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Biomarkers of selenium status in the amazonian context: Blood, urine and sequential hair segments

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Selenium (Se) is an essential element and deficit or excess of dietary Se is associated with health disorders. Relatively elevated Se levels have been reported in the Brazilian Amazon, where there are also important annual variations in the availability of different foods. The present study was conducted among six riparian communities of the Tapajós River to evaluate seasonal variations in blood and sequential hair cm Se concentrations, and to examine the relationships between Se in blood and hair, and blood and urine. Two cross-sectional studies were conducted, at the descending water (DWS, $n = 259$) and the rising water (RWS, $n = 137$) seasons, with repeated measures for a subgroup ($n = 112$). Blood Se (B-Se), hair Se (H-Se) and urine Se (U-Se) were determined. Match-paired analyses were used for seasonal comparisons and the method of best fit was used to describe the relationships between biomarkers. B-Se levels presented a very large range (142–2447 μg/l) with no overall seasonal variation (median 284 and 292 μg/l, respectively). Sequential analysis of 13 cm hair strands showed significant variations over time: Se concentrations at the DWS were significantly lower compared with the rising water season (medians: 0.7 and 0.9 μg/g; ranges: 0.2–4.3 μg/g and 0.2–5.4 μg/g, respectively). At both seasons, the relationships between B-Se and H-Se were linear and highly significant ($r^2 = 67.9$ and 63.6, respectively), while the relationship between B-Se and U-Se was best described by a sigmoid curve. Gender, age, education and smoking did not influence Se status or biomarker relationships. Variations in H-Se suggest that there may be seasonal availability of Se sources in local food. For populations presenting a large range and/or elevated Se exposure, sequential analyses of H-Se may provide a good reflection of variations in Se status.
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Introduction

Selenium (Se) is an essential element, hence deficit and excess in Se status are associated with health disorders (Rayman, 2000). Se is present in a number of foods, and the Se content in the food chain is highly dependent on local soil Se levels, which vary all over the world (WHO, 1986). The most important sources are fish and marine mammals, organ meat, eggs, meat, mushrooms and some comestible plants such as cereals, *Brassica sp*. and *Allium sp*. vegetables, and Brazil nuts (*Bertholletia excelsa* Humb. and Bonpl.) (reviewed by Dumont et al., 2006). However, the bioavailability of Se in a particular food varies with the digestibility of the Se-containing food proteins and the pattern of Se-amino acids, such as selenocysteine and selenomethionine (Combs, 2001). Thus, Se status in humans reflects soil composition (Fordyce et al., 2000), agricultural practices, preferences and availability of foods grown in the area, food imports and the bioavailability of Se forms in the diet (Combs, 2001).

Because of underlying geological variations, Se content of the same food item can have more than a 10-fold difference (Institute of Medicine, 2000). For example, in the Enshi District of China, Se-deficient and Se-toxic environments occur within 20 km of each other (Fordyce et al., 2000). Consequently, estimates of the Se exposure through dietary assessment may under- or overevaluate Se status for some populations. For this reason, epidemiological studies rely on biomarkers rather than dietary estimates (Mayne, 2003).

Plasma and serum are the favored biomarkers for comparison of Se status among countries (Thomson, 2004).

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However, most studies of Se status have focused on Se-deficient or Se-adequate populations. There are few reports on the validity of biomarkers in populations with high Se. Plasma or serum biomarkers may be inadequate for populations with high Se, as plasma Se tends to saturate at whole-blood Se levels between 300 and 900 μg/l, while erythrocytes continue to accumulate Se (Yang et al., 1989b; Hansen et al., 2004). Urinary Se may also constitute a relevant biomarker, as urine is the most important route for Se excretion (Robberecht and Deelsta, 1984) and reflects the organism's capacity to regulate and eliminate excess Se. Several authors have reported high correlations between Se in urine (U-Se) and Se intake, in whole-blood, plasma or serum (Valentine et al., 1978; Yang et al., 1989a; Alaejos and Romero, 1993; Longnecker et al., 1991, 1996).

Recent studies in the Brazilian Amazon show highly variable and relatively elevated whole-blood Se (B-Se) levels (Lemire et al., 2006), as well as hair Se (H-Se) levels in the upper normal range (Campos et al., 2002; Pinheiro et al., 2005). In this region, there are important annual variations in the availability of different foods (Passos et al., 2001), which may result in seasonal differences in Se intake. In China, Yang et al. (1989a) reported that significant seasonal fluctuations in measured Se intake were not detected in whole-blood Se levels, whereas hair Se biomarker was more sensitive and may act as an excretory organ at higher intakes. As hair grows approximately 1 cm a month (Robbins, 2002), segmental hair analysis can provide a retrospective profile over several months of exposure depending of the length of the hair (Cernichiari et al., 1995; Lebel et al., 1997; Dolbec et al., 2001).

The present study sought to better understand the variations in biomarkers of Se in a population with a wide range of Se levels. It was conducted in the Brazilian Amazon at two different seasons with a view to (i) evaluate seasonal variations of Se concentrations in whole-blood and hair and (ii) examine the relationships between Se concentrations in hair and urine with respect to blood.

Methods

Study Design

This study is part of a larger project that uses an integrated approach to examine factors modulating mercury transmission through aquatic ecosystems, human uptake and toxicity in the Lower Tapajós River Valley (CARUSO, 2007). To evaluate seasonal variations in biomarkers of exposure, the study took place over two different periods of time: (i) June and July 2003, during the descending water season (DWS) and (ii) January and February 2004, during the rising water season (RWS). A schema of the hydrologic cycle of the Tapajós River region is presented in Figure 1. Two cross-sectional studies were carried out and repeated measures were obtained for a subgroup of persons.

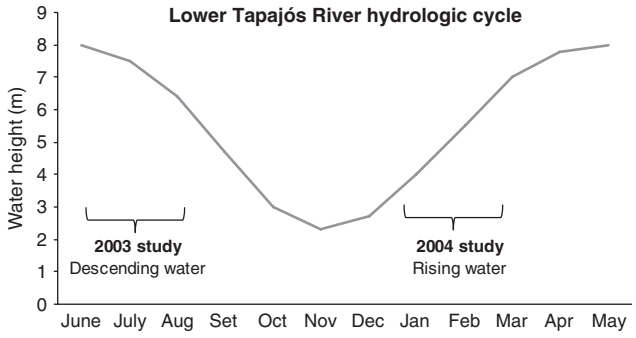

Figure 1. Schema of the annual Lower Tapajós River hydrologic cycle and the associated seasons.

Six riparian communities of the Tapajós River, one of the major tributaries of the Amazon (Figure 2) were targeted. The communities were selected so as to reflect the mosaic of the local ecosystems and diversity of riverside human populations. The valley of the Lower Tapajós comprises villages established on the riverbanks for more than a century, and immigrants, mostly from northeast states of Brazil, who began arriving in Amazon region in the early 1960s. The communities of São Luis do Tapajós (SLT) and Nova Canaã (NC) are located on the south of the municipality of Itaituba, on the east and west shores of the Tapajós River, respectively. The community of Santo Antônio (SA) is located on the shores of the Itapacurazinho River, a small tributary of the main stream. The communities of Vista Alegre (VA) and Mussum (MU) are neighbors and located on the west shore of the Tapajós River, close to the small municipality of Aveiro. The community of Açaituba (AC) is located on the south shore of the Cuparí River, another tributary of the Tapajós.

Recruitment

At DWS, a house-to-house socio-demographic survey was undertaken, during which the study was explained to each household and villagers were invited to participate. Meetings were also held in each community to further explain the study. Inclusion criteria for the present study were fish-eating, age $\geqslant$15 years, not pregnant or breastfeeding. In all, 259 persons (39% of the total adult population of these villages) accepted to participate. Reasons provided for non-participation included time constraints, lack of interest and religious beliefs.

Six months later, during RWS, the results were returned to the communities. Village meetings were held to explain the aggregate results and house-to-house visits served to provide the confidential individual results. Villagers were likewise invited to provide further samples and respond to a questionnaire. A total of 12% of the participants had moved away from their community, and because of flood, it was difficult to reach many of the houses. As RWS sampling was

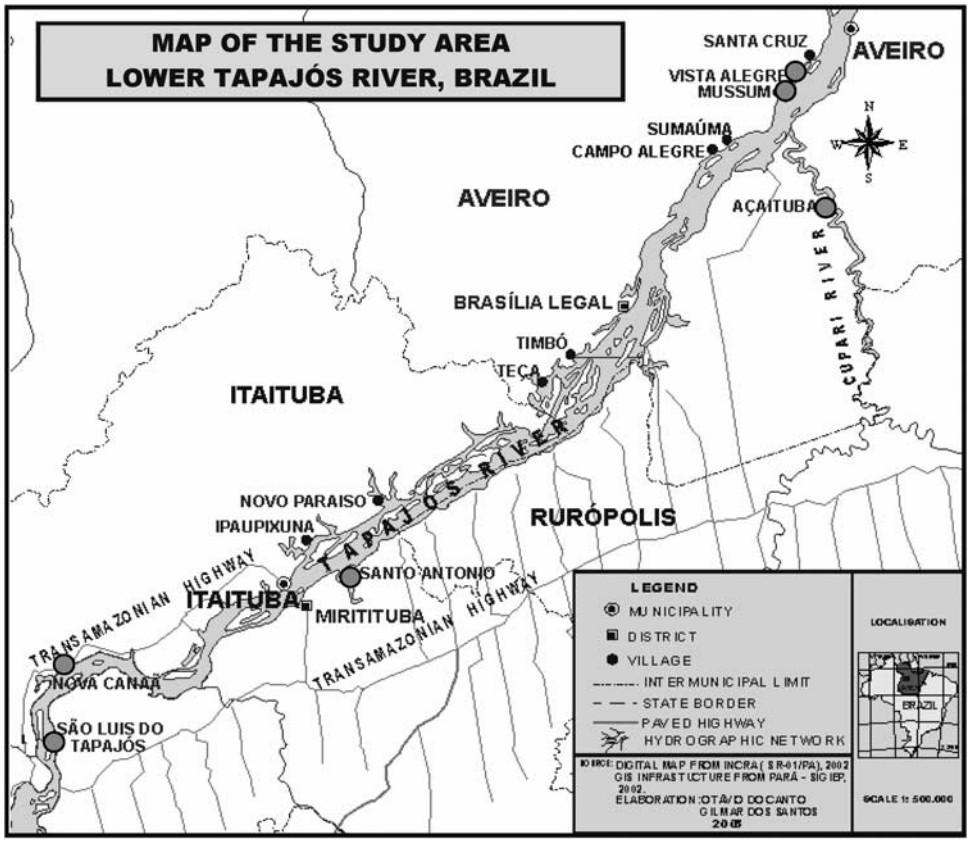

Figure 2. Map of the study area, the Lower Tapajós River Valley. Participating communities are identified by a large dot (●).

carried out during a period of high precipitation, we were only able to contact and re-sample 43% of the original participants. Twenty-five persons asked to be included, as they had not been available at DWS. At RWS, there were 137 participants, with 112 participating in both periods. The age and sex distribution of participants was similar to the underlying population (Lemire et al., 2006; Passos et al., 2007).

The study was approved by the Ethics Review Boards of the University of Quebec at Montreal (Canada) and the Federal University of Rio de Janeiro (Brazil). For both studies, all participants signed an informed consent form, which was read to them.

Socio-demographic Data

Socio-demographic characteristics including age, sex, smoking habits, alcohol consumption, years of education and subsistence practices (e.g. fishing, farming, etc.) were evaluated by an interview-administered questionnaire.

Sampling and Analyses of Biomarkers of Se Status

For each phase of the study, participants provided at least one of the biomarkers: (i) DWS: hair and blood and (ii) RWS: hair, blood and urine.

A nurse collected whole-blood samples by venipuncture into 6 ml heparinized Becton Dickinson Vacutainer® (BD7863). Spot-urine samples were collected in polypropylene bottles (Nalgene 125 ml, Cat. no. 2104-0004) and then transferred to screw cap tubes with conical base (RPK PPGWB 15 ml, SARSTEDT™) for transport purposes. All blood and urine samples were kept frozen at –20°C on the research boat and were later sent to the laboratory of the Quebec Toxicology Centre of the Quebec Public Health Institute (CTQ-INSPQ), Canada, for analysis of total Se. Collection materials were pre-screened for internal (plastic bottles and tubes) and external Se contamination. Whole-blood and urine samples were analyzed by inductively coupled plasma mass spectrometry (ICP–MS) on a Perkin-Elmer instrument (Elan 6000), according to the methods described by Baskett et al. (1994) and Labat et al. (2003), respectively. The detection limit for Se analysis was 7.9 μg/l in solution. Urine gravity was analyzed by refractometry (Cambridge Instruments Inc., Cat. no. 3461) to normalize samples for inter-individual dilutions caused by random micturition sampling. Spot-urine Se results were then adjusted for specific urine gravity (SG) to the overall average gravity of the study population of 1.018 g/ml (Spot-urine Se*(1.018−1)/(SG−1)). This method was preferred to creatinine adjustment,

as it is less sensitive to age, gender, body size and nutritional status (Miller et al., 2004; Suwazono et al., 2005). Fifteen individuals (12%) had urine gravity under 1.010 g/ml, which is considered low, but as no individual presented highly diluted urine samples ($\leqslant$1.001 g/ml), no one was excluded (Vahter et al., 2006). Analytical quality control was ensured by routine checks of accuracy and precision, using reference materials from CTQ-INSPQ Inter-Laboratory Comparison Programs and participation in the periodic evaluations of the same programs.

Se deficiency was set at B-Se $<44\,\mu$g/l and upper cut-offs were set at 500 μg/l, which corresponds to the tolerable upper intake level (Institute of Medicine, 2000) and at 1000 μg/l, which is the no observable adverse effect level (U.S. EPA, 2002), based on the study of Yang et al. (1989b). For urine, there are fewer guidelines, and the absence of symptoms of toxicity is usually associated with U-Se $<100\,\mu$g/l, while $>$400–700 μg/l are considered excessive (WHO, 2001). Here, we used $>400\,\mu$g/l as a high level and $>1200\,\mu$g/l as a very high level.

Hair strands from the occipital region were cut next to the scalp with stainless steel scissors and then placed in plastic bags, with the root end stapled. The samples were analyzed at the Geochemical Laboratories of the Earth and Planetary Sciences Department of McGill University (Canada), by hydride-generation atomic absorption spectrometry on a Perkin-Elmer instrument (Analyst 100, FIAS-400 flow injection system) using sodium borohydrate solution ($NaBH_4$ 0.2% w/v and NaOH 0.05% w/v) as the hydrogen source and HCl solution (HCl 10% v/v) as the carrier stream. Hair strands were placed on a stainless steel module, standardized for 1 cm length, and cut with a stainless steel scalpel. Mineralization and reduction of the samples was done using a technique adapted from Campos et al. (2002). From 5 to 23 mg of hair were weighed in a 20 ml beaker. Then, 0.5 ml of $HClO_4$, 0.5 ml of HNO_3, 0.5 ml of H_2SO_4 and 0.5 ml of high-purity water (Ω18 M) were added and heated at 90°C on a hotplate for 30 min with beaker covers. After cooling, the digested sample was made up to 10 ml in a volumetric flask with high-purity water (Ω18 M). A 2 ml volume of HCl and 1 ml of sulfamic acid (15% w/v) were added and left overnight. The sample was then carefully boiled for 10 min to reduce Se(VI) to Se(IV). All glassware were washed with neutral detergent (Micro, model 8790-00, Cole-Parmer), rinsed twice in bidistilled water, left in 10% HCl for 12 h, rinsed twice with bidistilled water and dried at 300°C. The detection limit for analysis was 0.2 μg/l in solution. Precision and accuracy of the analytical quality control of Se determination was ensured by the use of reference material (Human Hair 086) provided by the International Atomic Energy Agency (IAEA). The accuracy of the results was checked daily by running three replicates of the reference material and variation was observed in the recovery of the reference material. Overall, mean results ($\pm$SD) for the reference material triplicates was $0.70 \pm 0.13\,\mu$g/g ($n = 65$) of Se, whereas the reference material contained $1.00 \pm 0.20\,\mu$g/g. As there were significant differences between the daily sets of analyses (Wilcoxon Rank Sums test χ^2, $P = 0.04$), segmental cm hair Se results were corrected on the basis of the mean results of the IAEA three daily replicates. The normal range for hair Se (H-Se) concentration was considered to be between 0.1 and 5.0 μg/g (WHO, 1994).

Statistical Analyses

Descriptive statistical analyses were used to characterize the study population and biomarkers of Se status at the two seasons. Gender, smoking and alcohol consumption status, subsistence practices and villages were included as categorical variables.

Biomarker differences with respect to socio-demographic variables and community groups were tested by non-parametric Wilcoxon/Kruskall-Wallis tests (χ^2, $\alpha_{error} = 0.05$). Two-by-two comparisons were used to examine differences between multiple categories, such as communities. Student's match-paired t-test analyses were performed to test inter-seasonal differences for those who participated in both seasons. As most of the variables did not show a normal distribution, the correlations between biomarkers of Se were examined using non-parametric correlational statistics (Spearman's ρ).

For the simple and multiple regression models that were used to examine the relationships between Se biomarkers and factors influencing biomarkers' variability, logarithmic transformations ($\log_{10}$) were performed for variables with non-normally distributed residuals. The general linear model (GLM) univariate procedure provided linear regression analyses and Student's t-test analyses were used to obtain the regression parameters.

When the relationship between biomarkers was not linear, the best-fit for a non-linear regression model was estimated using the methods of least mean square and Student's t-test analyses for the parameters of the regression. We assessed threshold levels below and above which the increase of both biomarkers was no longer proportional (linear). For the relationship between B-Se and U-Se, three linear models seemed to be reasonable choices in the absence of information on toxicokinetic mechanisms (Wyzga, 1990). Thus, we used a subapplication of the change point problem in two-phase regression (χ^2 test of one degree of freedom), considering one phase as a constant line. In accordance with this model, the maximum-likelihood technique was used. This technique was previously described by Campagna et al. (1996). Results were defined as statistically significant for a P-value of $\leqslant 0.05$. Analyses were performed using JMP software, Sigma Plot and SPSS (version 5.0.1a, 6.00 and 8.02, respectively; SAS Institute Inc, Cary, NC, USA).

Results

Socio-demographic characteristics of the DWS study population are presented in Table 1. A large proportion (83.4%) was originally from the Tapajós region, and those who were not from the State of Pará were mostly immigrants from the northeastern states of Brazil, essentially from Maranhão (10.1%) and Ceará (2.0%). Socio-demographic data at RWS and the repeated measures subgroup had similar distributions.

The distribution of whole B-Se, Se in the first centimeter of hair (H_1-Se) and U-Se are presented in Table 2. For both seasons, no individuals showed B-Se or H_1-Se deficiency and both biomarkers presented a large inter-individual variation. At DWS, 24 participants (10.2%) had B-Se levels above 500 μg/l and 10 participants (4.2%) had B-Se levels over 1000 μg/l. No one had H_1-Se levels above 5 μg/g. At RWS, 15 participants (12.7%) presented B-Se levels above 500 μg/l and 5 persons (4.2%) above 1000 μg/l. Two participants (1.6%) had H_1-Se levels higher than 5 μg/g. For both seasons, all of those who presented the highest Se levels (B-Se levels >1000 μg/l and H_1-Se levels >5 μg/g) were part of an extended family from SA. Their mean H_1-Se and B-Se levels were significantly higher (Wilcoxon/Kruskall-Wallis test χ^2, $P<0.0001$) than those of the rest of the population (DWS B-Se: 1156 ± 617 μg/l *vs.* 305 ± 121 μg/l and H_1-Se: 3.1 ± 1.3 μg/g *vs.* 0.9 ± 0.4 μg/g; RWS phase B-Se: 1183 ± 466 μg/l *vs.* 313 ± 125 μg/l and H_1-Se: 2.6 ± 1.0 μg/g *vs.* 0.8 ± 0.3 μg/g). We thus considered the extended family separately from the others of this village: SA-1 and SA-2 (extended family).

Table 1. Socio-demographic characteristics of the study population.

	Descending water season (DWS) June and July 2003		
	X ± SD	*n*	%
Women		124	48.1
Men		135	51.9
Age (years)	35.7 ± 15.9 (15.0–89.0)		
Education (years)	3.6 ± 2.6 (0–11)		
Born in the region (state of Pará)		216	83.4
Current alcohol consumer		99	38.2
Current smoker		77	29.8
Fisher[a]		182	70.2
Farmer		159	61.4

[a]124 participants (47.9 %) were both fisher and farmer.

Table 2. Se biomarkers levels for both phases of the study.

	Biomarkers[a]		
	B-Se (μg/l)	H_1-Se (μg/g)	U-Se (μg/l)
Descending water season (DWS) June and July 2003			
X ± SD	361 ± 259	0.9 ± 0.5	
Median	284	0.8	
Range	142–2029	0.3–4.1	
n	236	235	
Rising water season (RWS) January and February 2004			
X ± SD	394 ± 340	1.1 ± 0.8	220 ± 355
Median	292	0.9	85
Range	142–2447	0.3–5.4	29–2579
n	118	127	125

[a]Whole-blood Se (B-Se), first hair cm Se (H_1-Se) and urine Se (U-Se) concentrations.

U-Se levels presented a larger interindividual variation, up to 100-fold, compared with B-Se and H_1-Se. Sixty-nine participants (55.2%) had U-Se levels <100 μg/l. However, 14 individuals (11.2%) had high U-Se levels, varying between 436 and 1184 μg/l, which included not only persons from SA-2 but also participants from SA-1 and AC. Two individuals had extremely high U-Se levels (1954 and 2579 μg/l, respectively) and came from the community of AC and MU.

Blood, hair and urine biomarkers did not vary with gender, age, years of education and smoking habits (Wilcoxon/Kruskall-Wallis test χ^2 and Spearman's ρ, $P>0.05$). At DWS, alcohol consumers had significantly higher B-Se (Wilcoxon/Kruskall-Wallis test χ^2, $P=0.01$), but no relationship was observed for H_1-Se. At RWS, no relationship was observed between alcohol intake and B-Se, H_1-Se or U-Se. For both seasons, farmers had consistently higher Se biomarker levels (Wilcoxon/Kruskall-Wallis test χ^2, $P<0.007$), whereas no difference was observed for fishing practices.

All Se biomarkers presented significant intercommunity differences (Wilcoxon/Kruskall-Wallis test χ^2, $P<0.0001$). Interestingly, except for SA-2, whose Se biomarkers remained consistently higher than other communities, two-by-two community comparisons showed that certain communities had higher Se levels compared with others. These differences were observed for all biomarkers (Wilcoxon/Kruskall-Wallis test χ^2, $P\leqslant 0.05$). At DWS, the communities AC, VA, MU and SA-1 had higher Se status than SLT, whose Se levels were lower but above NC. At RWS, the AC community had Se levels above VA, MU, SA-1 and NC, and SLT presented the lowest Se status (data not shown).

Interseasonal comparisons showed that, although total population H_1-Se levels were significantly higher at RWS (mean difference 0.15 μg/g, 95%CI [0.04 to 0.25]; Student's paired *t*-test, $P=0.004$), B-Se levels did not change significantly (mean difference 14.0 μg/l, 95%CI [−15.0 to 43.1]; Student's paired *t*-test, $P=0.341$) (Table 3). Within communities, B-Se and H_1-Se seasonal variations were in the same direction, but H_1-Se seasonal differences were more pronounced for most communities compared with those of B-Se. Mean hair to whole-blood ratio (μg/g *vs.* mg/l) were 2.67 ± 0.77 at the DWS and 2.88 ± 0.96 at RWS, and did not

differ between seasons (Student's paired t-test, $P = 0.216$). At RWS, mean U-Se to B-Se ratio (μg/l *vs.* μg/l) was 0.47 ± 0.57.

For 56 women, we were able to analyze a length of 13 cm of hair, cut into centimeters. Segmental H-Se monthly profiles of the women's hair by community are presented in Figure 3. H-Se levels of the SA-2 family were higher and significantly different from the rest of the study population over the whole year, from January 2003 to January 2004 (Wilcoxon/Kruskall-Wallis test χ^2, $P < 0.0001$). For some communities, there were too few women to statistically examine intercommunity differences, but visual observation suggest that H-Se levels were higher at the beginning of the rainy season (from January to March 2003) and were lower and presented less intercommunity variations during the dry season (from June to December 2003); there was a slight increase again in January 2004. Two individuals from the SA-2 family showed H-Se levels above 5.0 μg/g, one from January to June 2003 and the other during January and February 2003, reaching 7.1 μg/g.

Table 3. t-test results of match-pair analysis for DWS (June and July 2003) to RWS (January and February 2004) variations of selenium biomarkers.

Village	B-Se[a]		H_1-Se[a]	
	Results[b]	n	Results[b]	n
SLT	↓**	27	↓**	23
NC	↑*	10	↑*	12
SA-1	↑†	13	↑**	13
SA-2	NS	10	↑†	8
VA	NS	10	↑**	12
MU	NS	9	NS	14
AC	↑**	14	↑*	18
Subgroup population	NS	93	↑**	100

[a]Whole-blood Se (B-Se) and first hair cm Se (H_1-Se) concentrations.
[b]Probability: $**P \leqslant 0.01$; $*P \leqslant 0.05$; $^{\dagger}P \leqslant 0.10$; NS, nonsignificant results.

Paired comparisons (Student's match-pair t-test) of H_1-Se from DWS, carried out in June and July 2003, to the corresponding months of the sequential H-Se results of the women's hair (mean of June and July 2003 results), showed no difference ($P = 0.43$, $n = 42$). However, two-by-two pair analyses of the sequential hair concentrations showed that June 2003 results were significantly lower compared with January 2004 (Student's match-paired t-test $P = 0.001$), which is in the same direction as the results of H_1-Se seasonal variations presented in Table 3. The H-Se concentrations from June 2003 were also significantly lower than January 2003 (Student's paired t-test, $P < 0.0001$), and the same trend was observed for January 2004, where concentrations tended to be slightly lower than January 2003 results (Student's paired t-test, $P = 0.075$).

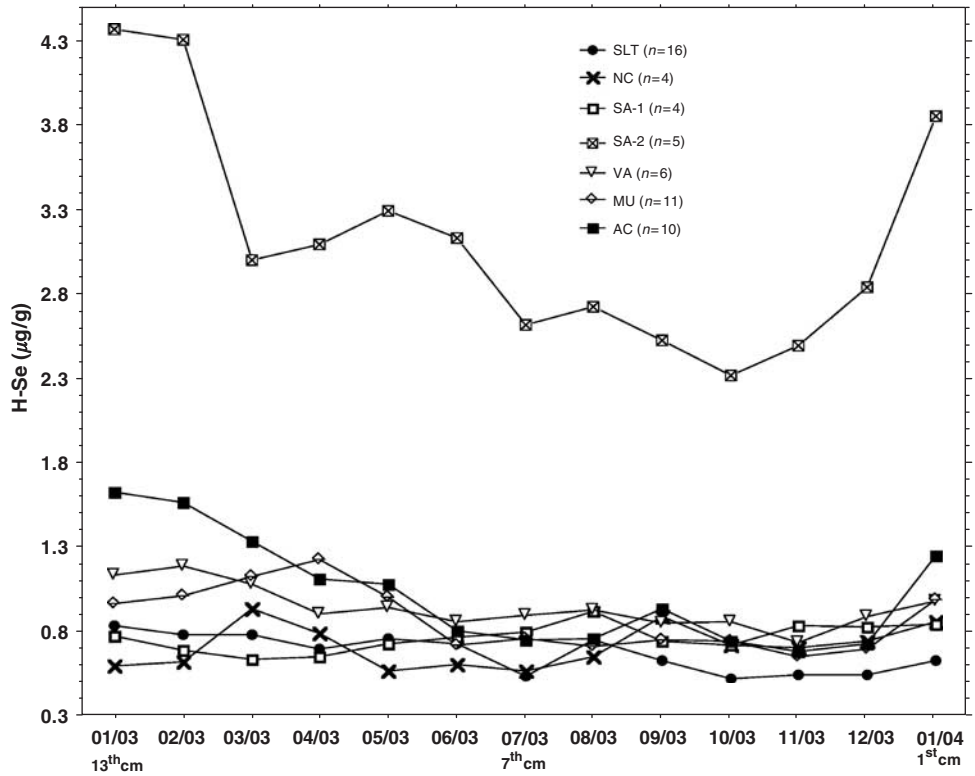

Figure 3. Sequential H-Se analysis for 13 cm of 56 women hair strands. Dots represents the community mean results for each month of analyse.

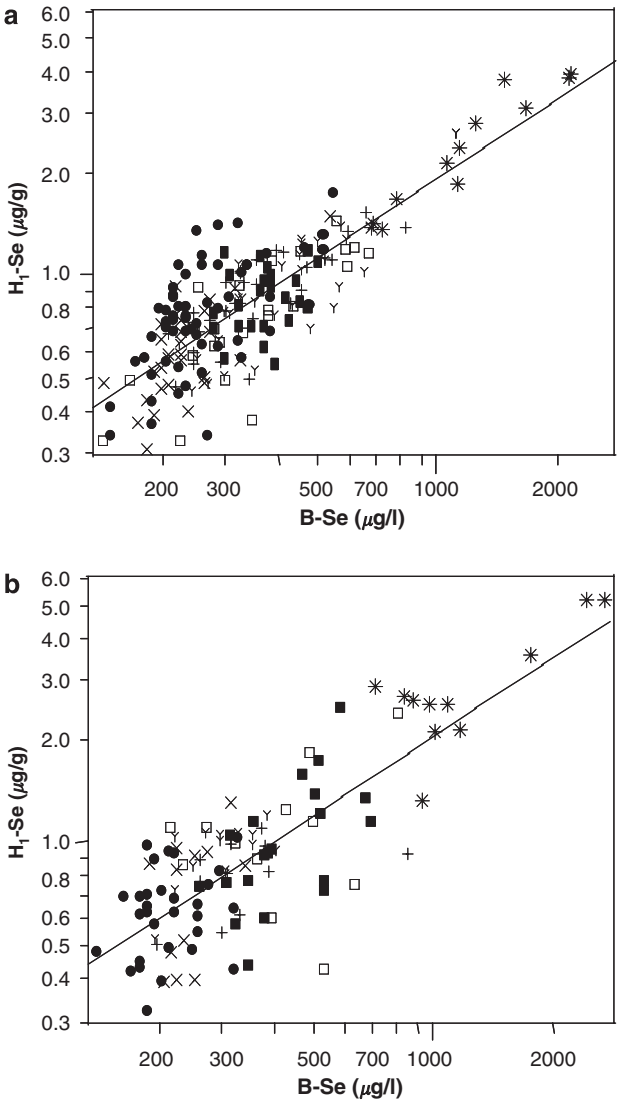

Figure 4. (a) Relationship between $\log_{10}$ B-Se and $\log_{10}$ H_1-Se biomarkers at the DWS ($n=212$); (b) relationship between $\log_{10}$ B-Se and $\log_{10}$ H_1-Se biomarkers at the rising water season ($n=110$). Different dots represented the studied villages: SLT: São Luis do Tapajós (●); NC: Nova Canaã (×); SA-1: Santo Antônio (□); SA-2: Extended family of Santo Antônio (*); VA: Vista Alegre (Y); MU: Mussum (+); AC: açaituba (■).

Figure 4a and 4b presents the seasonal relationships between whole blood and the previous month's hair Se biomarkers. The slopes of the linear regression model for $\log_{10}$-transformed B-Se and H_1-Se variables were similar for both seasons ([$f=ax+b$]; DWS: $a=0.799$, $P<0.0001$; and RWS: $a=0.800$, $P<0.0001$), even when the SA-2 family is excluded (DWS: $a=0.748$, $P<0.0001$; and RWS: $a=0.624$, $P<0.0001$). At DWS, B-Se levels explained 67.9% of the H_1-Se variation and at RWS, 63.6% of the H_1-Se variation. Spearman's correlations between B-Se and H_1-Se were also highly significant for both seasons ($\rho=0.737$, $P<0.0001$ and $\rho=0.663$, $P<0.0001$, respectively). The relationship

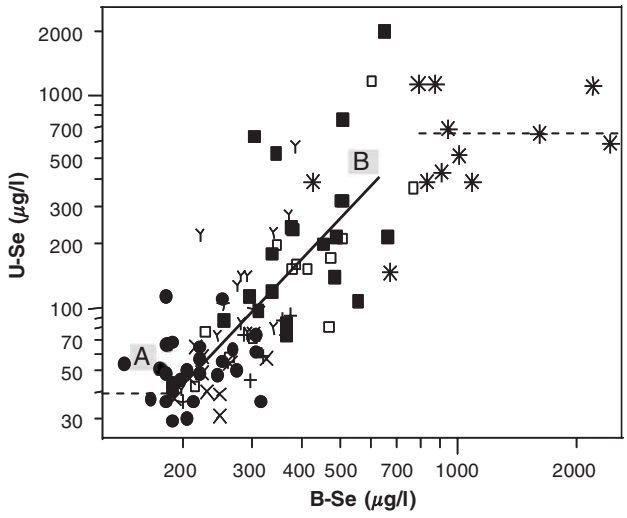

Figure 5. Relationship between $\log_{10}$ B-Se and $\log_{10}$ U-Se biomarkers at the rising water season ($n=107$). The strait line between the cut-offs A (212 µg/l, 49 µg/l) and B (601 µg/l, 377 µg/l) represents the segment of the regression where the relationship between B-Se and U-Se is linear. Pointed lines represent the two "pseudo-plateau" evaluated by the sigmoid curve model. Different dots represented the studied villages: SLT: São Luis do Tapajós (●); NC: Nova Canaã (×); SA-1: Santo Antônio (□); SA-2: Extended family of Santo Antônio (*); VA: Vista Alegre (Y); MU: Mussum (+); AC: Açaituba (■).

between B-Se and U-Se at the RWS is shown in Figure 5. The best non-linear relationship between B-Se and U-Se was described by a three parameter sigmoid curve ([$f=a/(1+\exp(-(x-x_0)/b))$]; $a=7.435$, $P<0.0001$; $b=1.100$, $P=0.002$; $x_0=5.205$, $P<0.0001$; $r^2=0.500$). The assessment of a threshold B-Se value at the lower part of the curve, for which no U-Se increase (plateau) was statistically detectable, was estimated at 212 µg/l of B-Se (corresponding to 49 µg/l of U-Se). This estimate was highly significant ($\chi^2=11.2$, $P<0.0001$; 95%CI [<142 to 295 µg/l]). At the upper part of the curve, a "pseudo-plateau" was estimated at 601 µg/l of B-Se (corresponding to 377 µg/l of U-Se). This estimate was also highly significant ($\chi^2=30.8$, $P<0.0001$; 95%CI [<426 to 988 µg/l]). Between 212 and 601 µg/l of B-Se (49 and 377 µg/l of U-Se), U-Se levels increased linearly with B-Se levels. Nineteen participants from SLT, NC, MU and VA (18%) had B-Se levels ⩾142 and <212 µg/l, 74 participants (69%) had ⩾212 µg/l and <601 µg/l (from all communities) and 14 participants (13%) from SA-2, SA-1, AC had ⩾601 µg/l. Participants' gender, age and education, as well as alcohol and smoking status, and farming and fishing practices did not influence blood-hair and blood-urine relationships (Figures 4 and 5).

Discussion

In this region of the Amazon, all biomarkers of Se are relatively high with important seasonal and intercommunity variations. High Se status is not common in human

populations and is generally associated with staple food consumption from seleniferous regions of the world for example, the Enshi district in China (Yang et al., 1989a), Nawan Shanhar district in India (Hira et al., 2004), North Dakota and Wyoming states in USA (Longnecker et al., 1991) and Portuguesa province in Venezuela (Bratter et al., 1991). For Inuit populations from Nunavik in Canada (Muckle et al., 2001) and Greenland (Hansen et al., 2004), high Se status has been related to consumption of marine mammals. Most cases of selenosis have been reported from the Enshi district of China (Yang et al., 1983, 1989a, 1994), where nail deformations were observed at B-Se concentrations $\geqslant 1054\,\mu g/l$, which are similar to the upper levels observed here. In the present study, over 10% of participants presented B-Se levels above $500\,\mu g/l$, which corresponds to the tolerable upper intake level (Institute of Medicine, 2000). However, a recent study on long-term Se supplementation and the incidence of type II diabetes suggests that this level may be too high (Stranges et al., 2007).

Overall, H-Se results from the present study are in the same range as H-Se levels of non-pregnant women from three villages of the Tapajós River (mean $2.5\,\mu g/g$, range $0.9–5.7\,\mu g/g$) reported by Pinheiro et al. (2005). This latter study did not specify the months of data collection, which could be important, as our results indicate important monthly fluctuations of Se status in the region. No previous data were available for U-Se levels in the Brazilian Amazon.

In the present study, the relationships for both seasons between B-Se and the 1st centimeter of H-Se were highly similar; the slope of the regressions were comparable and hair to blood ratios remained constant. Yang et al. (1989a) observed a similar to correlation for individuals with low to extremely high Se status. However, mean hair to blood ratios measured in the present population (2.67 and 2.88) were lower compared with results from Se-adequate and Se-high environments in China, which we estimated from their published data to be between 3.79 and 10.75. In the China studies, B-Se ranged from 95 to $3200\,\mu g/l$ (Yang et al., 1983, 1989a). There are several possible explanations for these differences: in the present study, the 1st centimeter of hair was used to calculate the ratio, while for the China studies, no information was provided about the number of centimeters that were considered. Other factors such as lower protein status and external exposure through Se-rich coal smoke in China (Yang et al., 1983), different bioavailability of Se in food sources, individual requirements and concomitant exposure to other metals such as mercury may explain the lower excretion ratio measured in the Amazon Tapajós riverside population.

According to several authors, urine excretion (24 h) is highly and linearly related to Se intake and plasma Se concentration (Yang et al., 1989a; Alaejos and Romero, 1993). In the present study, the range of Se exposure was large, and the relationship between B-Se and U-Se was best described by a sigmoid curve with two thresholds. There was a significant linear correlation between the lower and upper cut-offs at B-Se levels of 212 and $601\,\mu g/l$, respectively. As Se is an essential element, it is probable that at lower levels, homeostatic mechanisms retain Se in blood, while at normal levels, Se is excreted proportionally to its concentration in blood, and as blood levels further increase, excretory mechanisms may saturate so that excess Se remains in the blood. This upper level may be a threshold for possible Se toxicity, but this requires further study. On the other hand, the relationship between B-Se and H-Se remained linear, suggesting that H-Se would constitute a better biomarker for Se body burden.

Interseasonal differences suggest that sequential analyses for H-Se may be more sensitive to seasonal fluctuations in Se status than B-Se, although B-Se and H-Se were strongly correlated. This may be due to higher variability in B-Se compared to H-Se, which reflects a longer time period. Yang et al. (1989a), who showed that the consumption of unusually high Se food can occasionally and strongly influence B-Se content (bolus dose), suggest that, as global Se intake gets higher, whole-blood is generally a less sensitive biomarker than hair, nails and urine. Considering the seasonal variations of Se food sources, even if intrahair growth is variable and hair growth varies between individuals (Harkins and Susten, 2003), sequential hair analysis can provide relevant information on overall monthly Se status.

The Environmental Health Criteria of the International Program on Chemical Safety (WHO, 1986) has not set reference values for H-Se and U-Se. U-Se may be a useful biomarker to assess very recent intake, but there are several limitations: incomplete urine collection (<24 h) may not provide valid information about the Se status (WHO, 1986); the expression of U-Se per unit of volume of urine or adjustment techniques are not consistent between studies; and urine Se excretion is highly susceptible to variation (Yang et al., 1983) mostly because of current individual Se status, recent consumption of food with high Se content and bioavailability and/or interfering compounds in the diet (Robberecht and Deelsta, 1984).

It has been suggested that in circumstances where external Se contamination can be excluded, such as medicated shampoos and cosmetics (Leblanc et al., 1999; Senofonte et al., 2000), H-Se content could be a useful biomarker to assess Se status (WHO, 1986). Indeed, as a biomarker, hair has notable advantages compared with other media: sampling is less invasive; hair can be easily stored for long periods and transported; it can provide information over an extended period of time; it contains one order of magnitude more Se ($\mu g/l$ *vs.* $\mu g/g$) and it can provide an integrated measure, reflecting total body intake better than the more common biomarkers such as plasma and urine (Senofonte et al., 2000; Perreira et al., 2004). In this study, we saw no benefit in washing samples in a pre-treatment step before

digestion because of inconsistent data on washing methods, which can extract endogenous H-Se or fail to remove external contamination (Leblanc et al, 1999; Morton et al., 2002). None of the participants used medicated shampoo containing Se disulfide, one of the most common sources of external Se contamination, whose long-term use can increase individual H-Se levels by more than 100-fold (Leblanc et al, 1999).

For the population studied here, in general, H-Se status was consistently and significantly higher at the rising water season. However, within communities, seasonal variations were not necessarily in the same direction, which is probably related to local food availability and consumption patterns. In the Tapajós region, there are more than a hundred fish species, whose predominance varies over short distances and with seasons. Furthermore, Se content varies between Amazonian fish species and ecosystems (Dorea et al., 1998; Lima et al. 2005). There are also important seasonal variations in the availability of fruits and vegetables (Passos et al., 2001). Farmers present consistently higher Se status for both seasons, suggesting that there may be multiple local Se sources in the Tapajós ecosystems. Brazil nuts (*Bertholletia excelsa* Humb. & Bompl.), constitute an important potential source of dietary Se (Chang et al., 1995), but are not available throughout the year; the mature nut capsules usually fall from the trees from December to April, which corresponds to the RWS. In addition, the distribution of Brazil nut trees is not uniform throughout the Tapajós Valley. This nut is an excellent source of protein and fat (Chunhieng et al., 2004) and nuts can be stored for further consumption through the year. Most of those with the highest levels of Se live in an area surrounded by over 300 Brazil nut trees. We are currently examining Se content in local foods.

The findings of this study suggest that for populations with high Se status, H_1-Se may provide a more integrated measure of Se status than B-Se, as it is less sensitive than plasma, B- or U-Se to recent consumption of food with high Se content. Segmental H-Se may be a good biomarker of Se status for public health surveys and epidemiologic studies, particularly in remote areas where blood sampling and storage is difficult and in areas where there are important seasonal variations in diet. In addition, segmental H-Se can be a useful biomarker to provide a retrospective profile of the past Se status and its monthly or seasonal variations. Further studies should examine the relationships between Se intake and all of the Se biomarkers, as well as between biomarkers and health outputs.

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