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GPX1 Pro198Leu polymorphism and *GSTM1* deletion do not affect selenium and mercury status in mildly exposed Amazonian women in an urban population

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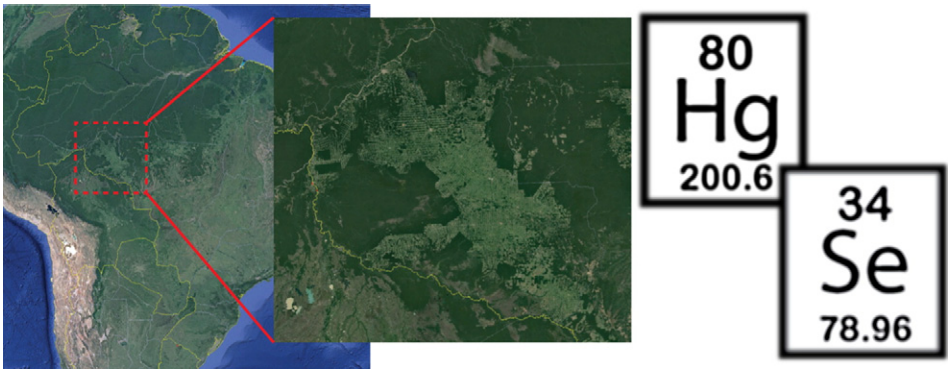
HIGHLIGHTS

- Study of effects of *GPX1* and *GSTM* polymorphisms on mercury levels in an urban female population in the Amazon
- Analysis of selenium in erythrocytes and mercury in hair, with oxidative stress markers and genotyping of *GPX1* and *GSTM*
- High prevalence of selenium deficiency despite ready access to selenium-rich produce
- Low levels of mercury toxicity despite historically high exposures in this region
- Polymorphisms to *GPX1* and *GSTM* do not appear to influence mercury levels in mildly-exposed populations

GRAPHICAL ABSTRACT



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ABSTRACT

Mercury is potent toxicant element, but its toxicity can be reduced by forming a complex with selenium for safe excretion. Considering the impact of mercury exposure in the Amazon region and the possible interaction between these two elements, we aimed to assess the effects of Pro198Leu polymorphism to *GPX1* and *GSTM1* deletion, on mercury levels in a population from Porto Velho, an urban locality in the Brazilian Amazon region. Two hundred women from the capital city of Rondônia state were recruited for this study with 149 deemed suitable to participate. We assessed dietary intake using 24-hour recall. Selenium levels in plasma and erythrocytes were measured using hydride generation quartz tube atomic absorption spectroscopy and total hair mercury using cold vapor atomic absorption spectrometry. Oxidative stress parameters (GPx activity, oxygen radical absorbency capacity [ORAC] and malondialdehyde [MDA]) were also analyzed. All participants were genotyped for Pro198Leu polymorphism and *GSTM1* deletion. We observed that this population presented high prevalence of selenium deficiency, and also low levels of mercury, likely due to food habits that did not include selenium-rich food sources or significant consumption of fish (mercury biomagnifiers) regularly. Univariate statistical analysis showed that Pro198Leu and *GSTM1* genotypes did not affect selenium and mercury levels in this population.

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Pro198Leu polymorphism and *GSTM1* deletion had no effect on mercury levels in mildly exposed people, suggesting these genetic variants impact mercury levels only in highly exposed populations.

1. Introduction

Mercury is a potent toxicant even in low doses, having adverse effects on the renal, immune, cardiovascular, reproductive and central nervous systems (Zahir et al., 2005). *In utero* transfer of mercury (inorganic or as the more toxic methylmercury species) from mother to fetus is of particular concern, as neurotoxic effects are most marked in the developing brain, and there is concern that current exposure advisories do not sufficiently take into account the potential damage of chronic, low-level exposure during pregnancy (Brown and Austin, 2012; Cardenas et al., 2015; Hui et al., 2016). Selenium is involved in mercury detoxification (Yoneda and Suzuki, 1997); therefore it is important to understand the genetic and biochemical factors that influence interactions between selenium and mercury in at-risk populations, such as women of childbearing age, as this can result negative health outcomes for the following generation.

Amazonian populations have some of the highest reported mercury exposure levels in the world (Passos and Mergler, 2008). While a small fraction of mercury in the environment occurs through natural geological events (*i.e.* volcanic activity), the majority of health effects arise from either occupational exposure (*e.g.* mining) or anthropogenic release (Holmes et al., 2009). Mercury retention in soil in the Amazon region is partially due to geographic factors, but is exacerbated by excessive and poorly-regulated gold-mining, soil cultivation methods and industrial contamination (Comte et al., 2013; Fostier et al., 2015; Miretzky et al., 2005; Pouilly et al., 2013). Biomagnification and the reliance on the aquatic environment for food make local fish intake the most relevant source of methylmercury contamination in this area for subsistence farmers (Barcelos et al., 2013; de Oliveira et al., 2014; Mazzaron Barcelos et al., 2012). Methylmercury exerts the most significant negative health effects through chronic neurotoxicity, particularly during development (Grandjean and Landrigan, 2006; Grandjean and Landrigan, 2014).

Concurrently, Amazonian populations are also exposed to high levels of selenium, also though agriculture, as the soil in this area is the most selenium-rich in Brazil (Cintra and Cozzolino, 1993; Favaro et al., 1997). The most concentrated and widely-consumed selenium food source are Brazil nuts *(Bertholletia excelsa, H.B.K)*, which are widely cultivated in the Amazon basin (Rita Cardoso et al., 2016; Souza and Menezes, 2004). Selenium is an essential nutrient, though it has a therapeutic window that can be exceeded *via* high dietary intake (MacFarquhar et al., 2010; Martens et al., 2015). Concomitant mercury and selenium exposure in areas such as the Amazon are of significant interest, as selenium is able to partially mitigate mercury toxicity in both animals and humans (Falnoga and Tušek-Žnidarič, 2007). The proposed protective mechanism involves the formation of an inert selenium-mercury complex (Naganuma and Imura, 1983; Yoneda and Suzuki, 1997) that is effective against both inorganic and methylmercury intoxication (Whanger, 1992). However, studies have demonstrated that certain polymorphisms to genes encoding the selenoproteome, a collection of 25 identified selenium-containing proteins (Cardoso et al., 2015) may alter response to dietary intake, thus increasing susceptibility to both mercury intoxication and nutritional selenium deficiencies. In regions like the Amazon, where environmental exposure is practically endemic, assessing these previously identified effects in at-risk populations is an important population health concern.

The Pro198Leu polymorphism (rs1050450) to the selenoprotein glutathione peroxidase 1 gene (*GPX1*) is characterized by a variant *T*-allele, encoding leucine (Leu) instead of proline (Pro) at codon 198 (Forsberg et al., 1999; Ravn-Haren et al., 2006). This polymorphism has been associated with changes to selenium status and the selenoprotein hierarchy (Combs et al., 2011; Jablonska et al., 2009; Karunasinghe et al., 2012; Xiong et al., 2010), although its effects in response to selenium supplementation remain unclear (Jablonska et al., 2015; Miller et al., 2012; Soares et al., 2016).

Additionally, glutathione-*S*-transferases (GSTs) are a family of phase II biotransformation enzymes that are also involved in mercury detoxification and elimination by mediating conjugation of mercury and glutathione (GSH), which is then excreted in bile and by the kidneys (Clarkson and Magos, 2006). The human GST family is subdivided into eight separate classes: alpha, mu, kappa, omega, pi, sigma, theta and zeta; which are encoded by the *GSTA*, *GSTM*, *GSTK*, *GSTO*, *GSTP*, *GSTS*, *GSTT*, and *GSTZ* genes, respectively (Hayes and Strange, 2000; Josephy, 2010). These genes are highly polymorphic, and deletion of *GSTM* (*GSTM1*) and *GSTT* (*GSTT1*) are significant as they result in a complete loss of activity of the enzymes, and studies have associated this deficiency with increased cancer risk and mercury retention (Barcelos et al., 2013; Barcelos et al., 2015; de Oliveira et al., 2014; Goodrich et al., 2011; Jain et al., 2006; Klautau-Guimarães et al., 2005), as well as increased maternal blood mercury levels (Lee et al., 2010). However, other reports have failed to find a link between GSTM1 concentration or activity and an outcome-biomarker of mercury retention (Wang et al., 2012), and thus further studies are required to determine the precise role of this deletion polymorphism on mercury detoxification.

Therefore, considering the importance of mercury exposure in the Amazon region and the interaction between this toxic metal and selenium, this pilot study aimed to evaluate the effect of Pro198Leu polymorphism and *GSTM1* deletion on mercury levels, taking into account the previously reported role these mutations play in generalized selenium metabolism. In the context of the Amazonian region, where isolated populations are fast becoming juxtaposed with densely populated urban regions, it is valuable to understand the interaction between exposure to this environmental toxicant and individual genetic susceptibility; a necessity highlighted at the National Institutes of Health Gene-Environment Interplay Workshop (Bookman et al., 2011). Because of the established toxic effect on fetus in addition to the mother, we conducted this study in a female cohort from Porto Velho, representing a diverse urban population in Amazon region.

2. Methods

2.1. Study participants

This study was conducted according to the Declaration of Helsinki guidelines and approved by Ethics Committee of the Faculty of Pharmaceutical Sciences at the University of São Paulo (protocol number 574). Written informed consent was obtained from all participants. Sample size calculation, based on prevalence of the variant allele of Pro198Leu (32%) estimated that c.a. 200 participants would be required to test the effects of *GSTM1* deletion and Pro198Leu variants of *GPX1* on biological selenium and mercury levels. We recruited 200 women who fulfilled the aforementioned criteria and were included in this study. However, 51 participants were excluded due to problems during sampling and transport, leaving 149 subjects. All participants were recruited for this transversal pilot study from Porto Velho, the capital city of the state of Rondônia, located in the western Amazon region (Fig. 1). This

Fig. 1. Location of Porto Velho (8°45′43″S 63°54′14″W) on the Madeira river in the State of Rondônia, Brazil. Image from Google Earth Pro (v 7.1.5.1557).

city has approximately 500,000 inhabitants, and is the most populated city in Rondônia state and 42nd in Brazil. Subjects were recruited from public advertisements in gyms, universities, bus stops and municipal medical centers. Eligible women were 18 to 48 year-old (mean age = 26.60 ± 7.75) residents of Porto Velho, and self-reported as non-smokers (including ex-smoking behaviors), pre-menopausal, and with no clinical history of chronic and/or infectious diseases. Participants did not practice intense physical activity and did not take vitamins and mineral supplements.

Blood and hair samples were collected and a 24-hour dietary recall was completed, as were anthropometric evaluations. These assessments were performed in an outpatient clinic in Porto Velho, and analysis of collected data was performed at the University of São Paulo.

2.2. Anthropometric evaluation

Anthropometric assessment consisted of height, weight and waist circumference measurements. Weight was measured using a standard digital weight scale (Filizola, Brazil) with an accuracy of ±100 g and maximum capacity of 150 kg. Height was recorded using an anthropometer coupled to the weight scale, measuring up to 2000 ± 5 mm. Waist circumference was assessed with a flexible and non-elastic tape (Sanny, Brazil) and screened according to Brazilian Society for the Study of Obesity and Metabolic Syndrome recommended cutoff of <80 cm for women (Associação Brasileira para o Estudo da Obesidade e da Síndrome Metabólica, 2009).

2.3. Dietary assessment

Diet evaluation was completed using a 24-hour dietary recall requested in three non-consecutive days (2 weekdays and 1 weekend). Recall of fish intake over the past 12 months (frequency, types, and origin) was included to qualitatively identify exposure to mercury *via* diet. The fish consumption questionnaire asked: a) frequency of fish intake; b) type of consumed fish; c) origin of the consumed fish (name of the river); d) frequency of other seafood intake.

Selenium intake was measured by using NutWin software (EPM-UNIFESP). A database of selenium content from analyzed food was provided by the Laboratório de Nutrição-Minerais at the University of São Paulo. Selenium intake was adjusted for energy intake, according to Willet (1998), using linear regression of nutrient intake on total caloric intake and addition of a constant (mean energy intake of the group). This was compared against dietary reference intake (DRI) recommendations for selenium (Institute of Medicine, 2000).

Selenium adequacy was calculated using *z* scores according to estimated average requirement (EAR) and upper tolerable intake level (UL) (Institute of Medicine, 2000):

$$z = (\mathrm{EAR}–\mathrm{M_i})/\mathrm{SD}$$

where M_i is mean selenium intake per day and SD is standard deviation.

2.4. Blood and hair sampling

Samples (20 mL) of fasting blood were collected in the morning by venepuncture in EDTA evacuated tubes. A fraction of total blood (1 mL) was stored in autoclaved microtubes for genotyping of *GPX1* and *GSTM*. Plasma was separated by centrifugation at 3000 rpm for 15 min at 4 °C and stored in demineralized EDTA microtubes for retrospective analysis of plasma selenium levels, oxygen radical absorbency capacity (ORAC) and malondialdehyde (MDA) concentration. The erythrocyte pellet obtained from whole blood by centrifugation was washed three times in 5 mL of sterile 9 g L^{-1} NaC1 solution, slowly homogenized by inversion and then centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant was discarded and the erythrocyte pellet was stored in EDTA microtubes for selenium and glutathione activity analysis. Samples were stored on dried ice for transportation to the analytical laboratory and kept at −80 °C until analysis.

Total mercury levels were measured in hair samples (1 g) cut from the occipital scalp. Samples were stored in sealed and labeled envelopes at room temperature until analysis.

2.5. Selenium and mercury assay

Selenium concentration in plasma and erythrocytes was measured by hydride generation atomic absorption spectroscopy (HG-AAS) (HITACHI®, Z-5000) (Cardoso et al., 2010). Samples of plasma and erythrocyte were prepared for analysis by digestion with 68% nitric acid (Merck, Darmstadt, Germany) and heating to 150 °C. After volatilization of the organic material, solutions were reduced from Se(VI) to Se(IV) by the addition of 1.2 normal hydrochloric acid and heating at 100 °C for 2 h. The samples were diluted to 25 mL in ultrapure water. A standard 7-point calibration curve was prepared using Tirisol with a concentration range of 0 to 5.0 μg L^{-1}. Recovery was determined by analysis of lyophilized human reference control Seronorm Trace Elements Serum and Whole Blood (Sero AS, Billingstad, Norway); and all samples were measured in triplicate.

Mercury determination in hair samples was performed by cold vapor atomic absorption spectrometry (CV AAS) using a FIMS-100 (Perkin Elmer, USA) (Farias et al., 2008). Samples were prepared

according to International Atomic Energy Agency recommendations and previously used methods (Farias et al., 2006, 2008). Briefly, small pieces of hair were immersed in acetone followed by washing with ultrapure water, then dried at room temperature and stored in envelopes until analysis. Aliquot of samples (0.15 g) were digested with concentrated nitric acid and sulfuric acid in a sealed Teflon container (Savillex, Minnetonka, MN, USA) at 90 °C. To stabilize mercury, we added 250 μL of potassium dichromate. Validation of total hair mercury measurement confirmed by analyzing certified reference materials (Human Hair–IAEA 086, GBW 09101 – Human Hair, and BCR CRM 397 – Human Hair). All reagents were of analytical grade or higher (Merck). Nanopure water diluent was used for all standards and samples.

2.6. GPx activity assay

Glutathione peroxidase activity was determined in erythrocyte lysates using a commercial kit (505-RANDOX Laboratories, United Kingdom) adapted for biochemical analyzer (Liasys, AMS Diagnostics, Italy), according to the method developed by Paglia and Valentine (Paglia and Valentine, 1967). GPx activity was expressed in units per gram of hemoglobin (U g^{-1} Hb).

2.7. Malondialdehyde assay

Malondialdehyde levels in plasma were determined by high-performance liquid chromatography (HPLC) according to the method developed by (Rezaei et al., 2013) on a Shimadzu HPLC system (Japan) with a C18 reverse-phase column (4.6 × 240 mm, 5 μm pore size; Phenomenex, USA). Standard 8-point calibration curves were prepared from an MDA stock solution with a range of 0–12.0 μM.

2.8. Oxygen radical absorbency capacity assay

Oxygen radical absorbency capacity was measured in plasma deproteineted with perchloric acid (Prior et al., 2003). Samples were analyzed in triplicate by microplate reader (BioTek Synergy H1; USA). A 5-point standard calibration curve was prepared from a stock solution of trolox (range 6.25–100 μM). Results were expressed in μM trolox equivalent per mL plasma (μmol TE mL^{-1}).

2.9. Pro198Leu and GSTM1 genotyping

Isolation of DNA from whole blood was carried out using a PureLink Genomic DNA kit (Invitrogen, Life Technologies Inc.; USA), and the concentration was measured using a NanoDrop ND 1000 spectrophotometer (Thermo Scientific, USA). All samples were adjusted with autoclaved water to achieve a standard concentration of 10 ng mL^{-1} in the 20 μL final volume. Detection of the Pro198Leu polymorphism and *GSTM1* deletion was determined by real-time polymerase chain reaction (RT-PCR) using the Custom TaqMan Genomic Assays service (Life Technologies, USA).

The reaction mixture for the Pro198Leu RT-PCR reaction was prepared as described by (Soares et al., 2016). The amplification of the fragment containing the SNP site was performed using the following primers: forward 5′-CATCGAAGCCCTGCTGTCT-3′; reverse 5′-CACTGCAACTBCCAAGCA-3′. The Pro198Leu assay was run on a StepOne Real-Time PCR system with an initial enzyme activation step at 94 °C (10 min) followed by 40 cycles at 92 °C (15 s per cycle), then 60 °C (1 min) for annealing and extension.

The *GSTM1* deletion was identified using the following primers: forward 5′-AAGATTCGTGTGGACATTTTGGA-3′; reverse 3′-TCTGGATTGTAGCAGATCATGCC-5′. The assay for *GSTM1* RT-PCR reaction contained 2 ng of genomic DNA and 300 μg of each primer prepared in 1 × TaqMan Universal PCR Master Mix (Applied Biosystems, USA) to a final volume of 20 μL. B2M primers (forward, 5′-TTGTTTCACTGTCCTGAGGACTATTTAT-3′ and reverse, 5′-TGTTACTCTGTCAATGTTCTCCACAT-3′), and fluorescent product-specific probes *GSTM1* (FAM-CAGACCATGGACAACCA-MGB; 120 nM) and B2M (VICCTCTAACATGATAACCCTCACMGB; 30 nM) were used to normalize data. Samples were genotyped on StepOne Real-Time PCR system as follows: 50 °C (2 min); 95 °C (10 min); 40 cycles at 95 °C (15 s), then 60 °C (1 min). *GSTM1* deletion was identified when threshold cycle (Ct) values were above 35 cycles, as standardized by (Covault et al., 2003).

2.10. Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS) (v 20.0) for Windows (IBM, USA), and the figures were produced using Prism 6 (GraphPad, USA). A p value <0.05 was considered to be statistically significant. A descriptive analysis was performed by showing the results as mean ± SD and range. Kolmogorov-Smirnov tests were performed in order to verify distribution, and revealed that only MDA data was normally distributed. Levene's test was used to evaluate homogeneity of variables. Spearman's correlation coefficient tests were performed to determine correlation between variables.

The influence of Pro198Leu polymorphism and *GSTM1* deletion on selenium and mercury levels were analyzed by univariate general linear models. As a first step variables were log transformed when not normally distributed. Next, the univariate model considering possible variables influencing selenium levels in plasma and erythrocytes (waist circumference, age and hair mercury) and mercury in hair (waist circumference, age, erythrocyte and plasma selenium) was applied. Variables which influenced selenium and mercury levels were then included in the other univariate model which included the *GPX1* and *GSTM* genes. Literature has suggested that age and adiposity can be associated with selenium (Hosseini et al., 2016; Rasmussen et al., 2009) and mercury levels (Agusa et al., 2005; Park and Lee, 2012), and we have previously suggested a possible association between these two metals (Vieira Rocha et al., 2014). For the second model, which had the genotypes as fixed factor, only those variables which influenced selenium and mercury levels (according to the first model) were included. For all statistical analyses, variant homozygous and heterozygous genotypes of Pro198Leu polymorphism were considered together and compared to the wild type.

3. Results

3.1. General information

The mean age of participants was 26.6 ± 7.8 years. According to BMI classification (World Health Organisation, 2000), 80.5% were eutrophic, 15.4% were overweight and 4% were underweight. Waist circumference was adequate in 88% of the population indicating low risk for cardiovascular diseases. Full population demographics are shown in Table 1.

Dietary assessment suggested the observed selenium deficiency was due to inadequate intake in 42% of the participants. Dietary recalls revealed that the main natural selenium food source, Brazil nuts, were

Table 1
Demographic and anthropometric characteristics of study population.

Age (years)[a]		26.60 ± 7.75 (18.0–48.0)
Ethnicity[b,c,d]	Mulato	133 (89.3%)
	Caucasian	12 (8%)
	Afro-Brazilian	3 (2%)
	Asian	1 (0.7%)
BMI (kg m^{-2})[a]		22.19 ± 2.58 (16.75–29.56)
Waist circumference (cm)[a]		70.50 ± 6.98 (48–91)

[a] Mean ± SD (range).
[b] Self-reported.
[c] N (%).
[d] Categorical variable.

consumed rarely (less than once a month), and only one respondent reported ingestion of this nut on occasion. The main protein food source was beef (73% reported daily intake), followed by chicken (27% reported daily intake). Fish was not reported in the 24-hour dietary recall, and the fish questionnaire identified that all participants consumed it rarely (less than once a month).

3.2. Biochemical parameters

Both plasma and erythrocyte selenium levels indicated a high prevalence of deficiency (Fig. 2). Only 16.8% of participants presented the minimum plasma selenium concentration required for optimization of iodothyronine 5′ deiodinases (IDIs) activity, a standard measure of selenium in tissue (Thomson, 2004); and only 3.4% had levels adequate for optimization of GPx, according to the parameters established by (Thomson, 2004). Erythrocyte selenium levels indicated 62% of participants were deficient, according to the cutoff established by (Ortuño et al., 1997).

Glutathione peroxidase activity in erythrocytes was correlated with plasma selenium ($r = 0.220$; $p = 0.01$) and in erythrocytes ($r = 0.259$; $p < 0.001$). Although selenium deficiency was highly prevalent, only 12.8% presented insufficient enzyme activity (Fig. 2). Selenium erythrocyte concentration negatively correlated with ORAC ($r = -0.223$; $p < 0.01$), and plasma MDA was independent of all other parameters. Assessment of mercury in hair revealed that only four participants (2.7%) showed concentrations above the cutoff established by the World Health Organization (1990) (Fig. 2) and the univariate model showed that mercury levels were influenced by selenium erythrocyte concentrations when adjusted by age ($\beta = -0.610$; $p = 0.009$), which was not observed for selenium in plasma ($\beta = 0.433$; $p = 0.076$).

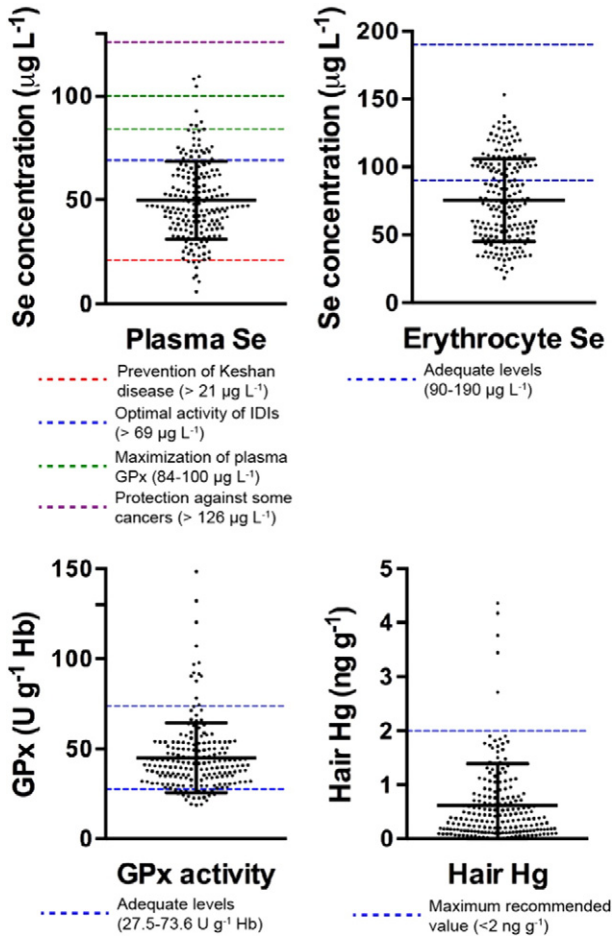

Fig. 2. Selenium (Se) status (plasma and erythrocytes), GPx activity and mercury (Hg) of study population in comparison to reference guidelines (limits from Ortuño et al., 1997; Thomson, 2004; World Health Organization, 1990) and RANDOX Laboratories Ransel kit. IDI = iodothyronine 5′ deiodinases; Se = selenium; SelP = selenoprotein P.

3.3. GPX1 and GSTM1 genotypes effects on selenium and mercury status

Genotype distribution of the Pro198Leu and *GSTM1* are shown in Table 2, with related biochemical parameters stratified according to genotype presented in Table 3. In the first univariate analysis, we observed that age ($\beta = 0.874$; $p < 0.05$) and erythrocyte selenium ($\beta = -0.768$; $p < 0.05$) were associated with hair mercury content. Plasma selenium was affected only by age ($\beta = 0.338$; $p < 0.05$); and erythrocyte selenium concentration correlated with hair mercury ($\beta = -0.080$; $p < 0.05$). Based on these latter data, we performed a second univariate analysis including the Pro198Leu polymorphism and *GSTM1* deletion, and we found that neither genotype affected selenium or mercury levels. We also looked at gene-gene interaction (*GPX1* × *GSTM1*), but again no significant effect was seen. However, our small sample size may mask small variances, as only 9 people (14.5%) presented *GSTM1* deletion associated with LeuLeu *GPX1* genotype (Table 4). As previous studies have shown that the association between erythrocyte GPx activity and selenium concentration may vary among genotypes (Jablonska et al., 2009; Ravn-Haren et al., 2006), we tested the correlation between these parameters and observed significant result only for the Leu carriers ($r = 0.362$; $p = 0.04$).

4. Discussion

Environmental exposure to mercury represents one of the major public health concerns related to chemical pollution and because this metal is especially toxic *in utero* (Hui et al., 2016; Karagas et al., 2012) it is important to evaluate women in a reproductive age from an area of known high mercury exposure (the Amazon basin). Furthermore, studies have demonstrated the role of gene-environment interaction in regard to susceptibility to mercury toxicity (Julvez and Grandjean, 2013; Llop et al., 2015). In this study we evaluated the effects of two polymorphisms involved in selenium metabolism and mercury detoxification in a female population representative of a diverse urban population in Amazon region that we predicted would have high exposure to both species. Rural populations in the Amazon region are typically exposed to significant mercury *via* fish intake (Barcelos et al., 2013; de Oliveira et al., 2014; Mazzaron Barcelos et al., 2012) or pollution from gold-mining (Klautau-Guimarães et al., 2005), while selenium exposure generally comes from the high content in agricultural soils (Martens et al., 2015). However, most of those studies were performed in estuarine areas with isolated populations, which contrast from the rapidly growing metropolitan areas such as Porto Velho. Access to remote communities is also typically limited to riverboats, and are thus are restricted to locally produced food, which includes fish as the main protein food source (Barcelos et al., 2015). We have shown in a previous study that closer proximity to developed cities provides additional access to industrialized food sources, which in turn lowers consumption of locally

Table 2
Prevalence of *Pro198Leu* and *GSTM1* genotypes[a].

Polymorphism	N (%)
Pro198Leu	
ProPro	86 (57.7%)
ProLeu	52 (34.9%)
LeuLeu	11 (7.4%)
Total	149 (100%)
GSTM1	
Present	87 (58.4%)
Null	62 (41.6%)

[a] Categorical variable.

Table 3
Selenium status, intake, hair mercury concentration, GPx activity, ORAC and MDA levels according to Pro198Leu polymorphism and *GSTM1* deletion.

Genotype	Plasma selenium (μg L^{-1})[a]	Erythrocyte selenium (μg L^{-1})[a]	Selenium intake (μg d^{-1})[a]	Hair mercury (μg g^{-1})[a]	GPx activity (U g^{-1} Hb)[a]	ORAC (μM TE mL^{-1})[a]	MDA (μM)[a]
All participants	49.76 ± 19.34 (5.85–109.37)	77.41 ± 30.51 (18.39–153.19)	48.82 ± 20.37 (10.38–185.2)	0.60 ± 0.74 (0.002–4.36)	45.24 ± 20.49 (18.86–148.49)	1.58 ± 1.32 (0.05–7.49)	0.86 ± 0.25 (0.28–1.55)
Pro198Leu							
ProPro	49.63 ± 21.09 (109.37–153.19)	77.94 ± 31.02 (25.34–153.19)	50.32 ± 23.03 (10.38–185.20)	0.64 ± 0.87 (0.02–4.36)	47.40 ± 23.57 (18.86–148.49)	1.64 ± 1.40 (0.05–7.49)	0.88 ± 0.27 (0.28–1.55)
ProLeu + LeuLeu	49.94 ± 16.83 (22.17–86.13)	76.69 ± 30.04 (18.39–133.20)	46.77 ± 16.01 (16.45–129.06)	0.57 ± 0.52 (0.02–1.91)	42.29 ± 15.01 (19.10–96.90)	1.51 ± 1.24 (0.10–7.17)	0.83 ± 0.23 (0.31–1.52)
GSTM1							
Present	50.46 ± 20.36 (12.47–109.37)	80.28 ± 30.97 (18.39–153.19)	49.69 ± 21.79 (18.00–185.00)	0.536 ± 0.798 (0.002–4.178)	45.79 ± 22.22 (18.86–148.49)	1.59 ± 1.42 (0.05–7.45)	0.84 ± 0.24 (0.31–1.44)
Null	48.79 ± 17.94 (5.85–92.69)	73.39 ± 29.65 (32.34–133.20)	47.60 ± 18.30 (10.00–142.00)	0.710 ± 0.795 (0.042–4.361)	44.48 ± 17.94 (19.10–106.96)	1.57 ± 1.21 (0.17–7.49)	0.90 ± 0.26 (0.28–1.55)

[a] Mean ± SD (min – max).

produced food (Vieira Rocha et al., 2014). Here, we used 24-hour dietary recall and a complimentary questionnaire to assess dietary intake. Despite the limitations associated to this method, as memory issues and difficulty in accurately estimating portion size (Biro et al., 2002), we performed the interview three times in order to improve reliability, and the questionnaire was reviewed by a trained nutritionist. We have observed that dietary habits in our population study were not influenced by high exposure to mercury and selenium through dietary means (though environmental exposure to mercury cannot be discounted). All participants reported limited fish intake and dietary selenium intake was well below our previously reported studies using an enriched food source. Further, when participants who consumed fish were asked about its origin, most respondents reported it was purchased from local supermarkets, which precludes tracking their source. The negative data reported here is useful to demonstrate that it is not possible to generalize Amazon region populations with regard to outcomes dependent on dietary intake considering the mixed remote, rural, semi-rural and urban populations.

Although our study population did not present high concentrations of either analyte, we observed a negative correlation between selenium and mercury levels in medium and long-term biomarkers. Selenium in erythrocytes corresponds up to 120 days' exposure, as the element is incorporated into erythrocytes during erythropoiesis (Cardoso et al., 2010). Hair mercury is considered a reliable long-term biomarker of methylmercury exposure as the metal is permanently incorporated into the hair after a short period in bloodstream (Barbosa et al., 2001; Nuttall, 2006; World Health Organization, 1990). To assure the quality of this biomarker, we collected samples from the same area, standardized the amount of analyzed sample and ensured consistent use of the washing process in order to eliminate exogenous contaminants as shampoo, hair oil and hair dye products (Airey, 1983; Barbosa et al., 2001). Association between selenium and mercury has been previously reported (Barany et al., 2002; Li et al., 2012; Lindberg et al., 2004; Vieira Rocha et al., 2014), and reflect the capacity of selenium to protect against mercury intoxication *via* formation of inert selenium-mercury complex. It is believed that this complex alters the distribution of mercury in tissues and induces binding of the mercury-selenium complexes to proteins for excretion (Joshi et al., 2010; Naganuma and Imura, 1983; Ralston and Raymond, 2010; Yoneda and Suzuki, 1997).

Table 4
Univariate regression parameters for the associations between *Pro198Leu* and *GSTM1* genotypes and selenium and mercury parameters.

Genotypes	Univariate analysis					
	Plasma selenium[a]		Erythrocyte selenium[b]		Hair mercury[c]	
	β	*p*	β	*p*	β	*p*
Pro198Leu	−0.063	0.379	0.009	0.896	−0.172	0.407
GSTM1	−0.078	0.285	−0.063	0.392	0.296	0.156
Pro198Leu × *GSTM1*	−0.143	0.325	−0.081	0.583	−0.137	0.739

[a] Age as covariate.
[b] Hair mercury as covariate.
[c] Age and erythrocyte selenium concentration as covariates.

Despite the small sample size of this pilot study we were able to identify, with statistical power, that Pro198Leu did not affect selenium status or GPx activity, although correlation between the micronutrient concentration in erythrocytes and enzymatic activity was positive only among ProLeu genotype subgroup, in contrast to other studies which have identified the effect of this polymorphism in selenium status (Combs et al., 2011) and verified an association between selenium in blood and GPx activity only in wild type samples (Cardoso et al., 2016; Cardoso et al., 2012). This data suggests that further studies regarding the effects of *Pro198Leu* on selenium status are required to conclusively ascertain if and how this polymorphism can interfere with selenium distribution in multiple tissue types and how it influences the established selenoprotein hierarchy.

As far as we know, this is the first study that aimed to assess the effect of Pro198Leu polymorphism in mercury levels, performed in conjunction with assessment of the effects of *GSTM1* deletion. Because this polymorphism is thought to influence selenium metabolism, we hypothesized that the Pro198Leu genotypes could also affect mercury levels due to its additional effects on selenium metabolism. However, we found no evidence that this polymorphism influenced mercury levels, though it should be noted that only 5 hair samples contained mercury levels considered above the WHO's recommended concentration. Surprisingly, we also did not observe an effect of the *GSTM1* genotype on hair mercury levels, refuting our first hypothesis that the null genotype would result in higher mercury levels by disrupting an established detoxification mechanism, as shown previously (Barcelos et al., 2013; Barcelos et al., 2015; de Oliveira et al., 2014; Klautau-Guimarães et al., 2005). Again, it must be noted that these studies were conducted with highly exposed populations whose mercury levels in blood ranged between 37 and 48 μg L^{-1}. Nevertheless, studies that assessed the effect of *GSTM1* deletion in mild mercury exposed populations (mercury levels in blood ranging from 1.34 to 3.6 μg L^{-1}) saw no impact of this polymorphism (Gundacker et al., 2007, 2009; Hanna et al., 2012; Lee et al., 2010). Gundacker et al. (2007) observed that in Austrian medical students with an average hair mercury level of 0.2 μg g^{-1}, the *GSTM1* null genotype had no effect when analyzed alone, but the double deletion polymorphism of *GSTM1* and *GSTT1* was associated with higher hair mercury concentrations. This information

raises an important question: does deletion of the GSTM protein reduce conjugation and excretion of mercury, resulting in a higher degree of retention? We predict that this outcome is relevant only in populations exposed to high levels, as mild exposure would be able to maintain sufficient mercury detoxification in the absence of GSTM. Secondary to this, does deletion of another GST protein, such as GSTT, combined with the *GSTM1* null genotype result in individuals being more vulnerable to mercury intoxication even under mild exposure conditions? As the sample size was the major limitation of this pilot trial, we were not able to analyze other *GST* polymorphisms, as well as the interaction between Pro198Leu polymorphism and *GSTM1* deletion. The identification of only two polymorphisms is also another limitation of our study. Thus we emphasize the need for more genotype interaction studies, with larger sample sizes, aimed at identifying their impact on mercury intoxication risks in both mild and highly exposed populations.

5. Conclusions

For the first time, our study showed that Pro198Leu does not influence mercury levels in an established biomarker of exposure within a mildly exposed population. We also observed that *GSTM1* deletion had no effect on mercury levels in mildly exposed people, suggesting that the isolate deletion of *GSTM* gene has impact only in highly exposed populations.

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