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A preliminary study of mercury exposure and blood pressure in the Brazilian Amazon

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Abstract

Background: Fish is considered protective for coronary heart disease (CHD), but mercury (Hg) intake from fish may counterbalance beneficial effects. Although neurotoxic effects of methylmercury (MeHg) are well established, cardiovascular effects are still debated. The objective of the present study was to evaluate blood pressure in relation to Hg exposure and fish consumption among a non-indigenous fish-eating population in the Brazilian Amazon.

Methods: The study was conducted among 251 persons from six communities along the Tapajós River, a major tributary of the Amazon. Data was obtained for socio-demographic information, fish consumption, height and weight to determine body mass index (BMI), systolic and diastolic blood pressure, and Hg concentration in hair samples.

Results: Results showed that overall, systolic and diastolic blood pressure, were relatively low (mean: 113.9 mmHg \pm 14.6 and 73.7 mmHg \pm 11.0). Blood pressure was significantly associated with hair total Hg (H-Hg), age, BMI and gender. No association was observed between fish consumption and blood pressure, although there were significant inter-community differences. Logistic regression analyses showed that the Odds Ratio (OR) for elevated systolic blood pressure (\geq 130 mmHg) with H-Hg \geq 10 μ g/g was 2.91 [1.26–7.28], taking into account age, BMI, smoking, gender and community.

Conclusion: The findings of this preliminary study add further support for Hg cardiovascular toxicity.

Background

Mercury (Hg), a worldwide pollutant transported by air and water throughout the planet, poses a particular chal-

lenge to global health. On the one hand, Hg is recognized as one of the most dangerous environmental contaminants [1]. On the other hand, fish, a very nutritious food,

is the major vehicle for its transmission to humans in its organic form, methylmercury (MeHg). For populations that rely on fish as their main source of protein, this represents an important public health dilemma [2,3], particularly since recent evidence suggests that fish can be both cardioprotective and cardiotoxic, depending upon their contribution to essential fatty acids and Hg body burden [4-8].

Fish is considered a very healthy food because it is rich in proteins, poor in saturated fats, and can be protective for coronary heart disease (CHD) [9,10]. Marine fish oils are rich in omega-3, an essential fatty acid, known to reduce CHD risk [11-14]. Populations that traditionally consume large amounts of marine fish generally experience lower rates of mortality from heart disease [15-18]. But Hg intake from fish may counterbalance beneficial effects [6,7]. Hg exposure has been associated with an increased risk of myocardial infarction, as well as death from cardiovascular disease and all causes [4,19]. Guallar et al. observed both the negative effects of Hg exposure and the positive effects of omega-3's in a case-control study involving a group of men with a first diagnosis of myocardial infarction and a reference group of healthy men; risk for myocardial infarction increased with Hg levels in toenails and decreased with serum omega-3 levels [4]. The United States Health Professionals Follow-up Study did not show an association between Hg and risk for coronary heart disease, but dentists, with elemental Hg exposure, made up 63.6% of controls [20]. Moreover, both of these studies used total Hg concentration in toenails and did not differentiate between organic and inorganic Hg.

Blood pressure, a good indicator of risk for cardiovascular disease, is a parameter relatively easy to measure, even in remote field conditions. Bulliyya et al. showed that fish consumption was associated with lower mean systolic and diastolic blood pressure among older men and women from coastal fishing villages in India [21]. In contrast, an increased incidence of hypertension and cerebrovascular disease has been reported among aging patients with chronic Minamata disease [22]. In the Greenland Inuit population, autopsies revealed that MeHg levels in organs are generally high, and blood pressure levels are similar to those in industrialized countries [23]. Epidemiologic studies relating MeHg and blood pressure have reported inconsistent findings [24,25]. In animal studies, fish proteins lowered blood pressure in spontaneously hypertensive rats [26-28], while in rats that had developed hypertension after sucrose administration, fish oils were able to reverse the alterations on metabolic parameters and blood pressure [29]. However, long term experimental studies suggest that low dose MeHg exposure can lead to irreversible hypertension that remains many months after cessation of exposure [30].

In the Brazilian Amazon, a number of studies have reported high levels of Hg in fish and in humans, and significant relations between fish consumption and bioindicators of Hg exposure [31-38]. Extensive interdisciplinary studies on the source, transmission and Hg contamination and its effects on populations of the Tapajós River have shown that Hg contamination is very widespread in this region and mainly derived from deforestation and 'slash and burn' agricultural practices [39-43]. When erosion drains soil sediments into the waterways, the inorganic Hg, naturally present in these soils, is transformed into MeHg through bacterial activity and enters the aquatic food chain [44-46]. For the traditional populations, living on the banks of the Amazon and its tributaries, fish is the dietary mainstay, with the large majority eating fish daily or several times a week [34-36,38].

Most communities living along the Tapajós River are not indigenous peoples, but traditional communities with mixed ethnic backgrounds [47]. Their diet consists primarily of fish, with manioc, rice, tomatoes, beans, fruit and some meat [36]. Common risk factors for high blood pressure, such as sodium intake, obesity and a sedentary life style are rare. As part of a global, interdisciplinary project on the sources, transmission and effects of Hg in a riverside Amazonian environment (State of Pará, Brazil), we conducted this preliminary study to examine blood pressure parameters with respect to Hg exposure and fish consumption among traditional communities living along the Tapajós River.

Methods

Population and sampling

The study population was from 6 communities (São Luiz do Tapajós, Nova Canaã, Santo Antônio, Mussum, Vista Alegre and Açaituba), living along the Tapajós River, a major tributary of the Amazon River (Figure 1). Since it was not possible to carry out a rigorous random sampling strategy in the conditions of the Amazon, a convenience sample was used and the age and sex distributions were compared to the underlying population, determined through a house-to-house survey. Recruitment into the study was carried out during the house-to-house survey and at village meetings, during which the research project was explained, and villagers were invited to participate on a voluntary basis. Inclusion criteria were persons above 15 years of age and for the present study, those with reported diabetes were excluded.

The study was approved by the Federal University of Rio de Janeiro, which has a mandate from the Ethics Review Board of the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) of Brazil, and the University of Quebec at Montreal and all participants signed an informed consent form, which was read to them.

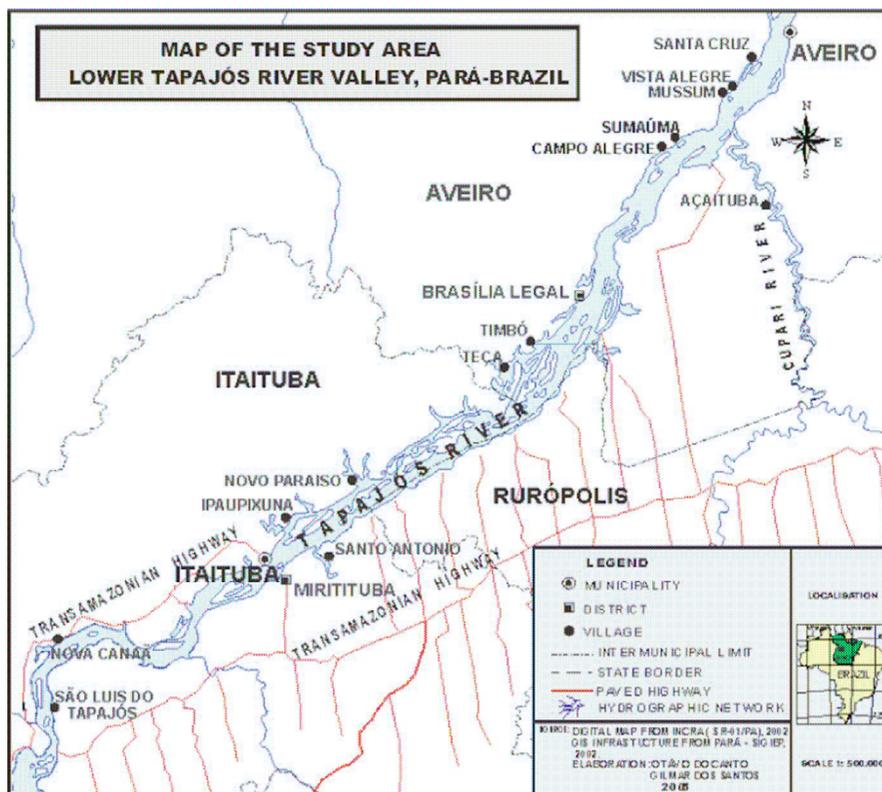


Figure 1
Map of the study area

Socio-demographic information

Two trained interviewers met the participants in their villages. All participants went through an interview of approximately one hour. A questionnaire was used to determine the socio-demographic variables: age, education, work and residence history, smoking and drinking habits, as well as fish consumption and medical history. At the end of the interview, all participants were weighed and measured, and body mass index (BMI = weight (kg)/height (m)²) was calculated.

Characterization of fish consumption

A seven-day recall of fish consumption was used. To facilitate the detailing of their fish consumption, a list of the fish species usually consumed in the Tapajós region was prepared [48]. For each of the past seven days, the participants indicated the number of fish meals and the species consumed at each meal. Fish that were not on the list were likewise noted.

Mercury exposure assessment

Hair has been extensively used as a bioindicator for current and retrospective evaluation of Hg [49]. This non-

invasive method provides samples that can be stored for a long time without deterioration before being analyzed. When hair grows, the intense metabolic activity at the follicle level exposes hair to elements present in the blood, including heavy metals [50].

In the present study, hair strands from the root were cut from the occipital region and stored in plastic bags, with the end root stapled. The first centimetre was analyzed for each sample. Hair mercury concentration (H-Hg) was determined by cold vapour atomic absorption spectrometry (CVAAS) after digestion with HNO₃, H₂SO₄ and K₂MnO₄ and spinning with hydroxylamine chlorhydrate before reading [51]. Hair samples were analyzed in the Laboratório de Traçadores, Centro de Ciências da Saúde, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brazil. Precision and accuracy of Hg determination were ensured using internal hair standards, provided by the International Atomic Energy Association.

Blood pressure assessment

The same nurse measured blood pressure throughout the study, after each participant had been sitting for five minutes, relaxed and not moving. Blood pressure was assessed in a seated position, with the arm supported at heart level, and without any tight clothing constricting the arm. The cuff was placed with the centre of the bladder over the brachial artery. Systolic and diastolic pressures, recorded in increments of 1 cmHg (10 mmHg), were assessed using a sphygmomanometer (Blood Pressure Monitor Kit, Mark of Fitness, Model MF-20, Stethoscope attached to cuff). The nurse had no knowledge of participants' Hg exposure, and since blood pressure was assessed prior to administration of the health questionnaire, nor was she aware of the potential risk factors such as smoking and diabetes.

Statistical analyses

Data was entered and analyzed in the StatView (Version 5.0.1) software (SAS Institute). Descriptive analyses were performed to characterize the population. Multivariate analyses were performed to identify the factors that influenced systolic and diastolic blood pressure. Logistic regression analyses, using categorized data, were used to determine the Odds Ratio (OR) for elevated arterial blood pressure.

Results

Relevant data were collected from 259 adults (≥ 15 years of age) representing 39.2% of the total adult population. Participants' age and sex distribution were similar to the underlying population (Table 1). The age distribution in the population is typical of that found in developing countries, with about 45 % of the population under 15 years of age (543 people < 15 years from a total population of 1204 persons).

Of the 259 adults who participated in the study, 7 were excluded from the present analyses for reported diagnosed diabetes, a known risk factor for hypertension and 1 for missing data. The characteristics of the remaining 251 participants are reported in Table 2. Mean age of the study population was 35.2 years (15 to 89 years); 19.9% were over 50. Only 17.5% of the population was overweight,

with a BMI above 25 kg/m², and 4% were considered obese (BMI > 30 kg/m²). Although 29.8% of the population smoked, the mean number of cigarettes/day was 8.0 (median = 5.0).

Fish consumption

Mean fish consumption was 6.8 ± 4.7 meals in the week preceding the interview, for an average of 1 fish meal/day, with 3.3 ± 3.6 piscivorous fish meals and 3.4 ± 3.2 non-piscivorous fish meals. The five species most commonly consumed were: Aracu (*Shizodon* sp.), Pescada (*Plagioscion* sp.), Tucunaré (*Cichla* sp.), Caratinga (*Geophagus* sp.) and Pacu (*Mylossoma* sp.). Fish consumption did not vary with any of the socio-demographic variables listed in Table 2.

Mercury exposure

Mean H-Hg was 17.8 µg/g ± 12.0 (0.21 µg/g – 77.2 µg/g) and 69.7% of the participants had H-Hg ≥ 10 µg/g. Multiple regression analysis of the variables that influence H-Hg showed that men had higher levels than women (p = 0.04) and decreased with educational level (p = 0.04). There were significant differences between communities (p < 0.001) and a positive association with consumption of piscivorous fish (β = 0.70; p = 0.002), but not non-piscivorous fish (p = 0.23).

Blood pressure

Mean systolic pressure was 113.9 mmHg ± 14.6, ranging from 90 mmHg to 170 mmHg and mean diastolic pressure was 73.7 mmHg ± 11.0, ranging from 60 mmHg to 110 mmHg.

Univariate analyses showed that systolic blood pressure was positively associated with age (r² = 0.12; p < 0.001), higher in men than in women (r² = 0.04; p = 0.001), positively associated with BMI (r² = 0.06; p < 0.001), higher among smokers (r² = 0.03; p = 0.01), and positively associated with H-Hg levels (r² = 0.02; p = 0.046). There were also borderline significant differences between communities (adjusted r² = 0.02; p = 0.05). For diastolic blood pressure, age (r² = 0.15; p < 0.001), sex (r² = 0.04; p < 0.001), BMI (r² = 0.08; p < 0.001) and smoking (r² = 0.03; p < 0.01) were correlated. The relation between H-Hg and diastolic pressure was not significant (p = 0.15).

Multivariate analyses showed that all of the previous variables, with the exception of smoking entered significantly into the regression model for systolic pressure: adjusted r² = 0.21; p < 0.001, with H-Hg explaining 1.7% of the total variance (β = 0.14; p = 0.03). For diastolic pressure, only age, sex and community entered significantly into the model. Fish consumption, measured by the total number of fish meals over the seven days previous to the interview, did not enter into the models (p = 0.33). Species-specific analyses with the five most commonly consumed fish

Table 1: Age distribution and participation in the study population

Age categories	Total population	# of participants	% participation
15 – 24	217	79	36.4
24 – 34	142	57	40.1
35 – 44	117	56	47.9
45 – 54	84	29	34.5
55 – 64	58	23	39.7
≥ 65	43	15	34.9
Total	661	259	39.2

Table 2: Socio-demographic characteristics of the study population

Characteristics	Women			Men		
	n	Mean ± SD	%	N	Mean ± SD	%
Age (years)	118	34.4 ± 15.3	100	133	35.8 ± 16.2	100
Education (years)	118	4.1 ± 2.6	100	133	3.3 ± 2.5	100
<i>Alcohol consumption</i>						
Drinks	27		22.9	67		50.4
No longer drinks	15		12.7	25		18.8
Never drank	76		64.4	41		30.8
<i>Smoking habits</i>						
Smokes	25		21.2	50		37.6
No longer smokes	22		18.6	28		21.0
Never smoked	71		60.2	56		41.4
Ever suffered malaria	56		47.5	94		70.7
Body mass index	118	22.5 ± 4.1	100	133	22.2 ± 3.0	100

(Aracu, Pescada, Tucunaré, Caratinga, Pacu) also showed no influence of fish consumption on systolic and diastolic blood pressures. Having suffered from malaria was not related to H-Hg or to blood pressure. Figure 2 shows a scatter plot of systolic blood pressure, adjusted for the significant covariates, in relation to H-Hg.

Only 8% of the study group suffered from hypertension (systolic pressure ≥ 140 mmHg). Thus, for logistic regression analyses, elevated blood pressure (≥ 130 mm Hg)

was used as a cut-off. A total of 52 persons (21.0%) had elevated systolic blood pressure while 42 (16.7%) had elevated diastolic pressure (≥ 90 mm Hg). Elevated systolic pressure was higher in those over 50 years (36.0% vs. 16.9%; Fisher's Exact test: p < 0.01), for those with BMI above 25 kg/m² (36.4% vs. 17.4%; p = 0.01) and for smokers (29.3% vs. 17.1%; p = 0.04); men had a higher prevalence than women (26.3% vs. 14.4%; p = 0.02), as did those with H-Hg levels ≥ 10 µg/g (24.0% vs. 13.2%; p = 0.06). Prevalence of elevated systolic pressure varied

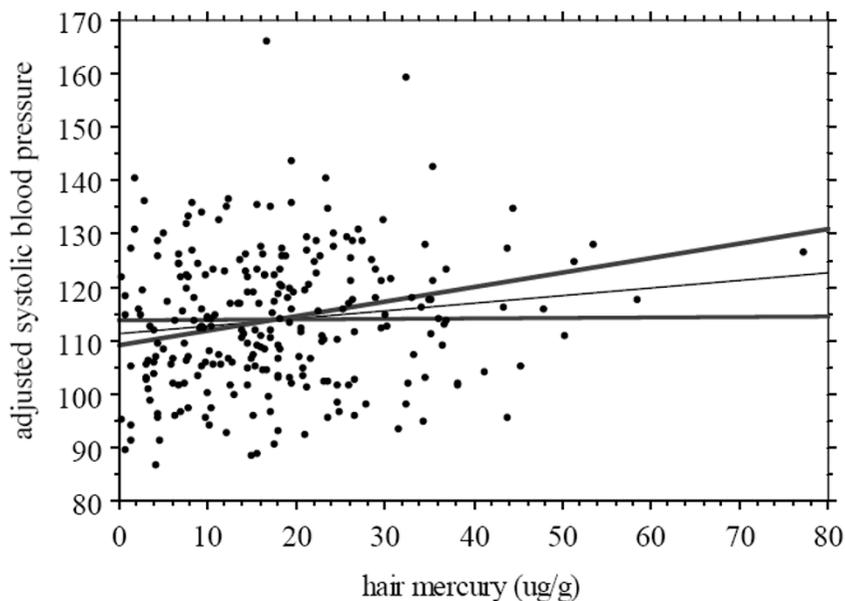


Figure 2 Scatter plot of systolic blood pressure (adjusted for covariates: age, sex, BMI, smoking and community) in relation to mercury exposure; regression line and 95% confidence interval (dotted lines)

between communities (Pearson χ^2 : $p = 0.03$), with the highest at 35.9% and the lowest at 10.8%. Elevated diastolic pressure was significantly associated with age over 50 years (30.0% vs. 13.4%; Fisher's exact test: $p = 0.01$), BMI over 25 (29.6% vs. 14.0%; $p = 0.02$), while differences between men and women showed a tendency (21.1% in men vs. 11.9% in women; $p = 0.06$) and the difference for H-Hg was not significant (11.8% for H-Hg < 10 $\mu\text{g/g}$ vs. 18.9% for H-Hg $\geq 10 \mu\text{g/g}$; $p = 0.20$). No difference was observed between communities ($p = 0.18$).

Logistic regression analysis, performed with the independent variables age, sex, BMI, smoking, community and H-Hg showed a significant risk with an Odds Ratio (OR) of 2.91 [1.26–7.28] for elevated systolic pressure with H-Hg $\geq 10 \mu\text{g/g}$ (Table 3); while elevated diastolic pressure showed a tendency ($p = 0.08$) (Table 4).

Discussion

This preliminary study shows that, in this population, where fish is a dietary mainstay, blood pressure is relatively low, with only 8% displaying hypertension (systolic pressure $\geq 140 \text{ mmHg}$). However, there is a significant dose-effect relation between Hg exposure and blood pressure. Even at these relatively low levels of blood pressure in a fairly young population, an increase was observed with age, BMI, and men had higher blood pressure as compared to women, confirming that the methods were sensitive enough to detect expected changes. These findings, in a population with minimal risk factors for hypertension and with an elevated environmental Hg exposure, offer strong support to a negative effect of Hg on blood pressure parameters.

Because of low blood pressure, we used 130 mmHg as the threshold for elevated blood pressure, as suggested by "The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure", whose guidelines indicate that individuals with a systolic blood pressure of 120 to 139 mmHg or a diastolic blood pressure of 80 to 89 mmHg should be considered as pre-hypertensive [52]. Others have suggested that the threshold of systolic blood pressure of 130

Table 4: Odd's Ratios from multivariate logistic regression model for elevated diastolic blood pressure ($\geq 90 \text{ mmHg}$). Mutual adjustment is included.

Variable	OR	p
Hair mercury ($\geq 10 \mu\text{g/g}$)	2.29 [0.95–6.06]	0.08
Age (≥ 50 years)	2.18 [0.96–4.83]	0.06
Sex (men/women)	2.16 [0.98–4.97]	0.06
BMI ($\geq 25 \text{ kg/m}^2$)	2.95 [1.23–7.03]	0.01
Smoking (smokers/non)	1.62 [0.75–3.51]	0.22
Community		0.04

mmHg is a limit for "borderline hypertension" [53]. In the present study, those with hair Hg levels of 10 $\mu\text{g/g}$ or more had over twice the risk of presenting systolic blood pressure of at least 130 mmHg.

A positive relation between Hg and blood pressure has been reported in animal studies [30], but human studies are less consistent [20]. In a recent study, Pedersen et al. [8] measured blood Hg and blood pressure among four groups of healthy subjects: 1) Danes living in Denmark consuming European food; 2) Greenlanders living in Denmark consuming European food; 3) Greenlanders living in Greenland consuming European food; 4) Greenlanders living in Greenland consuming mainly traditional Greenlandic food. They reported higher blood Hg in Greenlanders as compared to Danes, which they attributed to their higher fish consumption. Pulse pressure was higher and diastolic blood pressure lower in Greenlanders than Danes and blood Hg was positively correlated to pulse pressure. Another study showed that in middle-aged Finnish men, Hg accumulation in the body was associated with accelerated progression of carotid atherosclerosis [19]. In that study, the strongest predictors of the progression of atherosclerosis were elevated systolic blood pressure, high H-Hg content, treatment for dyslipidemia, high dietary intake of iron, cigarette smoking, and old age. The authors suggest that Hg may be a major environmental risk factor for atherosclerosis in humans, even at subtoxic levels, which have not been previously recognized as harmful.

Several studies have reported associations between prenatal exposure to MeHg and cardiovascular functions; however, manifestations vary from one study to another. Although a study on patients with foetal Minamata disease showed that parasympathetic nervous dysfunction might exist in these patients, they did not show elevated blood pressure [55]. Sørensen et al. [56] reported increased systolic and diastolic blood pressures among the Faroese 7 year olds, born from mothers without hypertension, in relation to prenatal MeHg exposure. At 14 years of age, there was no relation with blood pressure, although MeHg exposure was associated with decreased

Table 3: Odd's Ratios from multivariate logistic regression model for elevated systolic blood pressure ($\geq 130 \text{ mmHg}$). Mutual adjustment is included.

Variable	OR	p
Hair mercury ($\geq 10 \mu\text{g/g}$)	2.91 [1.26–7.28]	0.02
Age (≥ 50 years)	2.35 [1.07–5.13]	0.03
Sex (men/women)	2.27 [1.08–4.98]	0.03
BMI ($\geq 25 \text{ kg/m}^2$)	3.17 [1.35–7.22]	0.01
Smoking (smokers/non)	1.69 [0.81–3.51]	0.15
Community		0.01

sympathetic and parasympathetic modulation of heart rate variability [57].

Dórea et al. [25] reported a trend of lower increase in blood pressure with age among the higher fish consumers in the Amazonian region, but had no direct measure of fish consumption or of Hg levels. Although in the present study, we did not observe a dose-response relation between fish consumption and blood pressure, the relatively low blood pressure observed in the Tapajós riverine villagers may be related to their general diet of fish, coupled to other lifestyle factors. Some fish may have more of a positive effect than others, but this was not apparent in the present study, which relied on the number of fish meals over the past seven days as an indicator of fish eating habits. Cardio-protection has been reported in relation to omega-3 [15-17,58] and to fish consumption [10]. However, studies of freshwater sports fishers showed no relation between fish consumption, omega-3 levels in blood and/or blood pressure [59,60]. Freshwater fish have lower levels of omega-3 fatty acids compared to marine fish [2,61], which might explain the lack of relation between fish consumption and blood pressure in this study and others, but no data on fish omega-3 levels exist for Amazonian fish.

Studies have shown that fish cooking methods, such as deep frying and surface frying with oil, changes the lipid and moisture content as well as the fatty acid composition of fish products [62,63]. Deep-frying Baltic herring (*Clupea harengus membras*) in rapeseed oil changed the fatty acid compositions to that of the frying oil, increased the amounts of monounsaturated fatty acids and omega-6, while decreasing the levels of omega-3 [62]. Better fatty acid ratios have been observed for roasted salmon compared to fried samples; roasting did not modify the fat content, whereas frying increased the fat content 2-fold [63]. In the Tapajós region, fish is mainly fried or boiled, which possibly influences the fatty acid composition.

Another possible explanation of low blood pressure levels in this population could be low sodium intake. There are few prepared foods with high sodium content in these villages and salt intake comes principally from salting food. Further studies on cardiovascular function in these communities should assess urinary sodium concentrations.

There are several limitations to this preliminary study. First, a convenience sample was used. Although data collection on convenience samples has been shown to appropriately represent the underlying population in other settings [64,65], this sampling strategy may have introduced some selection bias in the present study. We did, however, achieve a participation rate of 35% in this adult population, well represented in most age categories. Sec-

ond, although persons did relax quietly for 5 minutes prior to taking blood pressure, only a single measure was used. This would, however, increase the variability of the response and tend to minimize any relation with Hg exposure.

In the Brazilian Amazon, our research group is working with local populations to identify factors that influence Hg uptake and metabolism in fish and humans in order to maximize the nutritional intake from fish and minimize toxic risk. This is an appropriate region to carry out case control studies to further knowledge on the positive and negative effects of fish consumption and Hg exposure on cardiovascular health. More extensive studies on cardiovascular parameters, including R-R variability and the role of fatty acids are currently under way.

Abbreviations

BMI – body mass index

CHD – coronary heart disease

Hg – mercury

H-Hg – hair total mercury

MeHg – methylmercury

OR – Odds Ratio

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

MF is a doctoral student. She participated in the fieldwork, data entry and analysis and writing of the present paper. DM is principal investigator of this study. As such, she participated in the design and the planning, data analysis and writing of the present paper. MF and DM contributed equally to this work. CJSP is a doctoral student. He coordinated the fieldwork and the laboratory analysis for hair mercury levels and participated in the data entry. FL is a professor in the mathematics department of Université du Québec à Montréal. He supervised the statistical analysis. ML is a doctoral student who participated in the preparation and realisation of the fieldwork. JRDG is a co-investigator in the study. He supervised the fieldwork and the laboratory analyses for hair mercury. All authors read and approved the final manuscript.

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