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

Irland B.G. Martens Ph.D. [a], Barbara R. Cardoso Ph.D. [b,c,*], Dominic J. Hare Ph.D. [c,d,e], Megan M. Niedzwiecki Ph.D. [e], Franco M. Lajolo Ph.D. [b], Andreas Martens Ph.D. [f], Silvia M.F. Cozzolino Ph.D. [b]

[a] Department of Nutrition, Federal University of Pará, Belém, Brazil
[b] Department of Food and Experimental Nutrition, University of São Paulo, São Paulo, Brazil
[c] The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, Victoria, Australia
[d] Elemental Bio-imaging Facility, University of Technology Sydney, Broadway, New South Wales, Australia
[e] Exposure Biology, Lautenberg Environmental Health Sciences Laboratory, Department of Preventive Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[f] Institute of Inorganic and Analytical Chemistry, Technical University of Braunschweig, Braunschweig, Germany

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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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* Corresponding author. Tel.: +55 11 30913625; fax: +55 11 3091-3657.
E-mail address: barbara.rita@florey.edu.au (B. R. Cardoso).

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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.