. Although some studies have 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.

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Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.nut.2015.05.005

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