Accepted Manuscript

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PII: S0899-9007(14)00147-6

DOI: 10.1016/j.nut.2014.03.013

Reference: NUT 9260

To appear in: *Nutrition*

Received Date: 26 November 2013

Revised Date: 28 February 2014

Accepted Date: 17 March 2014

Please cite this article as: Rocha AV, Cardoso BR, Cominetti C, Bueno RB, de Bortoli MC, Farias LA, Favaro DIT, Camargo LMA, Cozzolino SMF, Selenium Status and Hair Mercury Levels in riverine children from Rondonia - Amazonia, *Nutrition* (2014), doi: 10.1016/j.nut.2014.03.013.

Selenium Status and Hair Mercury Levels in riverine children from Rondonia - Amazonia

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Running head: Selenium and mercury status in riverine children

Word count: 4809

Number of tables: 5

Number of figures: 1

Statement of Author´s Contributions to Manuscript

AVR, LMAC and SMFC: designed research; AVR and RBB: conducted research; AVR, BRC, RBB, LAF, MCB, CC and SMFC: analyzed data; AVR, BRC and CC: wrote the manuscript; DITF: provided essential materials. All authors read and approved the final manuscript.

This study was supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), process number 478551/2008-8.

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Abstract

Objective: Riverine communities in Rondônia State are exposed to high selenium (Se) content in the diet because of the seleniferous soils identified in 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, we aimed to evaluate and to correlate Se status and hair Hg levels in riverine children (aged 3 to 9 years) living in two different locations in Rondônia State: Demarcação (DA) and Gleba do Rio Preto (GRP).

Methods: Se levels were assessed using hydride generation quartz tube atomic absorption spectroscopy (HGQTAAS); total hair Hg levels were assessed using cold vapor atomic absorption spectrometry (CV AAS). Dietary intake was evaluated through a 24-hour 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/day in DA and 179.0 μg/day in GRP. High hair Hg levels were observed in children from both localities (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.

Key-words: Brazil; Children; Mercury; Nutritional Status; Selenium.

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 Hg-intoxication symptoms by forming an inert complex with Hg (4).

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

However, Amazonian populations also have the highest reported mercury (Hg) exposure in the world. This metal is accumulated and 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 of great importance for the proper development and functioning of the body throughout childhood and because children have a 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 selenium intake and mercury 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 3 and 9 years old 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-hour 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 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 weighing scale to the nearest 0.1 kg, while height was measured with a portable stadiometer Sanny (São Paulo, SP, Brasil) to the nearest 0.1 cm.

Anthropometric status was classified according to WHO growth standards for weight-for-age, height-for-age and weight-for-height. The software Anthro v3.2.2 and AnthroPlus v1.0.4 were used to determine *z*-scores. The cut-off values for wasting, stunting and thinness were −2 SD; the cut-off values for overweight and obesity were 2 SD.

Dietary intake

The Se intake was evaluated using 24-hour 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 the Food Frequency Questionnaire. Because fish, peach palm (*Bactris gasipaes H. B. K.*) and Brazil nuts (*Bertholletia excelsa*) were the main foods consumed in the regions of study, we have analyzed the Se concentrations in these foods using hydride generation quartz tube atomic absorption spectroscopy (HGQTAAS) (17). Additionally, the fishes were analyzed in relation to Hg concentration using cold vapor atomic absorption spectrometry (CV AAS) (18,19). To evaluate the micronutrients in the fishes, they were fried with soy oil according to the manner in which these fish are prepared and consumed in the studied communities.

The 24-hour 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 x 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 10000 *x* g for 10 minutes at 4°C, and the supernatant was discarded. Se determination in biological 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 (a total of 9 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 a with certified value (*Human Hair* – IAEA 085).

Statistical analysis

A descriptive analysis was performed, and the results are shown as the mean ± standard deviation (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 thirty four of the children living in the DA and all fifteen living in GRP met the inclusion criteria; however, the parents of three children from the DA and four from GRP did not allow them to participate in the study. Nonetheless, the scope of the study was significant, because 91% of the children from the DA and 73% from GRP were included. The mean age of the participants from the DA was 5.5 ± 1.6, and the mean age of those from GRP was 6.0 ± 2.1 years; there was no significant difference between the communities (Table 1).

No differences regarding weight or height between communities were observed (Table 1). Our data showed that regarding weight-for-age (WA), height-for-age (HA) and weight-for-height (WH), most of the children were eutrophic. WH reflects body weight in proportion to attained growth in height, and in our study we observed that although most of children were eutrophic, 12% had a WH ≥2 *z*-score, which indicated overweight. The Food Frequency Questionnaire and 24-hour dietary recall showed that children from both communities experience food monotony, and the main foods they consumed were fish, rice, manioc flour, coffee and sugar. Children from the DA reported higher consumption of sweet popcorn, snacks, chewing gum, biscuits, artificial juices and soft drinks. We also observed that 36% of the children from GRP ate Brazil nuts weekly, while 41% of children from the DA used to eat Brazil nuts rarely. Together with fish, Brazil nuts are a good source of Se, while the peach palm has a low concentration of this mineral (Table 3).

Regarding Se intake, the children from GRP showed a higher Se intake compared with those from the DA (Table 4). In general, most of the children from both areas showed Se intakes varying between the recommended dietary allowance (RDA) (20 µg/d for 1-3 years; 30 µg/d for 4-8 years; 40 µg/d for 9-13 years) and the tolerable upper intake levels (UL) (90 µg/d for 1-3 years; 150 µg/d for 4-8 years; 280 µg/d for 9-13 years) (21). Twenty-one percent of the children from the DA had Se intake lower than the estimated average requirement (EAR) and 18% of the children from GRP showed an intake higher than the UL.

Table 4 illustrates the Se levels (µg/L) in the plasma and erythrocytes of riverine children from the DA and GRP. All of the children from GRP presented high plasma Se levels that surpassed reference values (>84–100 µg/L) (22). The children from the DA presented mean plasma Se levels within the reference range; however, 84% of them showed deficient levels. Regarding to erythrocyte Se level, 55% of children from DA presented adequate concentration and 45% were deficient, while 55% of children from GRP showed concentration that exceeded reference values (90–190 µg/L) and no one presented deficient level (1).

Table 5 shows the Hg levels in the main consumed fishes and in the hair of the children from the DA and GRP. Children living in GRP showed significantly higher Hg levels compared with participants from the DA. Only one child from the DA (10%) and three from GRP (27%) showed Hg values below 2 mg/kg, which is the reference value (23). The remaining children presented high hair Hg levels, with the highest concentrations found in the children living in GRP (6.2±5.9 mg/kg) compared with the DA participants (3.6±1.9 mg/kg). There was a positive correlation between erythrocyte Se levels and hair Hg levels in the children from GRP and no other significant correlations were observed (Figure 1).

Discussion

This study is the first to analyze Se nutritional status and Hg levels in riverine children in this specific Amazonian region. We showed that these children face a threatening nutritional risk from either deficiency or excess of Se and from high Hg levels.

The Food Frequency Questionnaire and 24-hour dietary recall revealed that children from both communities consume a monotonous diet containing few vegetables and fruits, as observed by Araújo (24). On the other hand, the children from the DA consumed more processed foods, a finding that can be explained by the fact that DA is closer to Calama, the most developed community in the region, which gives the DA easy access to beverages and processed foods. The presence of processed foods on the diet of children from DA may be responsible for the presence of some overweight children, as shown by WH. In contrast, GRP is located far from other communities and has limited access to them. Although the Amazon has a huge diversity of fishes, fruits and vegetables, we have observed that this abundance does not define the nutritional reality of the people in these communities because they have no knowledge about the importance of a healthy diet (25,26).

The assessment of Se intake from the diet presents many difficulties, namely the absence of specific Brazilian food composition tables (27,28). This is the main reason we have analyzed the Se concentration in the main foods consumed in these communities, including fishes, Brazil nuts and the peach palm. These foods were the main sources of Se for these communities, particularly for the GRP, because the children living in this community had a high intake of Brazil nuts, the main food source of this mineral (29). Limited data have been published about Se intake in groups of individuals from different Brazilian states. However, Farias (18) observed that the daily Se intake of riverine children living in Amazonia varied from 8.9 to 54 µg/day, and only 42% of the diets provided satisfactory Se amounts. Although our data are not sufficient to allow a more accurate interpretation, they suggest a particularly inadequate intake of Se from the diet, with a significant risk of deficiency for children from the DA and a significant toxicity risk for children from GRP.

The fact that children from GRP used to consume more natural foods can explain the higher levels of Se in their plasma and erythrocytes compared with DA children. Some authors suggest that to assess nutritional Se status, is important to evaluate at least two biomarkers. In our case, plasma was used as a marker of current exposure, while erythrocytes reflect longer-term nutritional status because of the incorporation of Se in erythrocyte synthesis (22,30).

Malnutrition resulting from the lack of specific micronutrients does not result in short-term clinical symptoms because it is not strictly related to poverty and deficient caloric intake. Micronutrient deficiency is not associated with visible health impairments; however, it can lead to long-term consequences, increasing the risks of developing severe disease and exerting harmful effects on the quality of life (31). This observation can be applied to the children from the DA, who presented adequate levels of growth and development based on anthropometric data despite presenting some level of Se deficiency. The children from GRP also presented adequate levels of growth and development; however, we encourage the monitoring of Se levels in this population to avoid possible risks of adverse effects. It is important to mention that Se toxicity does not usually cause dermal signs or sentinel symptoms, but it is related to cardio metabolic perturbations (insulin resistance, hyperglycemia, hypertension, hypercholesterolemia and cardiovascular diseases) (32).

Blood Hg levels reflect Hg exposure in both organic and inorganic forms, which can be biased by Hg-contaminated food intake. Hg remains in the blood stream for only a few days after exposure. Therefore, analyses must be performed quickly to detect recent exposure. In contrast, hair Hg levels are proportional to blood Hg concentrations. Because this metal is incorporated into the hair, its level is not modified (23). Consequently, hair analysis reflects sustained exposure to Hg, which enables the assessment of Hg levels over longer periods of time (33). A limitation of this study was that we did not measure Hg in all children and, in our case, recruitment was based on a convenience sampling procedure.

One explanation for the high Hg hair levels found in our study could be the frequent fish consumption, most notably by the children from GRP. However, despite the high fish consumption by the population from Manaus/AM, researchers found children in this area to present hair Hg levels below the recommended values (2.0 mg/kg) (23). Hair Hg and methylmercury (MeHg) levels were lower than 10 mg/kg in all evaluated districts, which indicated a low risk of Hg adverse effects on child development (18,33). Children from Jaú National Park (Amazon Region) had values ranging from 0.60 to 42.2 mg/kg (18). Likewise, children from two communities in Pará state (Brasília Legal and São Luiz do Tapajós) had high Hg hair levels. In the first community, the mean levels were 5.84±4.91 mg/kg; in the second one, the mean Hg hair levels were even higher (21.06±14.38 mg/kg) (28). Children <15 years old from Rio Negro in Amazonia also showed high mean hair Hg levels (18.52±10.04 mg/kg) (34).

Researchers have been trying to find an explanation for the absence of evident clinical symptoms in Amazonian populations who present high Hg levels. The presence of natural Se in the region has been considered a possible contributor to the apparent tolerance for chronic Hg intoxication (35-38). Some studies suggested that an adequate Se intake might provide effective protection against Hg and its toxic compounds (39). It is believed that when Se is combined with other metals, it can produce inert compounds (40,41). The interaction between Se and Hg forms the selenite-dimethylmercury (SDM) complex, which is unstable in blood and in other tissues (42). The protective effect of Se has been associated with higher Hg retention rather than higher Hg excretion (43). In comparison, in vitro and in vivo studies have shown that Hg compounds inhibit the activity of some selenoenzymes, such as thioredoxin reductases and glutathione peroxidases. These enzymes have an essential antioxidant role, and decreases in their activity are related to metabolic diseases (44-46).

Although there were some limitations, such as the small sample size and the collection of only one 24-hour diet record, this research allowed us to conclude that our results are considerably relevant. Certainly, studies including larger populations are necessary to confirm our results.

In summary, we can conclude that the children from both of the studied riverine communities are likely to present an altered Se status, represented either by a deficiency or excess of blood Se levels and dietary Se intake. Environmental factors, such as Hg contamination, may influence Se levels and present serious health risks to this population. It is urgent that public policies be adopted to improve the living conditions of these populations, which also includes dietary interventions to improve the nutritional Se status.

FIGURE 1. Correlations between selenium levels in plasma and erythrocyte and mercury hair concentration in children from DA and GRP. Se: selenium; Hg: mercury; A and C: Children from DA; B and D: Children from GRP.

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TABLE 1. The characteristics of the participants.

Parameters	**Demarcação area** (n=31)	**Gleba do Rio Preto** (n=11)
Age (years)	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
Gender		
Male (%)	55	70
Female (%)	45	30

All data are given as mean±SD

TABLE 2. Nutritional status of the children from DA and GRP according to z-score.

z-score	**Demarcação** (n=31)			**Gleba do Rio Preto** (n=11)		
	W/A	**W/H**	**H/A**	**W/A**	**W/H**	**H/A**
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

TABLE 3. Centesimal composition and Se content (µg/g) of food samples collected in the DA and GRP.

Foods	Energy (kcal/100g)	Protein (g/100g)	Lipid (g/100g)	Carbohydrate (g/100g)	Se (µg/g)
Brazil nuts	692.49	15.94	65.33	10.19	5.83
Peach palm	302.10	19.10	69.00	214.10	0.03
Fishes					
Pacu *(Piaractus brachypomus)**	490.10	36.80	38.10	0.00	0.31
Traíra *(Hoplias malabaricus)**	449.90	33.50	35.10	0.00	0.62
Jatuarana *(Bricon cephalus)***	504.40	38.80	38.80	0.00	0.12
Cachorro *(Hydrolycus scomberoídes)***	478.40	35.90	37.20	0.00	0.83
Piau *(Leporinus steindachneri)***	435.00	32.70	33.80	0.00	0.48
Pacu *(Piaractus brachypomus)***	493.30	42.10	36.10	0.00	0.65

* Machado River, **Preto River

TABLE 4. The biochemical parameters and intake of Se among the participants.

Parameters	**Demarcação** (n=31) mean±SD (min-max)	**Gleba do Rio Preto** (n=11) mean±SD (min-max)
Plasma Se (µg/L)	41.9±18.7 (16.1-84.4)	189.1±58.7* (96.3-278.5)
Erythrocyte Se (µg/L)	97.6±26.3 (58.8-165.4)	235.0±105.6** (117.4-474.4)
Se dietary intake (µg/day)	41.8±33.4 (0.0-146.0)	179.0±207.0** (27.5-767.0)

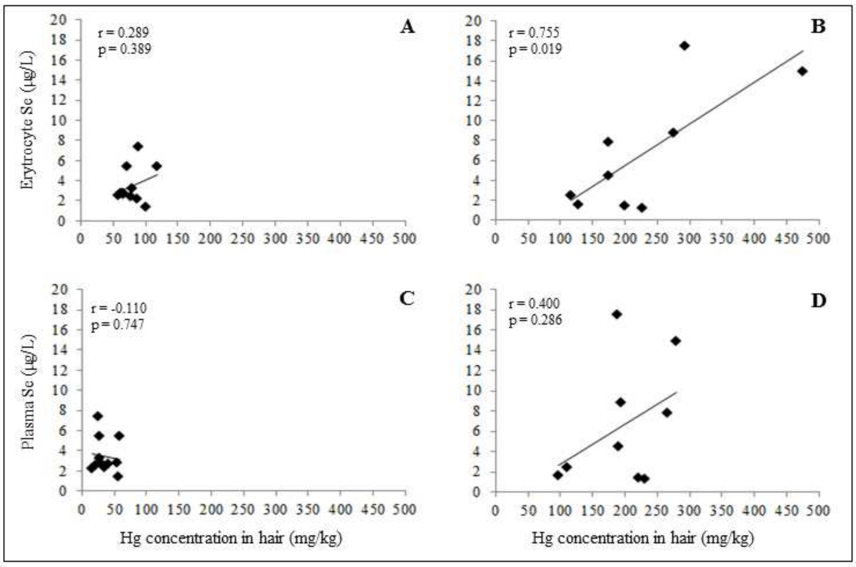
All data are given as mean±SD

* Significantly different from DA, P < 0.05 (Student's t-test).

** Significantly different from DA, P < 0.05 (Mann-Whitney U test).

TABLE 5. Hg concentration in fishes and in children from the DA and GRP.

	Hg concentration (mg/kg)
Demarcação – Machado River	
Pacu *(Piaractus brachypomus)*	0.02
Traíra (*Hoplias malabaricus)*	2.61
Children´s hair (n=10)	3.57±1.86
Gleba do Rio Preto – Preto River	
Jatuarana *(Bricon cephalus)*	1.73
Cachorro *(Hydrolycus scomberoídes)*	0.42
Piau *(Leporinus steindachneri)*	0.11
Pacu *(Piaractus brachypomus)*	0.03
Children´s hair (n=10)	6.24 ± 5.89


Erytrocyte Se (μg/L)
Plasma Se (μg/L)
Hg concentration in hair (mg/kg)
A
r = 0.289
p = 0.389
B
r = 0.755
p = 0.019
C
r = -0.110
p = 0.747
D
r = 0.400
p = 0.286
0 2 4 6 8 10 12 14 16 18 20
0 50 100 150 200 250 300 350 400 450 500