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# Selenium status and hair mercury levels in riverine children from Rondônia, Amazonia

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*Objective:* Riverine communities in Rondônia State are exposed to high selenium (Se) content in their diet because of the high-selenium soils identified in the Amazon. However, the Amazonian population has a high mercury (Hg) exposure because this metal accumulates in the soil. Because children are more vulnerable to Hg toxicity, the aim of this study was to evaluate and correlate Se status and hair Hg levels in riverine children (aged 3–9 y) living in two different locations in Rondônia State: Demarcação area (DA) and Gleba do Rio Preto (GRP).

*Methods:* Se levels were assessed using hydride generation quartz tube atomic absorption spectroscopy; total hair Hg levels were assessed using cold vapor atomic absorption spectrometry. Dietary intake was evaluated through a 24-h food record and a food frequency questionnaire.

*Results:* Forty-two children participated in this study. Eighty-four percent of the children from DA showed low plasma Se. Conversely, all children from GRP presented plasma Se levels above the reference values. Forty-five percent of the children from DA presented low erythrocyte levels, and 55% of the children from GRP showed concentration in erythrocyte above the reference values. The mean Se intake was 41.8 μg/d in DA and 179.0 μg/d in GRP. High hair Hg levels were observed in children from both the DA and GRP (3.57 ± 1.86 and 6.24 ± 5.89, respectively).

*Conclusions:* Children from both riverine communities are likely to present altered Se status according to their dietary intake. Additionally, these children are highly exposed to Hg, mainly through fish consumption, and the toxicity of this metal may cause metabolic damage.


## Introduction

Selenium (Se) is an essential nutrient in the diet because it is necessary to make the selenocysteine found in some selenoproteins. Several physiological functions are attributed to Se, such as antioxidant properties, immune system potentiation, and heavy metal detoxification [1–3]. It is believed that Se is able to delay mercury (Hg) intoxication symptoms by forming an inert complex with Hg [4].

The northern and northeastern regions of Brazil are considered to be the most selenium-rich in the country [5,6], and they are among the leading producers of the richest Se food source, the Brazil nut *(Bertholletia excelsa, HBK)* [7,8]. Studies show that the Se status of riverine populations ranges from normal to very high and is directly related to the consumption of large amounts of Brazil nuts [9,10].

However, Amazonian populations also have the highest reported Hg exposure in the world. This metal is accumulated and

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trapped in the soils along the geological foundation of the basin, and it also comes from exogenous sources related to gold mining or industrial uses [11,12]. In this way, Amazonian people who consume local fish as their main dietary protein source may be seriously threatened by Hg contamination [13]. Several studies have shown that Se may protect against the toxic effects of Hg, as the interactions between Hg and Se are normally antagonistic [14,15].

The levels of Se and Hg in children are of particular interest because an adequate intake of Se is important for the proper development and functioning of the body throughout childhood and because children have greater vulnerability to Hg, which has important toxic effects on developing systems (particularly the cardiovascular, neurodevelopmental, and immune systems) that may persist throughout later life [16].

Given the expected variation in Se intake and Hg exposition between the two populations, we aimed to characterize the Se status in children who live in two riverine communities in Rondônia State, Brazil and to correlate this data with Hg status.

## Material and methods

This study included children living in the Demarcação area (DA) and in Gleba do Rio Preto (GRP). DA is located within the municipality of Porto Velho on the right border of Machado River, approximately 30 km upstream of its confluence with the Madeira River, geographically located at S 8° 10 ’16:20′ W 62° 46 ’45.30′, 140 km from the city of Porto Velho, Rondônia State. GRP is formed by approximately 20 families of approximately 100 people who do not form a housing project because they are spread throughout an approximately 30-km space along the Preto River. The targeted communities were selected to represent different characteristics of lifestyle. DA is closer to the most developed community in the region and has easy access to products coming from outside the community. GRP presents a typical subsistence lifestyle because it is isolated from other communities, which limits the access to industrialized foods.

All of the children between the ages of 3 and 9 y who were living in these communities and were followed in our hospital boat between December 2006 and March 2007 were included in the study. We excluded children who were receiving or had received vitamin and mineral supplementation and those who presented acute inflammation, infection, fever, diarrhea, cancer, diabetes, or autoimmune disease.

Blood and hair samples and a 24-h food record were collected, and anthropometric evaluations were conducted. These assessments were performed in an outpatient clinic on a hospital boat.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving humans/patients were approved by the 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 weighing scale to the nearest 0.1 kg, while height was measured with a portable stadiometer Sanny (São Paulo, SP, Brazil) to the nearest 0.1 cm.

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). The software Anthro v3.2.2 (Geneva, Switzerland) and AnthroPlus v1.0.4 (Geneva, Switzerland) were used to determine $z$ scores. Cutoff values for wasting, stunting, and thinness were $-2$ SD; cutoff values for overweight and obesity were 2 SD.

### *Dietary intake*

Se intake was evaluated using 24-h dietary recall. The dietary recall interview was conducted on the day of blood collection, and it collected detailed information about the entire dietary intake on the previous day. The dietary intake was also assessed using a food frequency questionnaire (FFQ). Because fish, peach palm (*Bactris gasipaes HBK*) and Brazil nuts (*Bertholletia excelsa*) were the main foods consumed in the regions of study, we analyzed the Se concentrations in these foods using hydride generation quartz tube atomic absorption spectroscopy (HGQTAAS) [17]. Additionally, the fish were analyzed in relation to Hg concentration using cold vapor atomic absorption spectrometry (CV AAS) [18,19].

**Table 1**
The characteristics of the participants

| Parameters | Demarcação area (n = 31) | Gleba do Rio Preto (n = 11) |
|---|---|---|
| Age (y) | 5.5 ± 1.6 | 6.0 ± 2.1 |
| Weight (kg) | 21.0 ± 5.4 | 19.9 ± 5.4 |
| Height (m) | 1.14 ± 0.1 | 1.13 ± 0.1 |
| Male (%) | 55 | 70 |
| Female (%) | 45 | 30 |

All data are given as mean ± SD

To evaluate the micronutrients in the fish, they were fried with soy oil according to the manner in which they are prepared and consumed in the studied communities.

The 24-h recall data were analyzed using the software NutWin (Escola Paulista de Medicina/UNIFESP/Brazil), which was supplemented with the data obtained by analysis.

### *Biochemical assays*

Fasting morning blood samples were collected by venipuncture in EDTA evacuated tubes to determine the Se concentration in plasma and erythrocytes. Plasma was separated by centrifugation at 3000*g* for 15 min at 4°C. The erythrocyte pellet that was obtained from the whole blood by centrifugation was washed three times with 5 mL of sterile 9 g/L NaCl solution, slowly homogenized by inversion and centrifuged at 10 000*g* for 10 min at 4°C, and the supernatant was discarded. Se determination in biologic material was performed using HGQTAAS [17]. The method reproductivity was achieved by analyzing the samples in triplicate (technical replicates to average out the technical variation) and performing readings in triplicate (nine readings per person), and *SERONORM (SERO*®)-certified material was adopted as a reference to serve as a control for the methodology. All reagents received analytical grade or higher purity from Merck. Nanopure water was used to prepare all of the solutions and to dilute the samples.

Hair Hg level was determined in a sample of 10 children from each locality. In these children, a hair sample was cut from the back of the head (occipital area) close to the scalp. The hairs of each sample were bundled together and placed in a labeled envelope. The total Hg level was determined using CV AAS [18,19]. The methodology validation for total hair Hg was performed by analyzing reference material with a certified value (*Human Hair*–IAEA 085).

### *Statistical analysis*

A descriptive analysis was performed, and the results are shown as the mean ± SD for continuous variables. The Shapiro-Wilk W test was performed to verify data normality. When normal distribution was present, data from both communities were compared using the unpaired Student’s $t$ test; the Mann-Whitney $U$ test was used when the data were skewed.

Se intake was adjusted for energy to describe the relationship between aspects of food consumption and biochemical characteristics independent of energy intake. This procedure was performed according to the residual method [20].

Analyses were performed using the statistical software package GraphPad Prism Version 5.0. The level of significance was established at $P < 0.05$ for all tests.

## Results

All 34 children living in the DA and all 15 living in GRP met the inclusion criteria; however, the parents of 3 children from the DA

**Table 2**
Nutritional status of children from the Demarcação area and Gleba do Rio Preto according to $z$ score

| $z$ score | Demarcação (n = 31) | | | Gleba do Rio Preto (n = 11) | | |
|---|---|---|---|---|---|---|
| | WA | WH | HA | WA | WH | HA |
| $z < -2$ | 3 | 0 | 2 | 0 | 0 | 0 |
| $z$ −2 a +2 | 27 | 27 | 29 | 10 | 10 | 10 |
| $z > 2$ | 1 | 4 | 0 | 1 | 1 | 1 |

HA, height-for-age; WA, weight-for-age; WH, weight-for height