Association of mercury exposure with blood pressure among adults near artisanal and small-scale gold mining in Madre de Dios, Peru

by

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Abstract
There have been few studies investigating the association between mercury exposure and blood pressure, with inconsistent results. In this study, the association between hair mercury concentration with mean arterial pressure (MAP) and hypertension were evaluated using data collected in a 2015 cohort study, which sampled 23 communities in Madre de Dios, Peru. This area has recently experienced a rapid increase in artisanal and small-scale mining, which is the main anthropogenic source of mercury emissions. Generalized estimating equations were used to account for correlation within communities. Analyses for MAP and hypertension were performed using linear and logistic models, respectively, and confounding variables were included in both models. Due to the significant (p-value < 0.05) interaction between sex and mercury in both models, the analysis was stratified by sex. In women, there was an inverse association between hair mercury concentration with hypertension (OR: 0.84; 95% CI: 0.50–1.41) and MAP (gMR: 0.99; 95% CI: 0.98–1.008), but these associations were not significant at a 5% significance level. In men, the associations between hair mercury concentration with hypertension (OR: 3.07; 95% CI: 1.36–6.92) and MAP (gMR: 1.024; 95% CI: 1.01–1.04) were positive and significant at a 5% significance level. Differences observed between sex could be attributable to differences in exposure, men eating greater amounts of mercury-contaminated fish, or sex hormones, which regulate the distribution and excretion of mercury in the body.

Executive Summary

Background

Few studies have investigated the association between mercury exposure and blood pressure, and even then, results are inconsistent. We evaluated the association of hair mercury concentration with mean arterial pressure (MAP) and hypertension using data from the Amarakaeri Reserve Cohort Study conducted in 2015 among residents of 23 communities in Madre de Dios, Peru. This area has recently experienced a rapid increase in artisanal and small-scale mining (ASGM). ASGM is the number one source of anthropogenic mercury emissions globally, releasing toxic mercury into soil and waterways during gold extraction or emitting mercury into the air when the gold amalgam is burned. In aquatic environments, mercury undergoes methylation and biomagnifies up the food chain as methylmercury (MeHg), accumulating in predatory fish in concentrations up to 100 million times greater than that of the water (Chen et al. 2012).

Methods

The greatest source of human mercury exposure in our study area is the consumption of mercury-contaminated fish and wildlife. The majority (80%) of mercury in hair is MeHg which is stable over time, making hair the best long-term measure of mercury exposure (Berglund et al. 2005). Three tufts of hair (~0.5 cm in diameter) were cut from the root, stored at ambient conditions, and analyzed using a Milestone DMA-80 in Dr. Helen Hsu-Kim's laboratory at Duke University (Durham, NC). Blood pressure was measured using an Omron blood pressure monitor (10 Series, BP785). Body mass index (BMI) was calculated using height and weight measurements. Height and weight were measured using a portable height measuring device and Omron HBF-514 scale, respectively. Smoking status and physical activity were self-reported on the distributed survey. Serum was collected and analyzed for fatty acids, cholesterol and triglycerides at MedLab Peru, a laboratory in Lima, Peru. Dietary covariates assessed in our analysis, such as extent of western diet and dietary energy and nutrient intake, were estimated using the food frequency questionnaire distributed to the sample population and the 2018 Norwegian Food Composition Table (NFSA 2018).

MAP and hypertension were the outcomes of interest: MAP is a continuous measure calculated using diastolic and systolic blood pressure measurements; Hypertension is a binary outcome defined using the 2017 American Heart Association guidelines (average systolic blood pressure greater than or equal to (≥) 130 mmHg and/or diastolic blood pressure ≥ 80 mmHg). To account for correlated data within community, generalized estimating equations were used to assess the association between hair mercury concentration and the two blood pressure measures. Analysis of hypertension was performed using logistic regression and expressed as odds ratios (ORs). MAP was natural-log transformed and analyzed using linear regression, generating geometric mean ratios (gMRs). All measures of association were accompanied by 95% confidence intervals (CI) and p–values and adjusted for sex, age, BMI, and whether or not the community was native. In addition, the MAP model controlled for smoking status, serum dihomo-γ-linolenic acid concentration (mol%) and serum triglycerides (mg/dL). The hypertension model, on the other hand, controlled for dietary protein intake and serum stearic acid concentration (mol%). All models were examined for collinearity and the interaction between mercury and sex.

Results

In total, our dataset included complete information for 1,432 participants. The majority of our sample population was female (71%). Compared to the most recent census (2017), the distribution across age group in our sample population reflects that of Madre de Dios, with 50% of our sample population being between the ages of 18 and 34, and 41% being between 34 and 50 years old. Average hair mercury concentrations were higher in men (3.0 µg/g) compared to women (2.4 µg/g). The average hair mercury concentration was significantly higher in natives versus non-natives (4.56 µg/g versus 1.98 µg/g, respectively, p-value<0.0001). The average hair mercury concentration increased with age, peaking in native women aged 65 and up (7.7 µg/g).

According to our analysis, 14% of our sample population is hypertensive. Of the hypertensive participants 53% were female and 47% were male. There were 337 (24%) natives in our sample population and 22% of them were hypertensive, which was double the prevalence of hypertension among non-natives (11%). The average MAP for the entire sample population was within normal range (84 ± 10 mmHg). When stratified by both sex and native status, the average MAP was still within normal range. MAP was lower among females (82 ± 9 mmHg) compared to males (89 ± 10 mmHg), with the highest MAP occurring among the native male population (91 ± 11 mmHg).

In both models, there was a significant interaction between mercury levels and sex. After stratifying by sex, no variables were associated with hypertension at a 5% significance level among women. The association between mercury levels and hypertension in women, although non-significant, was inverse (OR: 0.84; 95% CI: 0.50–1.41; p-value=0.512). Among men, the association was positive with the odds of hypertension increasing 3-fold (OR: 3.07; 95% CI: 1.36–6.92; p-value=0.007) with each 2.7-fold increase in hair mercury concentration. No other variables were significant at a 5% significance level. In the MAP model, mercury levels were also inversely associated with MAP in women (gMR: 0.99; 95% CI: 0.98–1.008; p-value=0.311), but positively associated in men (gMR: 1.024; 95% CI: 1.01–1.04; p-value=0.012). The only other variable that was significant at a 5% significance level in men was BMI. With a BMI $\geq$ 30 kg/m^2, MAP is expected to increase 8.9% (gMR: 1.089; 95% CI: 1.02–1.16; p-value=0.010). Among women, age (gMR:1.002; 95% CI:1.00–1.003; p-value=0.045) and being a resident of a native community (gMR: 1.04; 95% CI: 1.00–1.089; p-value=0.050) were significantly associated with MAP.

Conclusion

In this study, the association between hair mercury concentration with hypertension and MAP differed by sex, being positive in men and inverse in women. The associations between hair mercury concentration with hypertension and MAP were significant at a 5% significance level among men, but not women. If confirmed, these findings may be attributable to differences in diet, men eating more mercury-contaminated fish and wildlife, as well as, the effect of sex hormones, which regulate distribution and excretion of mercury in humans. Hypertension prevalence as well as mercury and MAP levels were higher in native communities compared to non-native communities, which may also be attributable to increased consumption of mercury-contaminated fish and wild-life. Average mercury and MAP levels were highest among native women aged 65 and up. Further investigation of the differences between native and non-native

communities, including diet and specifically fish consumption, is warranted. Differences in mercury level between the age groups in these communities should also be further investigated. Additionally, the only interaction evaluated in this analysis was between mercury and sex. The study of other effect modifications with mercury is warranted, including age, serum stearic acid, and dietary protein intake.

Introduction

In 2010, 1.39 billion (31.1%) adults worldwide aged 20 and up were estimated to have hypertension, which was a 5.2% increase in global prevalence from 2000 (Mills et al. 2016). The cut-points for hypertension have changed over the years. Most recently, the American Heart Association (AHA) and collaborators[1] published guidelines in 2017 distinguishing hypertension as systolic blood pressure (SBP) greater than or equal to (≥) 130 millimeter of mercury (mmHg), and/or diastolic blood pressure (DBP) ≥ 80 mmHg (Whelton et al. 2017). Before these guidelines, the cut-points for hypertension were 140 and 90 mmHg for SBP and DBP, respectively (Whelton et al. 2017). SBP is the pressure in your blood vessels when your heart beats, and DBP is the pressure in your blood vessels when your heart rests between beats (CDC 2017a). Hypertension is described as the "strongest or one of the strongest risk factors for almost all different cardiovascular diseases" as well as for premature cardiovascular disease (Kjeldsen 2018). Lim et al. (2012) assessed 43 risk factors for the 2010 adult burden of disease in 21 regions and found high blood pressure to be the number one risk factor globally. For 20 of the 21 regions investigated, high blood pressure was in the top five ranked risk factors. In 8 of these regions, high blood pressure was ranked the number one risk factor. See Appendix 1 for the rank of high blood pressure in all 21 regions. A number of cardiovascular and heart diseases associated with hypertension were considered in their estimation, including ischaemic heart disease, rheumatic heart disease, stroke, chronic kidney disease, myocarditis and peripheral vascular disease (Lim et al. 2012). According to their findings, 7% of global disability-adjusted life years (DALYs) and 9.4 million deaths were attributed to high blood pressure in 2010 (Lim et al. 2012). Figure 1 displays the proportion of DALYs for the top 20 risk factors and their associated burden of disease. In Figure 1, it is made evident that cardiovascular and circulatory diseases represent the greatest global burden of disease. Recently, a comparative risk assessment was published evaluating the risk factors for the global burden of disease in 2017 (Stanaway et al. 2018). This assessment showed that 7 years later high blood pressure is still the leading risk factor for global burden of disease (Stanaway et al. 2018). In addition, the estimated number of deaths attributable to high blood pressure in 2017 increased to 10.4 million (Stanaway et al. 2018). According to Rose Stamler (1991), decreasing mean population SBP by 5 mmHg would "result in 14% fewer deaths from stroke, 9% fewer deaths from [coronary heart disease], and 7% fewer deaths overall".

[1]American College of Cardiology, American Heart Association, American Academy of Physician Assistants, Association of Black Cardiologists, American College of Preventive Medicine, American Geriatrics Society, American Pharmacists Association, American Society of Hypertension, American Society for Preventive Cardiology, National Medical Association, and Preventive Cardiovascular Nurses Association.

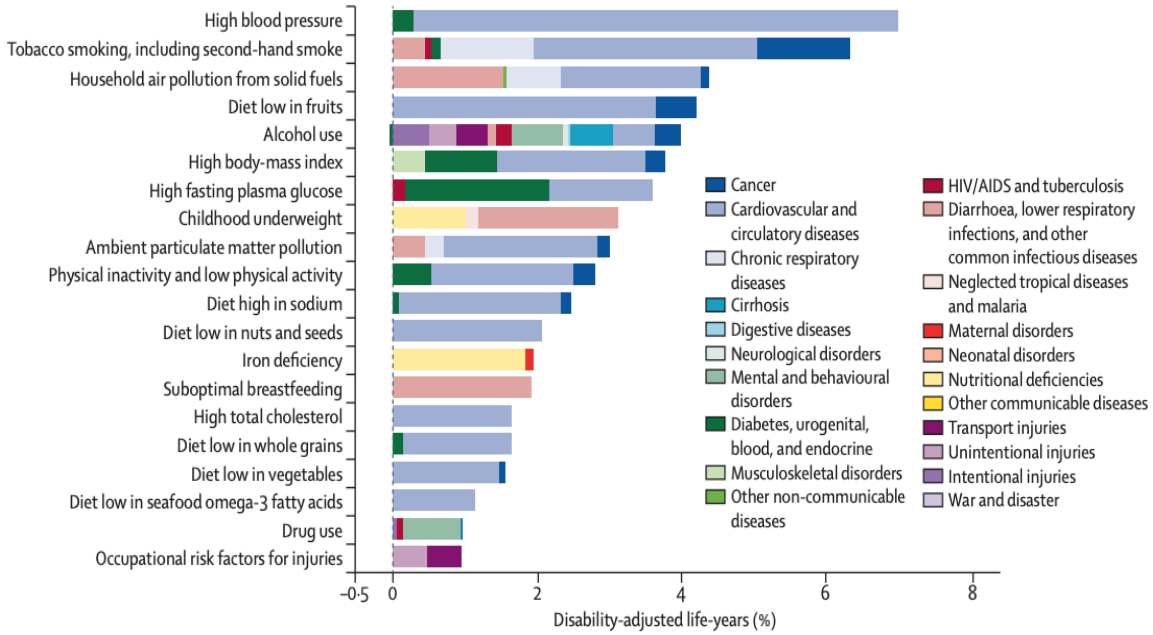

Figure 1. Burden of disease attributable to 20 leading risk factors in 2010, Lim et al. 2012

In recent years, more interest has been paid to the effect of chemical exposure, such as mercury, on cardiovascular health outcomes. Mercury is a persistent, heavy, silvery-white metal that occurs naturally in the earth's crust such as in the mineral cinnabar or in fossil fuels like coal and petroleum. This metal is released naturally into the environment by forest fires, volcanic eruptions and emissions from the ocean, as well as, through anthropogenic sources. The Global Mercury Assessment released by the United Nations Environment Programme (UNEP) in 2013 revealed the greatest proportion of global anthropogenic mercury emissions in 2010 were attributable to artisanal and small-scale gold mining (ASGM), representing 37% of emissions. The distribution of global anthropogenic sources of mercury emissions in 2010 are represented in Figure 2.

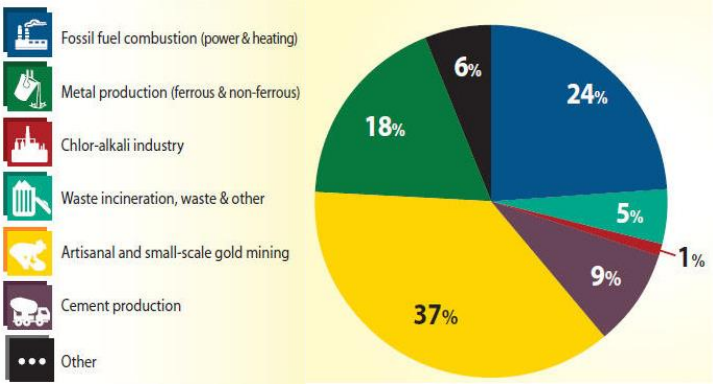

Figure 2. 2010 global anthropogenic sources of mercury emissions, UNEP 2013

Mercury (Hg) occurs in three forms: 1) elemental or metallic mercury, which exists in three oxidative states - elemental (Hg^0), mercurous (Hg^{+1}) and mercuric (Hg^{+2}); 2) organic mercury, which is mercury combined with carbon, forming mainly aryl or alkyl groups; and 3) inorganic

mercury, which is mercury combined with elements other than carbon, such as chlorine, sulfur and oxygen. Human absorption of mercury varies depending on its form but can occur via vapor inhalation, ingestion, injection and dermal absorption. The main route of human exposure to elemental mercury is in its vapor form once it has been heated, of which 70-85% is inhaled, 3% is dermally absorbed and only 0.1% is absorbed by the gastrointestinal tract (Bose-O'Reilly et al. 2010). On the other hand, 90-95% of organic mercury is absorbed by the gastrointestinal tract. Once absorbed, mercury is distributed throughout the body to the brain, kidney, liver, hair and skin. Elemental and organic mercury are more lipid-soluble than inorganic mercury, and therefore, can readily cross the blood-brain barrier and accumulate in the brain. Roughly 10% of methylmercury (MeHg), an organic alkyl mercurial, is distributed to the brain (Broussard et al. 2002). Although the half-life of mercury in humans will vary by individual, Broussard et al. (2002) reported the average biological half-life of inorganic mercury and MeHg to be 40 and 65 days, respectively. According to Broussard et al. (2002), the average half-life of elemental mercury in adults is 60 days. Although the half-life varies, all three forms of mercury are primarily excreted through urine and feces. Roughly 90% of organic mercury is excreted through feces alone (Bernhoft 2012). The human body burden of mercury is occupied mainly by organic mercury, of which 80% to 90% comes from the consumption of contaminated fish, seafood, and wildlife, and 75% to 90% of the mercury in these contaminated organisms is MeHg (Hong et al. 2012). Mercury in oceans, lakes, and rivers is transformed into MeHg by sulfate-reducing bacteria and molds. MeHg is bioaccumulative and biomagnifies through aquatic food webs. Therefore, the highest concentrations of MeHg are found in large predatory fish species such as tuna, swordfish, shark, and whale (CTEM 2000). In fact, concentrations of MeHg in predatory fish are up to 100 million times greater than concentrations in water (Chen et al. 2012). The biomagnification potential of mercury in humans makes the risk of its associated toxicity even more critical. Mercury is ranked as the third most toxic element or substance on the planet by the Agency for Toxic Substances and Disease Registry (ATSDR), and has been shown to have "cellular, cardiovascular, hematological, pulmonary, renal, immunological, neurological, endocrine, reproductive, and embryonic toxicological effects" (Rice et al. 2014).

Although the "mechanism by which mercury produces toxic effects on the cardiovascular system is not fully elucidated", there are a number of hypothesized biological mechanisms by which mercury exposure increases blood pressure (Fernandes Azevedo et al. 2012). One hypothesis is that mercury exposure increases blood pressure by inducing endothelial dysfunction, which is abnormal function of the blood vessels' inner lining (Furieri et al. 2011). Exposure to mercury reduces the "activity of antioxidant enzymes, such as glutathione peroxidase" and "increases the production of free radicals" (Fernandes Azevedo et al. 2012), such as superoxide anion, which decreases nitric oxide bioavailability, inducing endothelial dysfunction (Massaroni et al. 1995). Another hypothesis is that mercury exposure increases atherosclerosis, which is the buildup of substances such as fats and cholesterol in the arterial walls, forming plaques (Fernandes Azevedo et al. 2012; Salonen et al. 2000). These "plaques can narrow or completely block the arteries", increasing blood pressure (MedlinePlus 2019b). In this case, exposure to mercury inactivates the enzyme paraoxonase, which slows low-density lipoprotein (LDL) oxidation increasing atherosclerosis (Halbach 1990; Hulthe and Fagerberg 2002).

Hu et al. (2018) conducted a systematic review and meta-analysis investigating the effect of mercury exposure on hypertension. This study (Hu et al. 2018) included 29 studies, representing

55,000 participants and 17 countries. See Appendix 2, Table 1 for a list of these studies. "Comparing the highest and lowest mercury exposure categories" in fifteen of these studies, Hu et al. (2018) calculated a "pooled" odds ratio (OR) for hypertension of 1.24 [95% confidence interval (CI): 1.00-1.52; p-value <0. 0001; I^2 = 66:2%). See Appendix 2, Figure 1 for the ORs associated with each individual study. Nine of these fifteen studies reported a positive association between mercury exposure and hypertension (Bautista et al. 2009; Eom et al. 2014; Fillion et al. 2006; Guallar et al. 2002; Hu et al. 2017; Kobal et al. 2004; Shiue 2014; Virtanen et al. 2012a; Yorifuji et al. 2010). Three of the fifteen studies showed a negative association (Lee and Kim 2013; Mozaffarian et al. 2012; Nielsen et al. 2012 [Males]) and three showed no association (Nielsen et al. 2012 [Females]; Park et al. 2013; Siblerud 1990). Independent of this study, we conducted our own literature review on the association between mercury exposure and blood pressure. Of the 12 studies found, 5 showed no association (Grandjean et al. 2004; Park et al. 2013; Valera et al. 2011a; Valera et al. 2011b; Vupputuri et al. 2005) and 7 showed a positive association (Choi et al. 2009; Fillion et al. 2006; Pedersen et al. 2005; Salonen et al. 2000; Sørensen et al. 1999; Wells et al. 2017; Yorifuji et al. 2010). See Appendix 1, Table 2 for the summary characteristics of these studies. The findings of our literature review along with those conducted by Hu et al. (2018) confirm that studies on the association between blood pressure and mercury exposure are inconsistent, and therefore, warrant further study.

The United Nations Under-Secretary General and Executive Director of UNEP, Achim Steiner, exclaims that "there are no safe limits in respect to mercury and its organic compounds" (UNEP 2013). Yet the United States Environmental Protection Agency (USEPA) established an oral reference dose (RfD), defined as the numerical estimate of daily oral human exposure which is not likely to cause harmful effects during a lifetime, of 4-5 micrograms (µg) Hg per liter blood and 1 µg Hg per gram (g) hair (USEPA 1997), and an RfD for MeHg of 0.1 µg per kilogram body weight per day (USEPA 2001). Achim Steiner emphasizes that the "impacts of mercury on human health have been known for centuries if not millennia" (UNEP 2013). However, it was not until the discovery of severe mercury poisoning in Minamata City in Japan in 1956, that the human health impacts of mercury received international recognition. Between 1932 and 1968, MeHg was released via Chisso Corporation's chemical factory wastewater, which bioaccumulated in the fish and shellfish eaten by the residents of Minamata. This exposure resulted in 2,265 victims of the neurological syndrome now known as Minamata disease (Tulchinsky and Varavikova 2014). Named after this poisoning event, the Minamata Convention on Mercury, an international treaty, started in 2013 and entered into force in August 2017. The objective of this global environmental agreement is to "protect the human health and the environment from anthropogenic emissions and releases of mercury and mercury compounds" (UNEP 2017). The UNEP (2013) predicts that most uses of mercury will decline in coming years; however, the use of mercury in ASGM is projected to continue increasing.

Peru is the sixth-largest producer of gold in the world and produces roughly 148.8 metric tons of gold annually from ASGM (AGC 2018). The majority (54%) of Peru's artisanal gold production comes from Madre de Dios (MDD), one of Peru's twenty-five regions (AGC 2018). Located in southeastern Peru, this region's 85,301 square kilometers contain 15% of Peru's forested area and is considered one of the world's most active biodiversity hotspots, containing 13 species of primates and 10-15% of all the bird and butterfly species known on the planet (ACA 2017). According to the 2017 National Census (INEI 2019), 141,070 people live in MDD, of which

26.7% (37,726) are miners (AGC 2018). ASGM expanded in the region by 540% between 2006 and 2015, increasing mercury use (Cuzcano 2016). In 2000, it was estimated that 34 tons of mercury were imported into Peru, whereas in 2009, 195.6 tons were imported (AGC 2018). Mercury is used in ASGM to extract gold from ore and sediment, forming mercury-gold amalgams. These amalgams are burned for the gold yield at shops where gold is sold, emitting mercury into the air. The USEPA has found mercury emissions in Peruvian gold shops to be significantly above the American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) for occupational exposure to mercury of 25 ug/m^3 (USEPA 2018). Roughly 88% of ASGM in Peru uses mercury, producing 362.5 tons of mercury emissions annually (AGC 2018). Half of these emissions (181.5 tons) occur in MDD, where mercury is used in all gold extraction. It is estimated that roughly 30-40 tons of mercury are entering waterways in MDD each year (Fraser 2016), contaminating fish and wildlife. In fact, Fraser (2009) reported that a 2008 study by Peru's Ministry of Production found mercury levels in MDD river water to be 3 to 25 times the national standard (0.0001 milligrams per liter) set to conserve the aquatic environment. In MDD, the main route of human mercury exposure is through the consumption of mercury-contaminated fish. In 2016, the Peruvian government estimated that at least 48,000 people in MDD were exposed to high levels of mercury (Fraser 2016). There have been five studies (Ashe 2012; Langeland et al. 2017; Wyatt et al. 2017; Weinhouse et al. 2017; Yard et al. 2012) conducted in MDD assessing human mercury levels (See Appendix 3 for a summary of these exposure studies). All four of these studies reported mean hair mercury concentrations above the USEPA 1.0 µg/g standard. In the study by Wyatt et al. (2017), 231 individuals were sampled along riverine communities and 86% of these individuals were found to have hair mercury concentrations exceeding 1.2 µg/g, which is the USEPA provisional level that could result in child developmental impairment. Although these studies all demonstrate the prevalence of high mercury levels in this population, assessment of the potential health effects associated with this exposure are scant, including cardiovascular health.

Methods

Study population

The data analyzed in this report came from the "Amarakaeri Reserve Cohort Study" conducted between March and May of 2015. This study included 4,083 study participants across 23 communities selected *a priori* surrounding the Amarakaeri Communal Reserve in MDD. Of the 23 communities sampled, 11 are communities of indigenous peoples, referred to here throughout as native (See Figure 3). The sampling rate in each community depended on the population size; however, regardless of size, 90% of households enrolled in each community had to include a woman of childbearing age (WCBA). In communities with over 1,000 residents, 50% of households were sampled, 75% of households were sampled in communities with at least 350 residents, and all households were sampled in communities with less than 350 residents. After household selection, "sentinel groups" were formed which consisted of a WCBA, her partner, and youngest child under the age of 12. If the WBCA did not have a partner, the relative economic head of household was included in the sentinel group. Of the adult sentinels, 50 were randomly selected in each community for testing of serum cholesterol, triglycerides and fatty acids. All respondents self-reported their community of residence, sex and age on the surveys distributed.

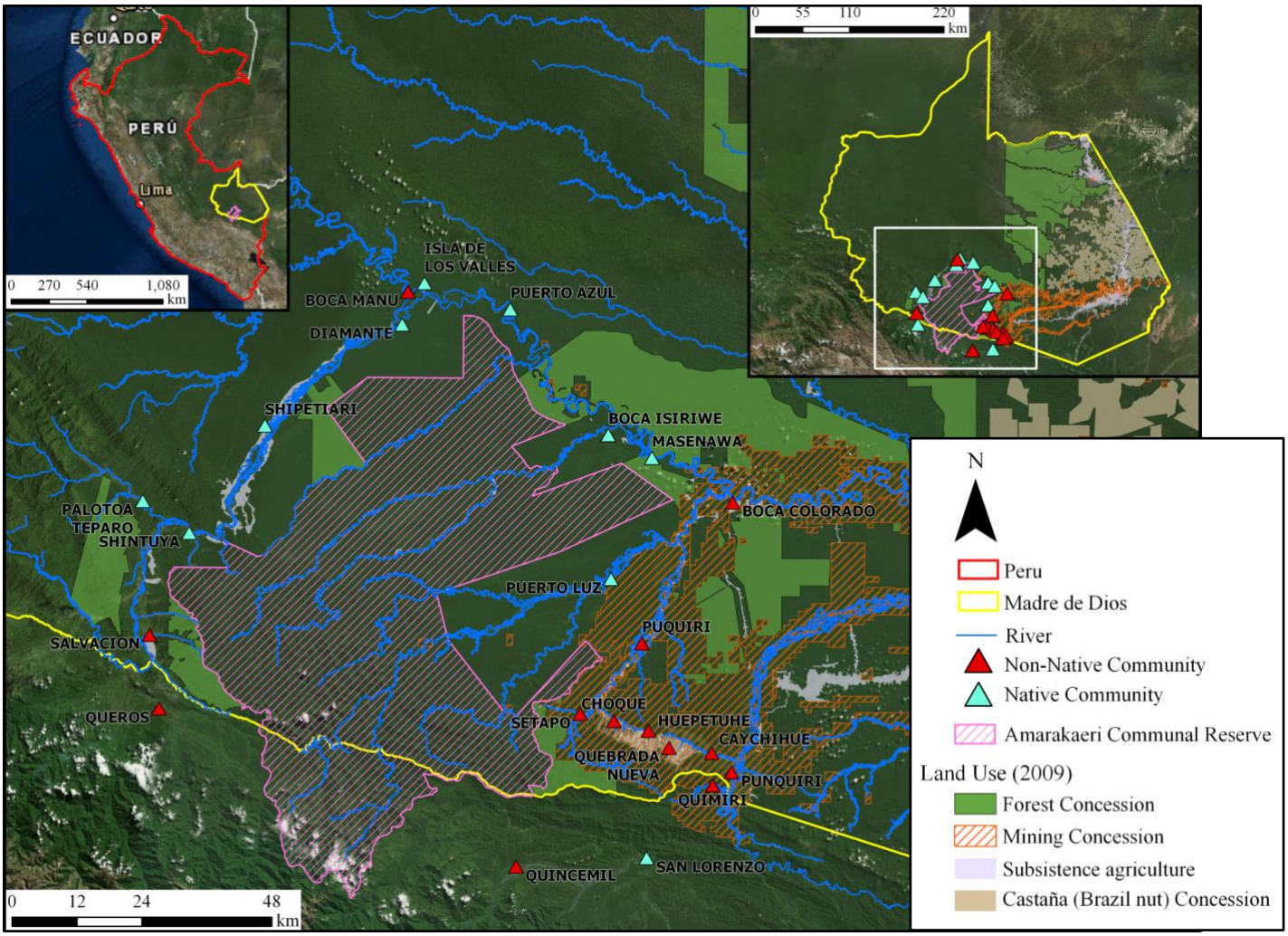

Figure 3. Amarakaeri Reserve Cohort Study Sites

Data and Covariates

Mercury

There are a number of biomarkers used to measure mercury exposure. The most frequently used being hair, urine and blood. Berglund et al. (2005) evaluated these biomarkers and "the physiology on which they are based, to explore the inter-individual variations and their suitability for exposure assessment". As aforementioned, the majority of the human body burden of mercury exists as MeHg. According to Berglund et al. (2005), over 80% of mercury in hair is MeHg and 9% is inorganic. However, a fraction of this inorganic mercury is demethylated MeHg. Therefore, the authors (Berglund et al. 2005) conclude that measuring total mercury in hair "is a better measure of MeHg exposure than MeHg in hair", and that hair is the "best measure of long-term average MeHg exposure". Due to the stability of MeHg in hair over time, weeks to years of mercury exposure can be measured in hair, allowing for sequential analysis and providing information on seasonal (or other peak) variations (NRC 2000). For these reasons, we measured total mercury in hair. Three tufts of hair about 0.5 cm in diameter were cut from the root at the back of each individual's head near the neck (Nutall 2004). The hair samples included in our final analysis (N = 1,432) ranged from 0.7 to 2.9 centimeters (cm) in length, but both the mean and median hair length was 2 cm, representing roughly 2 months of mercury exposure

(Nutall 2004). Hair samples were stored at ambient conditions in individual paper envelopes and analyzed in Dr. Helen Hsu-Kim laboratory at Duke University (Durham, NC). Total mercury content was determined in each 2-cm segment of hair by direct combustion, gold amalgamation, atomic absorption spectrometry (Milestone DMA-80). The "instrument was calibrated with aqueous Hg^{2+} acidified with 1[Mole] nitric acid, and calibration was verified by the analysis of a hair certified reference material (DB001, European Reference Materials) once per 10 hair samples in the analysis batch" (Weinhouse et al. 2017). A total of 2,335 total hair mercury samples were processed.

Blood Pressure Outcomes

Systolic and diastolic blood pressures were measured using an Omron blood pressure monitor (10 Series, BP785). Blood pressure measurements were recorded for 1,674 adult participants. Ideally, two sets of measurements were taken for SBP and DBP. However, for some participants, only one set of blood pressure measurements were recorded. If two sets were available, the measures were averaged and used as the SBP and DBP values in our analysis. These SBP and DBP values were used to establish our two blood pressure outcomes: 1) hypertension (binary outcome), and 2) mean arterial pressure (continuous outcome). Participants were identified as hypertensive using the 2017 AHA clinical guidelines for hypertension. If a participant had an average SBP of 130 mmHg or greater and/or a DBP of 80 mmHg or greater, they were considered hypertensive and assigned a value of 1. Those who were not considered hypertensive were assigned a value of 0. Mean arterial pressure (MAP) represents the average pressure in the arteries during one cardiac cycle and is considered a "better indicator of perfusion to vital organs" than SBP (Bonsall 2011). According to Cheung et al. (2019), MAP should not be lower than 65 mmHg or higher than 100 mmHg. With a low MAP, blood "may not be reaching your major organs", which can result in organ damage or failure (Nall 2018). A high MAP can lead to blood clots or cause damage to the heart muscle (Nall 2018). Papaioannou et al. (2016) evaluated seven different methods for calculating MAP, and recommended the following equation:

$$MAP = [DBP] + 0.412 * [SBP - DBP].$$

This method was used to calculate MAP values for our sample population. Following univariate analysis, the MAP values were transformed to achieve a more normal distribution for more interpretable results. The transformation used was natural logarithm ("natural log"), which has base *e*, equating approximately 2.718.

Health and Lifestyle Factors

Health and lifestyle factors considered in our analysis included body mass index (BMI), smoking status, and physical activity. Due to the correlation between BMI and a number of body fat measures as well as with "metabolic and disease outcome", BMI was selected as our indicator of weight and body fat (CDC 2017b). According to the Centers for Disease Control and Prevention (CDC 2017b), BMI is calculated by dividing a person's weight in kilograms (kg) by the square of their height in meters (m^2). The weight status categories associated with adult BMI ranges observed by the CDC are listed in Table 1.

Table 1. Weight status categories associated with adult BMI ranges

BMI (kg/m^2)	**Weight Status**
< 18.5	Underweight
18.5 - 24.9	Normal
25.0 – 29.9	Overweight
≥ 30	Obese

The United States Department of Health & Human Services (HHS) recommends maintaining a "Normal" BMI to prevent and manage hypertension, and states that by reducing your weight by 10 kg, you can lower your SBP by 5 to 20 mmHg (HHS 2003). A number of studies (Drøyvold et al. 2005; Dua et al. 2014; Linderman et al. 2018; Mungreiphy et al. 2011; Stamler et al. 1996; Vuvor 2017) have elucidated the direct relationship between BMI and blood pressure, being positively associated. In our sample population, height was measured using a portable height measuring device and weight was measured using an Omron HBF-514 scale, respectively. Height measurements were missing for 16 participants in our dataset and 10 participants had recorded heights below 130 cm (or 4 feet 3 inches). These 26 participants were excluded from our analysis. For fear of data entry error, two participants with recorded weights 18.3 and 30.4 kg, respectively, were excluded from our analysis.

Smoking status is a factor commonly considered when evaluating the risk of hypertension. Studies have shown the association between smoking and hypertension to be both inverse (Li et al. 2010; Thuy et al. 2010) and positive (Bowman et al. 2007; Halperin et al. 2008; Li et al. 2017). Regardless, because smoking has been established as an important risk factor for hypertension, it was considered in our analysis. In the survey distributed to our sample population, respondents were asked about their smoking status and were given the following three response options: 1) "Never", 2) "In the past", and 3) "Currently".

Physical activity is another important factor in determining the risk of hypertension. Various studies (Brook et al. 2013; Chobanian et al. 2003; Kelley and Kelley 2000; Pescatello et al. 2004; Whelton et al. 2002a; Whelton et al. 2002b) have shown physical activity to prevent hypertension. According to the HHS, walking briskly "at least 30 minutes per day, most days of the week" can lower one's SBP by 4 to 9 mmHg (HHS 2003). This factor was also evaluated using the survey. Respondents were asked if they get at least 30 minutes of moderately intense activity at least 5 times per week and had the choice of three responses: 1) "No", 2) "Yes, Sometimes", and 3) "Yes, Always".

Dietary Covariates

A number of dietary covariates were developed for inclusion in our analysis, including nutritional content and extent of western diet. These dietary covariates were created using information from a food frequency questionnaire distributed to our sample population. The food frequency questionnaires were completed by the head of household or their spouse and were representative of the entire household. The surveyor asked the participants how often the family ate the listed foods in the past 12 months.

1. Bushmeat/Wild Game
2. Beef
3. Pork
4. Chicken
5. Junk Food
6. French Fries/Chips
7. Soda
8. Candy/Sweets
9. Ice Cream
10. Quinoa
11. Cruciferous Vegetables (broccoli, cauliflower)
12. Leafy Green Vegetables (spinach, lettuce)
13. Brazil Nuts
14. Citrus Fruits (orange, lemon, star fruit)

The respondents had the selection of four responses: 1) "Never", 2) "Most weeks", 3) "1-3 times per month", and 4) "Occasionally". These responses were assigned values to reflect consumption frequency, the lowest consumption frequency ("Never") being assigned the lowest value (0) and increasing with frequency (See Table 2 for frequency weights).

Table 2. Consumption Frequency Weights

Survey Response	**Frequency Weight**
Most Weeks	7
1-3 times per month	3
Occasionally	1
Never	0

Nutritional Content

To create our energy, macro- and micro- nutrient covariates, we needed to identify the nutritional content of all fourteen foods listed. In order to do so, we used the 2018 Norwegian Food Composition Table (NFCT) published in collaboration by the Norwegian Food Safety Authority and the Department of Nutrition at the University of Oslo (NFSA 2018). This food composition table was the most comprehensive, publicly available nutritional database. Values in the NFCT were either generated from "chemical analyses performed in Norwegian quality-assured laboratories", "provided by the industry or borrowed from foreign food composition tables", or "estimated based on similar food items and dishes" (NFSA 2018). Some of the food items listed on the questionnaire were more general food categories. Using best judgment as well as personal experience with cuisine in Peru, foods were selected from the NFCT to represent the fourteen questionnaire foods. A list of the NFCT representative foods can be found in Appendix 4, Table 1.

The macro- and micro- nutrients most frequently cited in association with blood pressure were included in our analysis. The macronutrients included were protein, starch and saturated fat.

Multiple authors (Djousse et al. 2006; Preuss et al. 1996; Stamler et al. 1996) found a positive association between dietary saturated fat intake and blood pressure. A positive association was also observed between dietary starch intake and blood pressure (Preuss et al. 1996; Stamler et al. 1996). Dietary protein intake, on the other hand, showed an inverse association with blood pressure (Preuss et al. 1996; Stamler et al. 1996). Due to the association between caloric intake and blood pressure, dietary calorie intake was also included (HHS 2003). Micronutrients considered in our analysis included potassium, magnesium, calcium, sodium, and selenium. Selenium was considered in our analysis due to the study of selenium as a protective or mitigating factor against mercury toxicity (Berry and Ralston 2008; Spiller 2018; Sumino et al. 1977). However, it should be noted that results have been inconsistent. For example, Choi et al. (2007) found no evidence that selenium was an important protective factor against MeHg toxicity. The other micronutrients were included in our analysis due to their association with blood pressure. Many studies (Cappuccio and MacGregor 1991; Langford 1983; Meneely et al. 1957; Meneely and Battarbee 1976; Preuss et al. 1996; Vollmer et al. 2001) have found an inverse association between potassium and blood pressure. Cappuccio and MacGregor (1991) showed potassium supplements significantly lowered SBP and DBP, by 5.9 mmHg and 3.4 mmHg, respectively. Protective effects of magnesium (Preuss et al. 1996; Stamler et al. 1996) and calcium (Preuss et al. 1996; Stamler et al. 1996; Vollmer et al. 2001) have also been observed. The association between sodium intake and blood pressure is well studied. Numerous studies (Chobanian et al. 2000; Dahl 1972; McCarronet al. 1982; Preuss et al. 1996; Sacks et al. 2001; Stamler et al. 1996; Tobian 1979; Vollmer et al. 2001) have shown a positive association between sodium and blood pressure. According to the HHS (2003), limiting your dietary sodium intake to 2.4 grams (g) per day can reduce SBP by 2 to 8 mmHg.

The micronutrient (See Appendix 4, Table 2), macronutrient and energy (See Appendix 4, Table 3) values for each food were used along with the food's typical serving size (See Appendix 4, Table 4) to calculate the nutritional scores for each food item (Appendix 4, Table 5) as follows:

$$\frac{\text{Nutritional Content}}{100\text{ g}} * \text{Typical Serving Size (g)}$$

The resultant nutritional score was then multiplied by the respective frequency weight listed in Table 2 for each household to obtain a weighted nutritional score.

Western Diet Index

Western diet was another dietary covariate evaluated in our analysis. Statovci et al. (2017) define the Western diet as "high dietary intake of saturated fats and sucrose and low intake of fiber". A study conducted by Sadakane et al. (2008) found the Western diet "was associated with higher systolic and diastolic blood pressures". In fact, "The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure" published by the HHS in 2003 suggests that following the "Dietary Approaches to Stop Hypertension" (DASH) eating plan which consists of a "diet rich in fruits, vegetables, and lowfat dairy products with a reduced content of saturated and total fat" can reduce SBP by 8 to 14 mmHg (HHS 2003). Using this information as well as our established macronutrient values (See Appendix 4, Table 3), we assigned each food in the questionnaire a Western diet score. These scores ranged from 10, being the most Western, to 1, being the least. These scores, listed in

Table 3, were multiplied by the frequency weights (See Table 2) for each food item for each household. The resultant weighted western diet score for each food item was summed to produce a weighted total western diet score for each household.

Table 3. Western diet scores

Food in questionnaire	**Western diet score**
Junk Food	10
Soda	10
French Fries/Chips	9
Candy/Sweets	9
Ice Cream	9
Pork	8
Beef	7
Chicken	5
Bushmeat/Wild game	4
Quinoa	2
Brazil Nuts	2
Cruciferous Vegetables	1
Leafy Green Vegetables	1
Citrus Fruits	1

Finally, the standard score (z-score) of each weighted nutritional score and total western diet score in our dataset was calculated to produce a distribution that has a mean of 0 and a standard deviation of 1. The resultant z-scores were used as the dietary covariates in our analysis. The z-score calculation is as follows:

$z = \frac{X - \mu}{\sigma}$, where X is the individual weighted score, μ is the mean weighted score of our dataset, and σ is the standard deviation of our weighted score dataset.

Blood Chemistry

Fatty acids, cholesterol and triglycerides were measured in serum. Serum was collected in BD Vacutainer serum collection tubes with clot activator (BD Worldwide, cat no. 367382) and analyzed at MedLab Peru, a Clinical Laboratory Improvement Amendments-certified laboratory in Lima, Peru. In order to test for cholesterol and triglycerides, 3 milliliters (mL) of venous whole blood (1 mL of serum) were drawn from participants (N = 713). Cholesterol is a type of lipid (fat) used by the body "to make hormones, vitamin D, and substances that help you digest foods" (MedlinePlus 2019b). However, excess cholesterol combines with substances in the blood, forming plaque and resulting in atherosclerosis. The influence of serum cholesterol on blood pressure has been established in multiple studies (Ferrara et al. 2002; Bønaa and Thelle 1991). Triglycerides are another type of lipid in the blood, which are stored in fat to be used for energy. Although the association between triglycerides and blood pressure is under-studied, studies (Kajikawa et al. 2015; Kajikawa et al. 2016) have shown there to an independent

association between triglycerides and endothelial dysfunction, distinguishing them as a predictor for high blood pressure. Serum cholesterol and triglyceride values, measured as milligrams per deciliter (mg/dL), were available for 645 (45%) participants in our final dataset (N=1,432). After univariate analysis of the serum cholesterol and triglycerides, only the serum triglycerides were natural log-transformed for a more normal distribution.

Fatty acids are major sources of energy as well as precursors for essential structural and metabolic substances in the body. To test for serum fatty acids, 5 mL of venous blood serum were drawn. Serum was analyzed for 13 different fatty acids (See Appendix 5). Of the 13 fatty acids analyzed, 3 were saturated and 10 were unsaturated, respectively. In existing literature, a positive association with blood pressure was found for the following six fatty acids:

1. Arachidonic (Tsukamoto and Sugawara 2018);
2. Palmitoleic (Cambien et al. 1988; Grimsgaard et al. 1999; Simon et al. 1996; Uusitupa et al. 1994);
3. Palmitic (Grimsgaard et al. 1999; Uusitupa et al. 1994);
4. Dihomo-γ-linolenic (Grimsgaard et al. 1999; Simon et al. 1996);
5. Oleic (Grimsgaard et al. 1999); and
6. Myristic acid (Uusitupa et al. 1994).

The following five fatty acids were inversely associated with blood pressure:

1. Stearic acid (Simon et al. 1996);
2. Linoleic acid (Grimsgaard et al. 1999; Tsukamoto and Sugawara 2018; Uusitupa et al. 1994);
3. Gamma-linolenic acid (Engler et al. 1998);
4. Docosahexaenoic acid (Bønaa et al. 1990); and
5. Alpha-linolenic acid (Tsukamoto and Sugawara 2018).

The association between eicosapentaenoic acid and blood pressure has been found to be both positive (Grimsgaard et al. 1999) and inverse (Bønaa et al. 1990; Miyajima et al. 2001). In our literature review, only one study was found that evaluated the association between elaidic acid and blood pressure. This study (Teres et al. 2008) was conducted in rats and showed elaidic acid had “little effect” on blood pressure. Due to a lack of information, we decided to include elaidic acid in our analysis, and therefore, all 13 fatty acids were considered in our analysis. Only ~25% (N = 364) of the participants in our final dataset (N=1,432) were analyzed for serum fatty acid. Like the dietary covariates, all serum fatty acid concentrations were converted into z-scores

Statistical Methods

Z-score conversions as well as univariate and multivariate analyses were conducted using R version 3.5.1 (R Core Team 2018) and Stata 15 software (StataCorp 2017). After merging all of the separate covariate datasets, we ended up with a dataset which included 1,433 participants. Descriptive statistics were also calculated using R and Stata in order to describe our final sample population, and to determine how many of our participants had high levels of mercury exposure, had a MAP outside the normal range, and were considered hypertensive. Using this statistical software, we explored the variables, assessing variable distribution, which allowed us to determine whether or not transformations were necessary. After transforming the applicable variables, we began our model creation process. We needed to create two separate models for

our blood pressure outcomes (hypertension and MAP). In a study on 251 individuals in the Brazilian Amazon, Fillion et al. (2006) found "significant inter-community differences" in blood pressure. Therefore, to account for potential correlation within communities, we applied generalized estimating equations (GEE) which is a population-averaged model, using community as our group variable. Although we used GEE for both models, the family type and link function differed. Hypertension is a binary outcome (Yes=1 or No=0), and therefore, logistic regression was used, setting the family to binomial and the link function to logit. MAP is a continuous outcome, and therefore, linear regression was used, setting the family to Gaussian and the link function to identity.

Due to the number of potential confounding variables, we created a base model to facilitate variable selection. Due to the observed sex (Choi et al. 2017; Gillis and Sullivan 2016; Joyner et al. 2016; Maranon and Reckelhoff 2013; Mirabito Colafella and Denton 2018; Reckelhoff 2018; Syme et al. 2009) and age (Franklin et al. 1997; Kotchen et al. 1982; Pinto 2007) differences in blood pressure and hypertension, these variables were included in our base model along with our variable of interest (mercury) and BMI because of its direct relationship with blood pressure and strong presence in blood pressure literature. The selection of these variables in our base model are supported by the results of Fillion et al. (2006), who found blood pressure to be significantly associated with age, sex, BMI, and hair mercury levels. Before introducing the potential confounding variables, we first checked for sample outliers in our base model. In order to identify any potential outliers, we used the "outlierTest()" function in R (version 3.5.1), which produces the Bonferroni p-value for the most extreme sample. The function determined one sample to have a significant Bonferroni p-value (p-value=0.012), and therefore, this sample was removed from our dataset. After removing this sample and re-running the outlier test, no other samples were identified as significant outliers. Therefore, our final dataset included 1,432 samples.

We then introduced the potential confounding variables one by one into our base MAP and hypertension models to assess the significance (p-value) of their point estimate. A total of 32 potential confounding variables were introduced into our models (See Appendix 6, Table 1 for the full list of variables). Those variables whose point estimate p-value was less than 0.2 in the base model were included in the full model (See Appendix 6, Table 2). Next, we assessed multicollinearity in our full MAP and hypertension models. In order to measure multicollinearity, we used the "variance inflation factor" (VIF) function in our statistical software. We used the rule of thumb that a VIF greater than 5 suggests "severe multicollinearity may be present" (SFU 2016). After assessing multicollinearity, one variable (Western diet z-score) was removed from our hypertension model and four variables (dietary magnesium and saturated fat intake z-scores as well as serum oleic and palmitic acid z-scores) were removed from our MAP model.

For the scope of this analysis, only the interaction between mercury and sex was considered. The p-values for the mercury-sex interaction were 0.013 and 0.015 in our MAP and hypertension models, respectively. In order to account for this effect modification, our sample population was stratified by sex. The sample size of the models before stratification were 305 and 304 in the MAP and hypertension model, respectively. However, stratifying by sex reduced the sample size in our male MAP and hypertension models to 95 and 94, respectively. With 13 independent

variables in the MAP model and 11 in the hypertension model, the male sample size no longer supported the number of variables. According to van Smeden et al. (2016), having 10 samples per variable is "a widely advocated minimal criterion for sample size considerations" in both linear and logistic regression analysis (Moons et al. 2014; Moons et al. 2015; Pavlou et al. 2016). Therefore, we had to perform backwards elimination to achieve a parsimonious model with a total of 9 variables including categorical levels. Backwards elimination was performed before sex stratification. As the main focus of our analysis is to assess the association between mercury exposure and our two blood pressure outcomes, we had to ensure that while reducing variables, the coefficient for mercury was within 10% of its value before variable elimination, as recommended by Kleinbaum et al. (2014). Figure 2 represents a simplified graphic of our model creation process. The final MAP model adjusted for sex, age, BMI, smoking status, serum triglycerides, whether or not the community was native, and serum level of unsaturated fatty acid, dihomo-γ-linolenic acid. The final hypertension model adjusted for sex, age, BMI, serum cholesterol, dietary protein intake, whether or not the community was native, and serum level of saturated fatty acid, stearic acid.

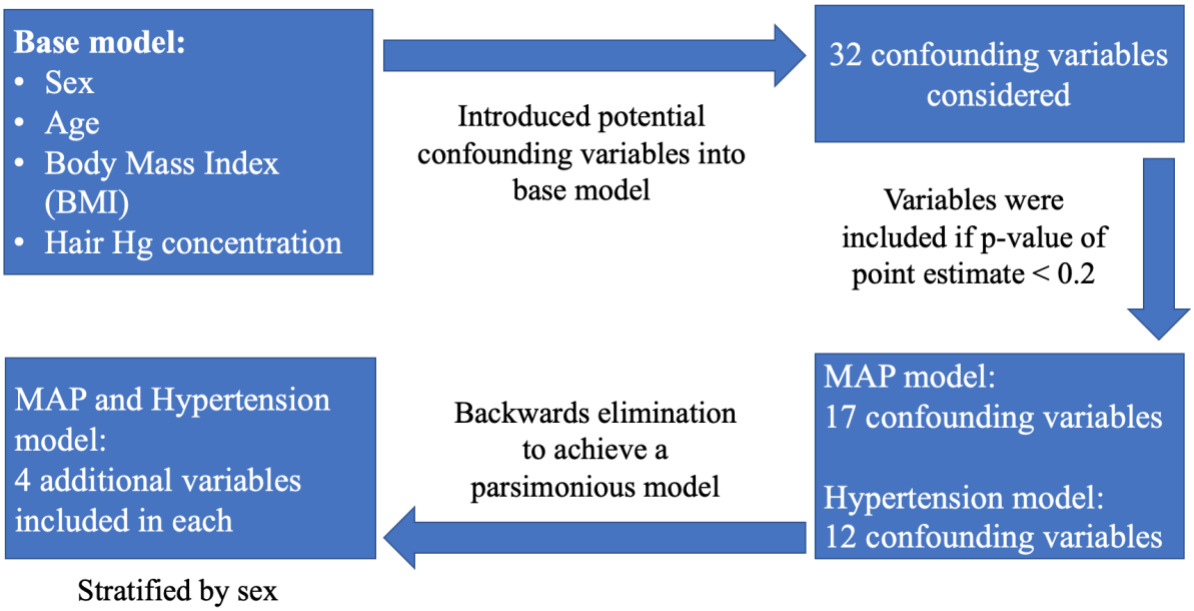

Figure 4. Model Creation Process

Results

In total, our dataset included information for 1,432 participants. The sex distribution of our sample population was not entirely representative of MDD. The 2015 population of MDD was estimated to be 137,316, of which 43% was female and 57% was male (INEI 2015). However, the sex distribution of our sample population was 71% female and 29% male (See Table 4). This could be attributed to the absence of males for occupational reasons or because the males in our study location were less willing to participate in the survey. Roughly 91% of our sample population was under the age of 50. The average age of the sample population was 34.9, 33.2, and 39.1 in the total, female and male populations, respectively (See Tables 5 and 5). Conducting a two-sample t-test with equal variances, referred to here throughout simply as "t-test", in Stata

showed there to be a statistically significant (p-value = 0.0000) difference in the average age of the male and female population, on average, females being younger than males. A statistically significant (p-value=0.0064) difference in the average age between natives and non-natives was also observed, natives, on average, being older. There was also a statistically significant difference in the mean BMI between the male and female population (p-value = 0.0000), the female population having an average BMI of 28.6 kg/m^2 compared to 27.0 kg/m^2 in the male population. Both of these averages are within the "overweight" category. In fact, 41.7% of the sample population was considered overweight and 30.7% obese, respectively. A greater proportion of the overweight and obese population was female, with 68% and 80% of the participants who were overweight and obese, respectively, being female (See Table 4). The t-test also showed a statistically significant (p-value=0.0003) difference in the average BMI between the native and non-native population. However, in this case, the average BMI in the non-native population (28 kg/m^2) is higher than the average of the native population (27 kg/m^2). The majority of the sample population (83%) self-reported that they never smoke. According to the survey data, 11% of the sample population smoked in the past and only 6% of the sample population self-reported that they currently smoke. Of the 81 current smokers, 46% are natives. The majority of the past and current smokers were male, with 60% of past smokers and 81% of the current smokers being male, respectively. According to our analysis, 14% of our sample population were hypertensive. Of the hypertensive participants, 53% were female, 47% were male and 63% were non-native. However, the proportion of hypertensive natives to non-natives was double. There were 337 (24%) natives in our sample population and 22% (N=72) of them were considered hypertensive in comparison to 11% (N=123) of non-natives. Figure 5 displays the proportion of the sample population considered hypertensive by sex (left) and native status (right).

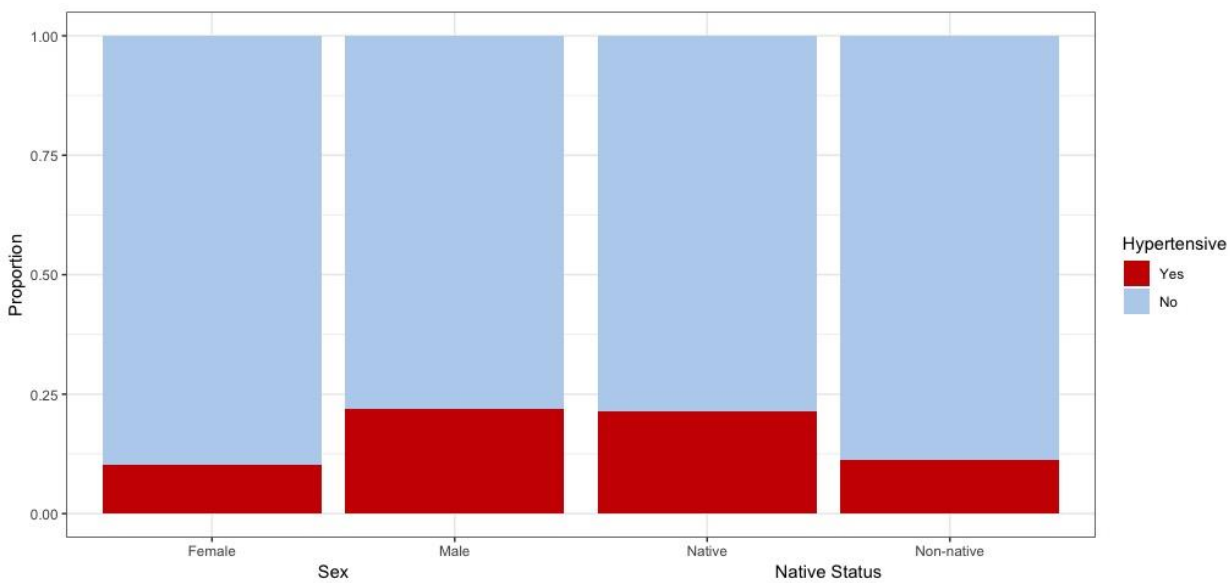

Figure 5. Proportion of sample population that is hypertensive by sex (left) and native status (right)

Table 4. Summary of categorical variables in our final models

Variable	All		Female		Male	
	N	**Proportion (%)**	**N**	**Proportion (%)**	**N**	**Proportion (%)**
Sex	1,432	100%	1,013	71%	419	29%
Age						
18-34	714	50%	567	79%	147	21%
34-50	580	41%	390	67%	190	33%
50-65	120	8%	50	42%	70	58%
65+	18	1%	6	33%	12	67%
BMI						
Underweight	6	0.4%	5	83%	1	17%
Normal	389	27.2%	248	64%	141	36%
Overweight	597	41.7%	408	68%	189	32%
Obese	440	30.7%	352	80%	88	20%
Smoking Status						
Never	1192	83%	933	78%	259	22%
In the Past	157	11%	63	40%	94	60%
Currently	81	6%	15	19%	66	81%
Native	337	24%	209	62%	128	38%
Hypertensive	195	14%	103	53%	92	47%

N = Sample Size

The continuous variables included in our final models are summarized in Table 5 for the entire sample population and by sex in Table 6, respectively. The two-sample t-test with equal variances showed no statistically significant difference in the average serum stearic acid concentrations (mol%) or dietary protein intake by sex. However, statistically significant differences in these variables were observed among the native and non-native population. We found dietary protein intake to be significantly (p-value=0.0000) higher among non-natives and serum stearic acid concentrations to be significantly (p-value=0.0006) higher among natives. The average serum dihomo-γ-linolenic acid (mol%) (p-value=0.0349) and serum cholesterol (mg/dL) (p-value=0.0412) concentrations were statistically higher among women than in men. The difference in average serum cholesterol (mg/dL) was also statistically significant (p-value=0.0152) between natives and non-natives, being statistically higher in the non-native population compared to the native population.

Table 5. Summary of continuous variables in our final models for the entire sample population

Variable	N	Mean	SD	Minimum (Min)	Maximum (Max)
Age	1,432	34.9	11.3	18.0	96.0
Hair mercury (µg/g)	1,432	2.6	3.2	0.0	44.8
BMI (kg/m^2)	1,422	28.1	4.9	16.2	62.5
Average SBP (mmHg)	1,432	105.7	13.2	74.0	159.5
Average DBP (mmHg)	1,432	69.2	9.2	43.5	116.0
MAP (mmHg)	1,432	84.2	10.2	56.1	122.3
Serum cholesterol (mg/dL)	645	172.4	36.2	62.0	317.0
Serum triglycerides (mg/dL)	645	149.0	86.4	30.0	694.0
Dietary protein intake	1,430	394.3	156.5	0.0	837.1
Serum stearic acid (mol%)	364	212.2	98.4	74.2	835.3
Serum dihomo-γ-linolenic (mol%)	364	49.2	24.1	15.0	204.1

N = Sample Size
SD = Standard Deviation

Table 6. Summary of continuous variables in our final models for female and male populations

Variable	Female					Male				
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
Age	1,013	33.2	10.4	18.0	96.0	419	39.1	12.2	18.0	86.0
Hair mercury (µg/g)	1,013	2.4	2.8	0.1	22.6	419	3.0	4.1	0.0	44.8
BMI (kg/m^2)	1,008	28.6	5.1	16.2	54.6	414	27.0	4.3	18.0	62.5
Average SBP (mmHg)	1,013	102.3	11.7	74.0	159.5	419	113.9	13.1	88.5	158.5
Average DBP (mmHg)	1,013	68.0	8.7	43.5	103.5	419	72.1	9.6	49.0	116.0
MAP (mmHg)	1,013	82.1	9.3	56.1	118.7	419	89.3	10.4	66.8	122.3
Serum cholesterol (mg/dL)	453	174.0	36.3	62.0	317.0	192	168.6	35.8	66.0	277.0
Serum triglycerides (mg/dL)	453	145.7	83.7	30.0	694.0	192	156.7	92.2	30.0	575.0
Dietary protein intake	1,012	396.9	156.2	0.0	835.0	418	388.1	157.4	0.0	837.1
Serum stearic acid (mol%)	253	216.2	106.1	78.0	835.3	111	202.9	77.8	74.2	552.7
Serum dihomo-γ-linolenic (mol%)	253	50.7	26.3	15.3	204.1	111	45.8	17.8	15.0	110.2

N = Sample Size
SD = Standard Deviation

In the sample population (N = 1,432), 58% of individuals had hair mercury concentrations that exceeded the 1.2 µg/g USEPA provisional level with a population average of 2.6 µg/g (See Table 5). The average hair mercury concentration in the women (2.4 µg/g) compared to the men (3.0 µg/g) was statistically significant (p-value=0.0006) (See Table 6). Average hair mercury

concentration was also statistically significant between natives and non-natives (p-value=0.0000). The average hair mercury concentration among natives was significantly higher, with an average of 4.56 µg/g compared to 1.98 µg/g in the non-native population. Figure 6 displays the hair mercury distribution by sex (left) and native status (right). Average hair mercury concentration (µg/g) also increased with age. Table 7 shows the average hair mercury concentrations in four age groups, demonstrating that the highest average concentration (3.16 µg/g) was observed among participants aged 65 and up. Among those aged 65 and up, 12 (67%) were men and 6 (33%) were women. However, the average hair mercury concentration for women aged 65 and up (4.6 µg/g) was almost double the average for men in the same age group (2.4 µg/g). The natives within these populations had averages twice as high, with native women aged 65 and up having an average concentration of 7.7 µg/g and native men aged 65 and up having an average concentration of 4.3 µg/g, respectively.

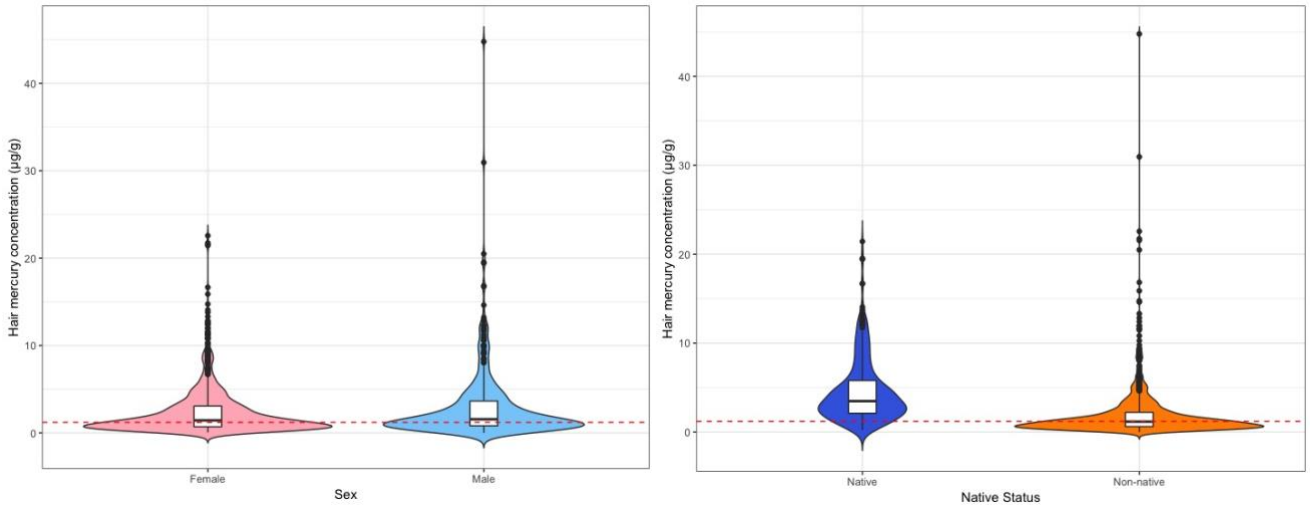

Figure 6. Left panel: hair mercury concentration (µg/g) by sex, Right panel: Hair mercury concentration by native status. The dotted red line represents the 1.2 µg/g USEPA provisional level.

Table 7. Hair mercury concentrations (µg/g) and MAP (mmHg) by age group

Age group	N	Hair mercury concentration (µg/g)				Mean arterial pressure (mmHg)			
		Mean	SD	Min	Max	Mean	SD	Min	Max
18-34	714	2.47	3.28	0.081	44.77	81.512	8.7	56.1	118.7
34-50	580	2.69	3.06	0.003	22.57	85.895	10.5	60.0	121.4
50-65	120	2.77	3.70	0.090	30.95	90.604	10.9	66.0	122.3
65+	18	3.16	3.78	0.137	12.35	96.611	11.0	80.3	115.2

N = Sample Size
SD = Standard Deviation

The average SBP, DBP and MAP for both males, females and natives were within the normal range. Average SBP and DBP were significantly (p-values=0.0000) higher in males compared to females and natives compared to non-natives. In total, 16 of the study participants had MAP

lower than 65, all of whom were female and two of which were native. Of the 110 participants who had MAP higher than 100, 61 (55.5%) were male, 49 (44.5%) were female, and 43 (39%) were native. The lowest average MAP was observed in the female population (82 mmHg), which was significantly (p-value=0.0000) different from the average MAP in males (89 mmHg). The t-test showed a statistically significant (p-value=0.0000) difference in the average MAP between natives and non-natives, the average MAP for the native population was 87 mmHg compared to 84 mmHg in the native population. The highest average MAP (91 mmHg) was observed among the native male population. Figure 7 displays MAP (mmHg) for the entire sample population by sex (left) and native status (right). Like with mercury levels, MAP increased with age group, the average being highest (96.6 mmHg) in the population aged 65 and up (See Table 7). Similar to mercury, MAP levels were highest among native women aged 65 and up with an average MAP of 96.5 mmHg compared to 90.3 mmHg in men.

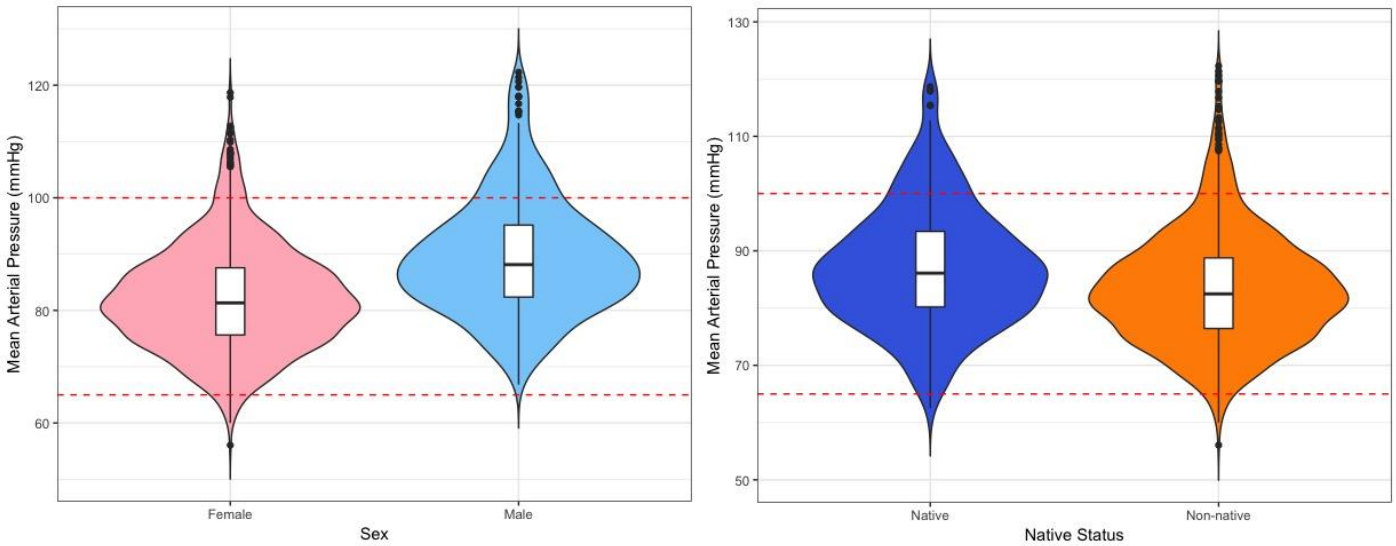

Figure 7. Left panel: MAP (mmHg) by sex, Right panel: MAP (mmHg) by native status. The dotted red line represents the 1.2 µg/g USEPA provisional level.

In both models, there was a significant interaction between mercury levels and sex. After stratifying by sex, no variables showed to be associated with hypertension at a 5% significance level among women (See Table 8). The association between mercury levels and hypertension in women, although insignificant, was inverse (OR: 0.84; 95% CI: 0.50–1.41; p-value=0.512). In contrast, this association was statistically significant and positive in men. Among men, the odds of hypertension for each 2.7-fold increase in hair mercury concentration increased 3-fold (OR: 3.07; 95% CI: 1.36–6.92; p-value=0.007). No other variables were significant at a 5% significance level (See Table 8). In the MAP model, mercury levels were also inversely associated with MAP in women (gMR: 0.99; 95% CI: 0.98–1.008; p-value=0.311), but positively associated in men (See Table 9). Again, this association was only significant among men (gMR: 1.024; 95% CI: 1.01–1.04; p-value=0.012). The only other variable that was significant at a 5% significance level in men was BMI. With a BMI $\geq$ 30 kg/m^2, MAP is expected to increase 8.9% (gMR: 1.089; 95% CI: 1.02–1.16; p-value=0.010). Among women, age (gMR:1.002; 95% CI:1.00–1.003; p-value=0.045) and being a resident of a native community (gMR: 1.04; 95% CI: 1.00–1.089; p-value=0.050) were significant at a 5% significance level (See Table 9).

Table 8. Odds Ratios (ORs) from multivariate logistic regression model for hypertension

	All (N = 304)		**Female (N= 210)**		**Male (N= 94)**	
Variable	**OR [95% CI]**	**p**	**OR [95% CI]**	**p**	**OR [95% CI]**	**p**
Sex: Male	2.49 [1.14–5.43]	0.021*	-	-	-	-
Age	1.03 [0.99–1.06]	0.141	1.01 [0.97–1.07]	0.562	1.05 [0.99–1.11]	0.089
Mercury	1.34 [0.89–2.03]	0.159	0.84 [0.50–1.41]	0.512	3.07 [1.36–6.92]	0.007*
BMI						
Obese	2.98 [0.88–10.11]	0.080	1.83 [0.34–9.71]	0.479	2.75 [0.41–18.58]	0.299
Overweight	1.5 [0.44–5.20]	0.519	1.12 [0.20–6.34]	0.895	1.31 [0.2–8.8]	0.781
Cholesterol	1.01 [1.00–1.02]	0.031*	1.01 [1.00–1.03]	0.123	1.02 [1–1.04]	0.100
Native Status	1.72 [0.69–4.26]	0.245	2.42[0.69–8.47]	0.168	0.96 [0.24–3.81]	0.957
Dietary protein intake	0.72 [0.46–1.14]	0.160	0.62 [0.34–1.14]	0.123	0.52 [0.23–1.2]	0.126
Serum stearic acid	1.17 [0.82–1.66]	0.388	1.10 [0.73–1.65]	0.649	1.66 [0.77–3.56]	0.195

*Significant at 5% significance level
N = Sample Size
CI: Confidence Interval
p: p-value

Table 9. Geometric mean ratios (gMRs) from multivariate linear regression model for MAP

Variable	All (N=308) gMR [95% CI]	p	Female (N=212) gMR [95% CI]	p	Male (N=96) gMR [95% CI]	p
Sex: Male	1.08 [1.05–1.12]	0.000*				
Age	1.001 [1.0002–1.003]	0.021*	1.002 [1.00–1.003]	0.045*	1.001 [1–1.003]	0.206
Mercury	1.01 [0.99–1.02]	0.393	0.99 [0.98–1.008]	0.311	1.024 [1.01–1.04]	0.012*
BMI						
Obese	1.06 [1.02–1.10]	0.002*	1.03 [0.99–1.084]	0.159	1.089 [1.02–1.16]	0.010*
Overweight	1.02 [0.98–1.05]	0.316	1.00 [0.95–1.042]	0.833	1.05 [1–1.11]	0.065
Underweight	0.95 [0.82–1.10]	0.503	0.95 [0.77–1.178]	0.654	0.962 [0.79–1.18]	0.710
Smoking Status						
In the past	1.04 [1.00–1.08]	0.055	1.05 [0.99–1.11]	0.122	1.035 [0.98–1.09]	0.191
Currently	0.99 [0.94–1.04]	0.643	1.02 [0.93–1.121]	0.700	0.977 [0.92–1.04]	0.449
Serum dihomo-γ-linolenic acid	1.01 [1.00–1.02]	0.168	1.01 [0.99–1.022]	0.278	1.012 [0.98–1.05]	0.478
Serum triglycerides	1.03 [1.00–1.06]	0.035*	1.03 [1.00–1.07]	0.053	1.021 [0.98–1.07]	0.341
Native	1.03 [1.00–1.06]	0.087	1.04 [1.00–1.089]	0.050*	1.001 [0.95–1.06]	0.964

*Significant at 5% significance level
N = Sample Size
CI: Confidence Interval
p: p-value

Discussion

Our study revealed 14% of the sample population to be hypertensive. The prevalence of hypertension in our study was slightly higher than that observed by Fillion et al. (2006) who investigated the association between blood pressure and hair mercury levels in 251 individuals aged 15 and up in the Brazilian Amazon and found 8% of these individuals to be hypertensive. Fillion et al. (2006) found similar proportions of men and women hypertensive. Their study (Fillion et al. 2006) found 21.1% of men and 11.9% of women to be hypertension, and we found 22% of men and 10.2% of women to be hypertensive, respectively. Fillion et al. (2006) did not distinguish the prevalence of hypertension between native and non-native communities. In our study, we found the proportion of hypertensive natives to be double that of hypertensive non-natives, with 22% of natives and 11% of non-natives considered hypertensive, respectively. None of the previous studies we found during our literature review compared mercury levels and blood pressure between natives and non-natives within their study area. Our analysis showed a statistically significant (p-value=0.0000) difference in the average hair mercury concentration

between natives and non-natives, with concentrations, on average, being higher in natives. The average hair mercury concentration in the native population was 4.56 µg/g compared to 1.98 µg/g in the non-native population. This could be attributed to differences in diet, natives relying more heavily on fish so eating more mercury-contaminated fish and wildlife. Therefore, in this case, the negative effect of mercury outweighs the positive effects of eating fish This notion is supported by numerous studies (Choi et al. 2009; Hu et al. 2017; Fillion et al. 2006; Nielsen et al. 2012; Pedersen et al. 2005; Valera et al. 2009; Valera et al. 2011b; Valera et al. 2013;Yorifuji et al. 2010), which were used by Hu et al. (2018) to calculate pooled ORs for hypertension using studies representing general and indigenous populations. Nine studies were used in each OR calculation for hypertension by population: general (Bautista et al. 2009; Park et al. 2003; Shiue 2014; Guallar et al. 2002; Siblerud 1990; Mozaffarian et al. 2012; Eom et al. 2014; Lee and Kim 2013; Virtanen et al. 2012a) and indigenous (Choi et al. 2009; Hu et al. 2017; Fillion et al. 2006; Nielsen et al. 2012; Pedersen et al. 2005; Valera et al. 2009; Valera et al. 2011b; Valera et al. 2013; Yorifuji et al. 2010). Hu et al. (2018) reported the OR for hypertension to be 1.08 (95% CI: 0.89–1.32; p-value=0.013) in the general population and 2.08 (95% CI: 1.28–3.38; p-value=0.016) in the indigenous population, respectively. This supports our findings that hypertension is more prevalent in native communities. See Appendix 7 for the study-specific as well as pooled ORs used in the analysis by Hu et al. (2018).

Fillion et al. (2006) is one of the nine studies that was included in the OR calculation for indigenous population by Hu et al. (2018). In Fillion et al. (2006), the average age of the women was 34.4 and 35.8 in men, respectively. Although the average age between our study and that of Fillion et al. (2006) was similar, the individuals in our study had a higher average BMI compared to Fillion et al. (2006), who reported the average BMI in women to be 22.5 kg/m^2 and 22.2 kg/m^2, in men, respectively. In our study the average BMI in women was 28.6 kg/m^2 and 27.0 kg/m^2 in men. These averages are slightly higher than that (26.0 ± 4.6 kg/m^2) reported by Huayanay-Espinoza et al. (2017), who used a 2012 health survey to evaluate BMI in 16,082 Peruvian women. We found 75% of the MDD women in our study to be overweight or obese. In the nation-wide study conducted by Huayanay-Espinoza et al. (2017), the highest rates of overweight and obese women were in MDD, where 87.3% of women were considered overweight or obese. In our analysis a BMI ≥ 30 kg/m^2 in men is expected to increase MAP by 8.9% (gMR: 1.089; 95% CI: 1.02–1.16; p-value=0.010). Although it is not surprising that BMI was significant (p-value=0.010) and positively associated with MAP, it was surprising that this was only the case among men. This study as well as that of Huayanay-Espinoza et al. (2017) warrant the continued attention to BMI and diet in this area. In addition, it is important to note that none of the previous studies we found during our literature review investigated the association between mercury levels and MAP. The average MAP for the entire (84 mmHg), male (89 mmHg), female (82 mmHg) and native (87 mmHg) populations were within the normal range. The highest average MAP was observed in native female population, with an average of 91 mmHg. Our study showed an insignificant inverse association between MAP and mercury levels among women (gMR: 0.99; 95% CI: 0.98–1.008; p-value=0.311) and a significant positive association among men (gMR: 1.024; 95% CI: 1.01–1.04; p-value=0.012).

Higher averages for SBP and DBP were reported by Fillion et al. (2006), the average SBP was 113.9 mmHg ± 14.6 and the average DBP was 73.7 mmHg ± 11.0, respectively. In our analysis the average SBP was 105.7 mmHg ± 13.2 and DBP was 69.2 mmHg ± 9.2, respectively. See

Appendix 8 for Table 2 of the Fillion et al. (2006) report which summarizes the study population's socio-demographic characteristics. Fillion et al. (2006) reported an OR of 2.91 [95% CI: 1.26–7.28] for SBP ≥ 130 mmHg, adjusting for age, BMI, smoking status, community and sex, with hair mercury concentrations ≥ 10 μg/g. Although our study did not calculate ORs using exposure quantiles and used 140 mmHg as the cut-point for elevated SBP, as recommended by the latest AHA hypertension guidelines, both studies still showed positive associations between mercury levels and blood pressure. In addition to Fillion et al. 2006, fifteen other studies reported a positive association between mercury exposure and blood pressure (Bautista et al. 2009; Choi et al. 2009; Eom et al. 2014; Guallar et al. 2002; Hu et al. 2017; Kobal et al. 2004; Nielsen et al. 2012 [Females]; Pedersen et al. 2005; Shiue 2014; Salonen et al. 2000; Sørensen et al. 1999; Virtanen et al. 2012a; Vupputuri et al. 2005; Wells et al. 2017; Yorifuji et al. 2010).

One of these studies, Vupputuri et al. (2005), investigated the association between mercury levels and blood pressure in women aged 16 and 49 in the United States, and found varying results based on fish consumption. Vupputuri et al. (2005) found a positive association in non-fish consuming women and no overall association in women consuming fish. Nielsen et al. (2012) measured mercury exposure in whole blood of the Inuit in Greenland, whose main source of mercury exposure is through MeHg in marine food. Both our study and that of Nielsen et al. (2012) showed an association between mercury and hypertension in men but not women. However, the direction of the association in the studies was contrary. In Nielsen et al. (2012), the OR for hypertension among men was reported as 0.99 (95% CI: 0.98-0.99; p-value=0.03). In our study, the odds of hypertension for each 2.7-fold increase in hair mercury concentration increased 3-fold among men (OR: 3.07; 95% CI: 1.36–6.92; p-value=0.007). It is important to note that although the main mercury exposure was to MeHg, Nielsen et al. (2012) measured mercury exposure in blood, and therefore, their mercury levels may not be as representative of long-term MeHg exposure. Sex differences in mercury levels are relatively under-studied. We could only find two studies that investigated sex differences in mercury, both of which were rodent studies published in 1986 (Hirayama and Yasutake 1986; Thomas et al. 1986). Hirayama and Yasutake (1986) administrated mice with MeHg and observed sex and age differences in mercury distribution and urinary excretion. Younger mice were found to have significantly lower levels of urine mercury compared to the older mice (Hirayama and Yasutake 1986). In addition, the youngest mice (2 weeks) in the study showed no significant sex difference in the distribution and excretion of mercury. However, at certain ages (4, 7, 10, and 45 weeks) male mice showed higher urinary mercury levels than female mice. Hirayama and Yasutake (1986) suggest that the differences in "urinary mercury excretion may be related to sex hormones, especially androgens" which increase hair follicles. Further study is required to assess whether these findings are applicable to the distribution of mercury in hair. Thomas et al. (1986) observed significant differences between males and females in the kinetics of "organic mercury retention" in "kidney, brain, skeletal muscle, pelt, and whole body". The authors (Thomas et al. 1986) found females to have more rapid elimination of organic mercury, "lower integrated" exposure of organic mercury, and higher average concentrations of organic mercury in the kidneys. This could explain the lower average of mercury levels found in our female population. In addition, Thomas et al. (1986) found that a significantly higher average proportion of the administered dose of organic mercury accumulated in the pelt and whole body of male rats compared to female rats. We could be witnessing a parallel in our study, with organic mercury accumulating

in higher proportions in hair of men compared to women. Hair is made up of fibrous protein known as keratin, which contains L-cysteine amino acids to which mercury binds (Nutall 2004). Accumulation differences in hair between sexes could potentially be related to differences in hair or hair protein structure. However, no information was found to either support or nullify this theory, and therefore, further research is needed. According to Guol and Katta (2017), protein malnutrition or decreased protein intake affects hair and can cause hair loss. However, the difference in average dietary protein intake between sex was not statistically significant, and therefore, cannot be used to evaluate the role of protein differences by sex. However, the lack of differentiation in dietary protein intake by sex is attributed to the design of the food frequency questionnaire which was used to create the dietary covariates. Only the head of household responded to the food frequency questionnaire, which was theoretically representative of the entire household. Therefore, men and women within the same household had the same dietary intake values. Evaluating the diet individually instead of for the household as well as incorporating consumption quantities would not only improve the overall accuracy of the results but would allow the evaluation of differences in consumption and diet between sexes, including the role of protein, which would improve our overall analysis.

Limitations of this study include the sample size. Although the sample size for the entire population was 308 and 304 in our MAP and hypertension models, respectively, after stratifying by sex the sample size was reduced to 96 and 94, respectively. This reduced the allowable number of variables in our models, potentially decreasing the accuracy of our results. In future study, a larger sample size will allow for the inclusion of more covariates. Another limitation in our study is the absence of the evaluation of alcohol consumption in our models. According to the "World Health Organization Global Burden of Disease 2000 Comparative Risk Analysis", 16% of all hypertensive disease is attributable to alcohol, making it an important predictor for hypertension (Rehm et al. 2003). Unfortunately, no information regarding alcohol consumption was recorded in the Cohort study. Future study should include this information. Other variables that could be added to improve our analysis include socio-economic indicators, such as income or education, and the proper assessment of fish consumption. Vupputuri et al. (2005) found opposite associations in fish and non-fish consuming women, emphasizing the importance in properly evaluating fish consumption when investigating the association between mercury levels and blood pressure. In addition, there were several interactions between mercury and other variables in our models that were not investigated our analysis. In assessing hypertension, the following interactions were found to be significant:

- The interaction between mercury and age in men (p-value=0.006);
- The interaction between protein and mercury among men (p-value=0.169); and
- The interaction between stearic acid and mercury among women (p-value=0.127) and men (p-value=0.175).

In assessing MAP, the following interactions were found to be significant:

- The interaction between mercury and age in men (p-value=0.163);
- The interaction between mercury and age in men who smoked in the past (p-value=0.131); and
- The interaction between mercury and Serum dihomo-γ-linolenic acid in women (p-value=0.105).

These interactions as well as seasonal variations in diet that could affect mercury exposure should be investigated in future study. However, it is important to note the many strengths of this study. These strengths include a relatively large sample size and the inclusion of a large number of relevant covariates, including dietary covariates and serum fatty acid concentrations. In addition, none of the previous studies comparing mercury levels and blood pressure we found during our literature review investigated differences between natives and non-natives within the same study area. Also, our study is the only one we could find evaluating the association between mercury levels and mean arterial pressure, specifically.

Conclusion

In this study, the associations between hair mercury concentration with hypertension and MAP differed by sex, being positive in men and inverse in women. The associations between hair mercury concentration with hypertension and MAP were significant at a 5% significance level among men, but not women. If confirmed, these findings may be attributable to differences in diet, men eating more mercury-contaminated fish and wildlife, as well as, the effect of sex hormones which regulate distribution and excretion of mercury in humans. Hypertension prevalence as well as mercury and MAP levels were higher in native communities compared to non-native communities, which may also be attributable to increased consumption of mercury-contaminated fish and wild-life. Average mercury and MAP levels were highest among native women aged 65 and up. Further investigation of the differences between native and non-native communities, including diet and specifically fish consumption, is warranted. Differences in mercury level between the age groups in these communities should also be further investigated. Additionally, the only interaction evaluated in this analysis was between mercury and sex. The study of other effect modifications with mercury is warranted, including age, serum stearic acid, and dietary protein intake.

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Appendix 1. 2010 attributable burden of disease rank for high blood pressure

Location	**Rank**
High-income Asia Pacific	1
Central Europe	1
East Asia	1
Tropical Latin America	1
Southeast Asia	1
Central Asia	1
North Africa and Middle East	1
Caribbean	1
Western Europe	2
Southern Latin America	2
Eastern Europe	2
Andean Latin America*	**2**
Southern sub-Saharan Africa	2
Australasia	3
High-income North America	3
South Asia	3
Central Latin America	4
Oceania	5
Eastern sub-Saharan Africa	5
Central sub-Saharan Africa	5
Western sub-Saharan Africa	6

*Peru, our country of study, is part of Andean Latin America

Appendix 2. Existing literature on the association between mercury exposure and blood pressure and hypertension
Hu et al. 2018, Table 1.

Table 1. Characteristics of studies included in the systematic review and meta-analysis.

Reference	Population	Exposure group	Mean age or age range (years)	Male (%)	N	Biomarker (unit)	Form	Mercury concentration	Outcome	Matrix available	Definition of HPT	Blood pressure measurement	Variables adjusted for
Bautista et al. 2009	Wisconsin, USA	General population	59.4	52.5	101	Hair (1g=g)	Total	Mean 0.27 (95% CI: 0.23, 0.32)	HPT, BP	H, B	140=90 mmHg, BP medication	Average of 2 measurements in 5 minutes interval using standard mercury sphygmomanometer, after seated for 15 minutes	Age, gender, BMI, fish intake, hypertension
Choi et al. 2009	Whaling men, Faroe Islands	Coastal and Indigenous population	54.8	100	42	Blood (1g=L)	Methyl	GM 29.5 (range: 5.19, 128.4)	BP	H, B, T	NA	Measured with standard mercury sphygmomanometers in seated position	Age, smoking, alcohol consumption, fish consumption, BMI
Daneshmand et al. 2016	Finland	General population	42–60	100	1828	Hair (1g=g)	Total[a]	Mean 1.90 (SD: 1.95)	BP	H	NA	Average of six measurements with zero mercury sphygmomanometer (after a supine rest of 5min, 3 in supine, 1 in standing and 2 in sitting position)	None
Eom et al. 2014	South Korea	General population	45.5	43.5	2114	Blood (1g=L)	Total	GM 3.90 (geometric SD: 1.88)	HPT, BP	B	130=85 mmHg, BP medication	Measured according to protocol	Age, gender, smoking, alcohol, residence area, seafood intake (+ income for HTN)
Fillion et al. 2006	Amazon, Brazil	Coastal and Indigenous population	35.2	53	251	Hair (1g=g)	Total	Mean 17.8 (range: 0.21, 77.2)	HPT	H	SBP ≥130 mmHg	Measured with standard mercury sphygmomanometers in sitting position	Age, gender, BMI, smoking, community
Goodrich et al. 2013	Dentist, Michigan, USA	Occupational population	52.3	38	262	Hair (1g=g)	Total	Median 0.28 (IQR: 0.14, 0.55)	BP	H, U	NA	Measured in sitting position with device (Omron HEM 432-C)	Age, gender, BMI, BP medication
Guallar et al. 2002	European & Israel	General population	53.2	100	724	Toenail (1g=g)	Total[a]	Mean 0.25 (IQR: 0.15, 0.40)	HPT	T	Self-report	NA	None, age balanced across quintiles
Hong et al. 2013	Smokers, South Korea	General population	16–75	62.7	236	Hair (1g=g)	Total	Mean 1.41 (SD: 1.1)	BP	H	NA	Measured after 10 min of rest in a sitting position using a model TM-2655P automatic sphygmomanometer	Age
Hu et al. 2017	Inuit, Canada	Coastal and Indigenous population	18–78	38.7	2169	Blood (1g=L)	Total	Median 7.8 (range: 0.3, 70)	HPT, BP	B	140=90 mmHg, BP medication, self-report	Average of 3 readings from a BpTRU™ Vital Signs Monitor	Age, sex, smoking status, systolic blood pressure, TC/HDL, BMI, physical inactivity, diabetes, marital status, education, income, red bloodcellomega-3 and omega-6 fatty acids, log transformed blood concentration of lead, cadmium, sum of PCBs and sum of PBDEs
Kobal et al. 2004	Mercury miners, Slovenia	Occupational population	45	100	120	Urine (1g=L)	Total	Mean 69.3 (range: 26, 158)	HPT, BP	B, U	140=90 mmHg	No detail	None
Lee and Kim 2013	South Korea	General population	≥20	100	3783	Blood (1g=L)	Total*	Mean 4.96 (SE: 0.07)	HPT	B	130=85 mmHg, BP medication	Average of 3 readings using a mercury sphygmomanometer in seated position	Age, BMI, residence area, education level, smoking, drinking status, exercise, AST, ALT, lead, cadmium
Mordukhovich et al. 2012	Elderly, Boston, USA	General population	72	100	639	Toenail (1g=g)	Total[a]	Median 0.22 (IQR: 0.07, 0.38)	HPT, BP	T	140=90 mmHg, BP medication	Average of both arms with standard mercury sphygmomanometers	Age, smoking, pack-years smoking, season of clinical visit, year of clinical visit, BMI, education, race/ethnicity, alcohol intake, fish intake

Note: BMI, body mass index; BP, blood pressure measurement; HPT, hypertension; GM, geometric mean; IQR, inter-quartile range; SD, standard deviation; SE, standard error; H, hair; B, blood; S, serum; U, urine; T, toenail; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFAs, polyunsaturated fatty acids; HDL, high density lipoprotein; TC/HDL, ratio of total cholesterol to high density lipoprotein. NA, outcome was not reported in the study.
[a]Assumed from study.

Appendix 2. Existing literature on the association between mercury exposure and blood pressure and hypertension
Hu et al. 2018, Table 1 (Continued.)

Table 1. (*Continued.*)

Reference	Population	Exposure group	Mean age or age range (years)	Male (%)	N	Biomarker (unit)	Form	Mercury concentration	Outcome	Matrix available	Definition of HPT	Blood pressure measurement	Variables adjusted for
Mozaffarian et al. 2012	Health professionals & Nurses, USA	General population	M 60.2 F 53.1	26.9	6045	Toenail (1g=g)	Total	Men Median 0.30 (90% CI: 0.07, 1.31) Women Median 0.21 (90% CI: 0.07, 0.76)	HPT, BP	T	Self-report	Self-reported usual blood pressure in 10 mmHg categories	Age, gender, race, month of toenail return, family history of hypertension, smoking status, BMI, diabetes, hypercholesterolemia, future cardiovascular disease status, physical activity, alcohol use, fish consumption, and consumption of whole grains, unprocessed meats, processed meats, fruits, and vegetables
Nielsen et al. 2012	Inuit, Greenland	Coastal and Indigenous population	46.9	43.6	1861	Blood (1g=L)	Methyl	Men Median 22 (IQR: 11, 41) Women Median 16 (IQR: 8.8, 34.1)	HPT	B	140=90 mmHg, BP medication	Average of the second and third reading using automatic BP apparatus (Kivex UA 779), after 5 minutes rest	Age, smoking, selenium, n-3/n-6 fatty acids, waist circumference
Park et al. 2013	USA	General population	46.6	48.6	6607	Blood (1g=L)	Total	GM 1.03 (95% CI: 0.95, 1.11)	HPT, BP	B, U	140=90 mmHg, BP medication, self-report	Average of up to 3 measurements in 5 minutes interval using standard mercury sphygmomanometer, disregarding the first measurement	Age, gender, race/ethnicity, education, BMI, alcohol, cotinine, omega-3, total caloric intake, BP medication
Park and Choi 2016	South Korea	General population	≥20	49.6	8371	Blood (1g=L)	Total[a]	GM 3.90 (geometric SD: 1.80)	BP	B	NA	Average of second and third time measurements in 5 minutes interval using standard mercury sphygmomanometer	Age, gender, smoking status, alcohol consumption, job status, education, residence, diabetes mellitus
Pedersen et al. 2005	Greenland & Denmark	Coastal and Indigenous population	20–60	43	186	Blood (1g=L)	Total[a]	Greenlanders Median 16.2 (range: 0.8, 117.7) Danes Median 2.2 (range: 0.8, 117.7)	BP	B	NA	Twenty-four hour ambulatory BP measurement (every 15 mins during daytime, every 30 mins during night-time)	Age, gender, BMI, residence
Rajaee et al. 2015	Gold miners, Ghana	Occupational population	30.6	60	70	Urine (1g=L)	Total	Median 4.24 (IQR: 1.24, 11.0)	BP	H, U	NA	Average of 3 readings using a mercury sphygmomanometer in seated position	Age, gender, smoking status
Shiue 2014	USA	General population	31.2	49.6	10537	Urine (1g=L)	Total[a]	Normal BP group Mean 0.68 (SD: 1.04) High BP group Mean 0.54 (SD: 0.75)	HPT	U	140=90 mmHg	NA	Age, gender, ethnicity, BMI, urine creatinine
Siblerud 1990	Dental amalgam, Colorado USA	Occupational population	23	40.6	101	Urine (1g=L)	Total	Non-amalgam Mean 1.23 (SD: 1.79) Amalgam Mean 3.70 (SD: 3.78)	BP	H, U		Several readings in sitting position by the auscultator method, mostly during evening time	None
Valera et al. 2009	Inuit, Nunavik, Canada	Coastal and Indigenous population	34.3	43.6	732	Blood (1g=L)	Total[a]	Mean 50.2 (95% CI: 46.6, 54.1)	BP	B	NA	Average of the second and third reading using mercury	Age, age^2, gender, EPA, selenium, alcohol consumption, waist circumference

Note: BMI, body mass index; BP, blood pressure measurement; HPT, hypertension; GM, geometric mean; IQR, inter-quartile range; SD, standard deviation; SE, standard error; H, hair; B, blood; S, serum; U, urine; T, toenail; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFAs, polyunsaturated fatty acids; HDL, high density lipoprotein; TC/HDL, ratio of total cholesterol to high density lipoprotein. NA, outcome was not reported in the study.
[a]Assumed from study.

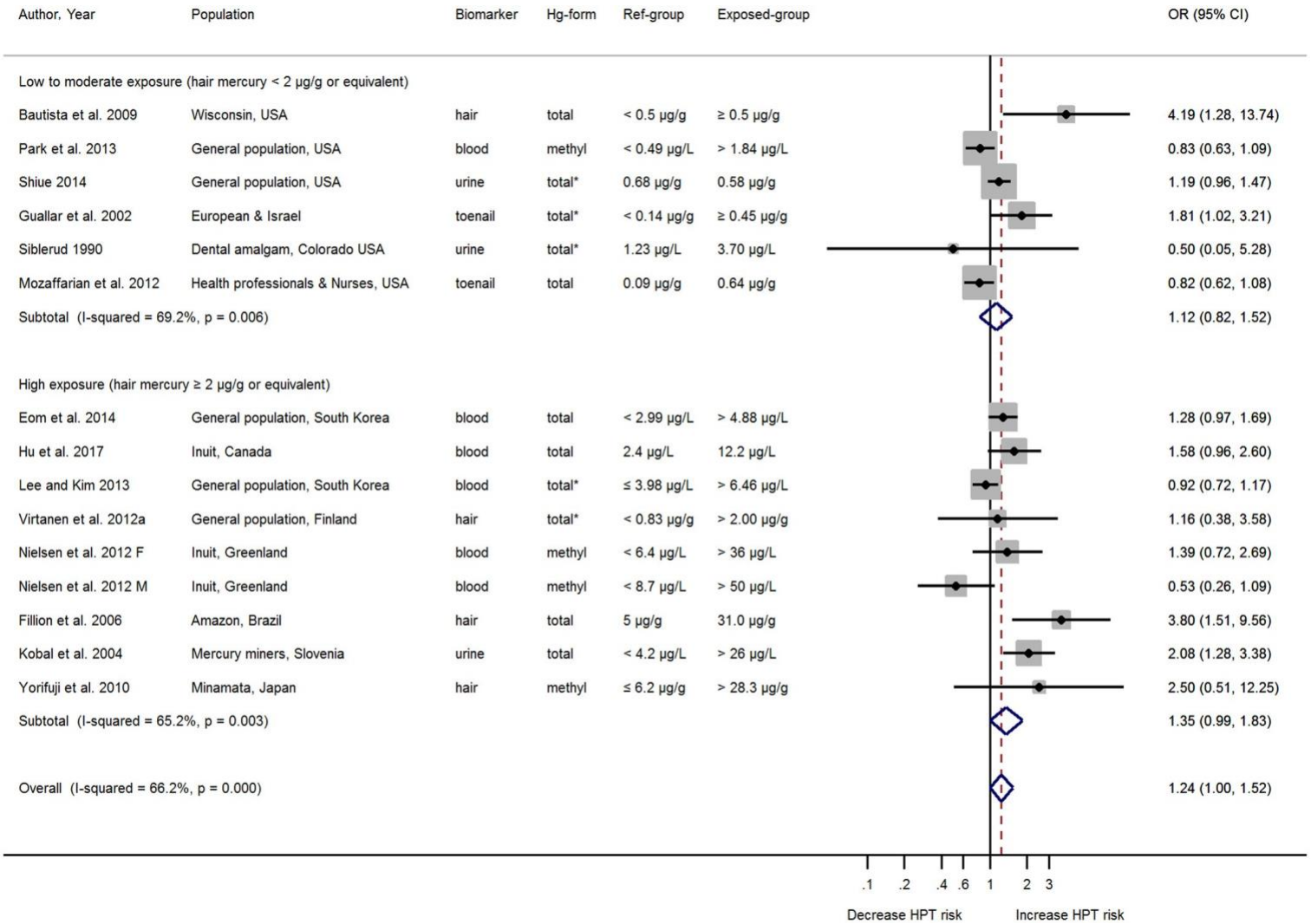
Appendix 2. Existing literature on the association between mercury exposure and blood pressure and hypertension
Hu et al. 2018, Table 1 (Continued.)

Table 1.(*Continued.*)

Reference	Population	Exposure group	Mean age or age range (years)	Male (%)	N	Biomarker (unit)	Form	Mercury concentration	Outcome	Matrix available	Definition of HPT	Blood pressure measurement	Variables adjusted for
												sphygmomanometers, after 5 minutes rest	
Valera et al. 2011a	The Cree, Quebec, Canada	Coastal and Indigenous population	35	53.2	791	Hair (1g=g)	Total[a]	Median 0.53 (IQR: 0.15, 1.62)	BP	H, B	NA	Average of the second and third reading using mercury sphygmomanometers, after 5 minutes rest	Age, sex, HDL-cholesterol, LDL-cholesterol, waist circumference, total n-3 PUFAs, triglycerides, fasting glucose, selenium, lead, PCB 153 and smoking.
Valera et al. 2011b	French Polynesia	Coastal and Indigenous population	48.6	47.2	180	Blood (1g=L)	Total[a]	Median 13.5 (IQR: 8.5, 22)	BP	B	NA	Average of the second and third reading using mercury sphygmomanometers, after 5 minutes rest	Age, sex, waist circumference, fasting glucose, triglycerides, anti-hypertensive treatment, selenium, total n-3 PUFA
Valera et al. 2013	Inuit, Quebec, Canada	Coastal and Indigenous population	38	42.2	313	Blood (1g=L)	Methyl	Median 17.0 (IQR: 9.0, 28.4)	BP	B	NA	Average of the second and third reading using mercury sphygmomanometers, after 5 minutes rest	Age, sex, waist circumference, DHA + EPA, and total PCBs
Virtanen et al. 2012a	Finland	General population	52.8	100	1857	Hair (1g=g)	Methyl[a]	Mean 1.91 (range: 0, 15.67)	HPT	H	No detail	NA	None
Virtanen et al. 2012b	Finland	General population	53–73	51.6	768	Hair (1g=g)	Methyl	Mean 1.42 (SD: 1.54)	BP	H	NA	Average of six measurements with zero mercury sphygmomanometer (after a supine rest of 5min, 3 in supine, 1 in standing and 2 in sitting position)	Age, gender, examination year, hypertension in family, smoking, leisure-time physical activity, alcohol consumption, BMI, education, employment status, 24-h urinary potassium and sodium excretion
Vupputuri et al. 2005	USA	General population	32.9	0	1240	Blood (1g=L)	Total	Mean 1.8 (range: 0.1, 21.4)	BP	B	NA	Average of up to 3 measurements in 5 minutes interval using standard mercury sphygmomanometer	Age, race, income, body mass index, pregnancy status, and dietary sodium, potassium, and total calories
Wells et al. 2017	Pregnant women, Baltimore, USA	General population	16.4–36.7	0	263	Blood (1g=L)	Methyl	GM 0.95 (95% CI: 0.87, 1.07)	BP	B	NA	Continuous blood pressure measurements were collected with a General Electric Corometrics model 120 series fetal monitor	Age, race/ethnicity, median neighborhood household income, pregnancy, body mass index, smoking during pregnancy, EPA + DHA and selenium.
Yorifuji et al. 2010	Minamata, Japan	Coastal and Indigenous population	≥10	41.7	120	Hair (1g=g)	Methyl	Low exposure <6:2 High exposure >28:3	HPT, BP	H	160=95 mmHg	Measured using a mercury sphygmomanometer by doctors in lying position	Age, occupation, past history of alcoholism, and past history of diabetes

Note: BMI, body mass index; BP, blood pressure measurement; HPT, hypertension; GM, geometric mean; IQR, inter-quartile range; SD, standard deviation; SE, standard error; H, hair; B, blood; S, serum; U, urine; T, toenail; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFAs, polyunsaturated fatty acids; HDL, high density lipoprotein; TC/HDL, ratio of total cholesterol to high density lipoprotein. NA, outcome was not reported in the study.
[a]Assumed from study.

Appendix 2. Existing literature on the association between mercury exposure and blood pressure and hypertension
Figure 1: Hu et al. 2018, Figure 2.



"ORs of hypertension by mercury exposure levels. The area of each square is proportional to the inverse of the variance of the estimated log OR. Black diamonds represent point estimates of OR and horizontal lines represent 95% confidence intervals (CIs). The open diamonds represent the combined OR for each subgroup and the overall OR for all studies. The solid line represents OR = 1. The dash line represents the point estimate of overall OR for all studies.
The "metan" package in Stata only outputs *p* value up to 3-digit numbers for the heterogeneity tests. We reported in the text "*p* < 0:0001" when the figures showed "*p* = 0:000". Note: CI, confidence interval; HPT, hypertension; OR, odds ratio. * indicates mercury form was not described in the Methods section and was assumed by authors of the present review."

Appendix 2. Existing literature on the association between mercury exposure and blood pressure and hypertension

Table 2. Characteristics of the studies in our literature review on the association between mercury exposure and blood pressure

Study	**Association with BP**	**Location**	**Exposure measure**	**Sample size**	**Sex**	**Sample Population**
Grandjean et al. 2004	No	Faroe Islands	Hair and cord blood	878	Both	Aged 7 and 14 years
Park et al. 2013	No	United States	Urine and blood	Urine: 2,201; Blood: 6,607	Both	Aged 20 and up
Valera et al. 2011a	No	Canada	Hair and Blood	549	Both	Aged 18 and up
Valera et al. 2011b	No	French Polynesia	Blood	281	Both	Aged 12 and up
Vupputuri et al. 2005	No	United States	Blood	1,240	Women	Aged 16-49 years
Vupputuri et al. 2005	Positive	United States	Blood	481	Women	Aged 16-49 years; Non-fish consumers
Choi et al. 2009	Positive	Faroe Islands	Blood	42	Men	Aged 30-70 years
Fillion et al. 2006	Positive	Brazil	Hair	251	Both	Aged 15 and up
Pedersen et al. 2005	Positive	Denmark	Blood	186	Both	Aged 20 to 60 years
Salonen et al. 2000	Positive	Finland	Hair	1,014	Men	Aged 42–60 years
Sørensen et al. 1999	Positive	Faroe Islands	Cord blood	917	Both	Aged 7 years
Wells et al. 2017	Positive	United States	Cord blood	263	Women	*WCBA; mean 25.8 years
Yorifuji et al. 2010	Positive	Japan	Hair	3,038	Both	Aged 10 and up

BP: blood pressure

*WCBA: Woman of childbearing age

Appendix 3. Summary of mercury exposure studies in Madre de Dios, Peru

Study	**Hg measurement**	**Sample Population**	**Sample Size**	**Mean Hg**	**Unit**	**Hg Range**	**Parameters evaluated**
Ashe 2012	Total Hair Hg	Both sexes, all ages	100	2.67	µg/g	0.36-20.26	Age, sex[*], residence location[*], duration of residence, and monthly fish consumption[*]
Langeland et al. 2017	Total Hair Hg	Both sexes, adults	80	3.4	µg/g	0.3-11	Age, sex and site[*]
Weinhouse et al. 2017	Total Hair Hg	Children, aged <12	83	1.18[∬]	µg/g	0.06-9.70	In this study, parameters were evaluated with respect to their association with hemoglobin levels not mercury.
Wyatt et al. 2017	Total Hair Hg	Both sexes, all ages	231	2.6[ϟ]	µg/g	0.4-10.5[∮]	Among all age groups only the association (positive) between body fat and mercury was statistically significant.
Yard et al. 2012	Blood MeHg	Both sexes, all ages	103	2.27[ϟ]	µg/L	0.6-10	Highest education attained ([*]university), drinking water source ([*]nonpublic system), mix mercury with gold([*]yes), and fish consumption([*]yes)
Yard et al. 2012	Total creatinine-corrected Hg	Both sexes, all ages	102	5.47[ϟ]	µg/g	0.7-151	Drinking water source[∯], heat gold-mercury amalgams[*]

[ϟ]Mean reported as geometric mean

[∮]95% Confidence Interval

[∬]Median value reported

[*]Statistically significant at an alpha level of 0.05

[∯]Statistically significant at an alpha level of 0.1

Appendix 4. Dietary Covariates

Table 1. Food representative from the Norwegian Food Composition Table (NFCT)

Food in questionnaire	**NFCT Food Representative**
Bushmeat/Wild Game	Wild boar, meat, raw
Beef	Beef, striploin, roasted
Pork	Pork, for roast, fat covered, roasted
Chicken	Chicken, with skin, raw
Junk Food	Cheese burger, single, with bread, cheese, dressing etc, fast food restaurant
French Fries/Chips	Potato Crisps
Soda	Soft drinks, cola, with sugar
Candy/Sweets	Sweets mix, chocolate included
Ice Cream	Ice cream, dairy
Quinoa	Quinoa, cooked
Cruciferous Vegetables	Cauliflower, cooked
Leafy Green Vegetables	Iceberg lettuce
Brazil Nuts	Brazil nuts
Citrus Fruits	Orange, raw

Appendix 4. Dietary Covariates

Table 2. Micronutrient values per 100 grams of edible food from the Norwegian Food Composition Table (NFCT)

Food in questionnaire	**Se (µg)**	**Calcium (mg)**	**Sodium (mg)**	**Potassium (mg)**	**Magnesium (mg)**
Bushmeat/Wild Game	13	4	66	367	26
Beef	7	4	54	471	29
Pork	19	6	89	486	24
Chicken	11	8	76	301	24
Junk Food	3	102	536	59	17
French Fries/Chips	0	8	455	1130	66
Soda	0	0	12.9	2	0
Candy/Sweets	0	175	84	365	55
Ice Cream	0	121	44	213	14
Quinoa	3	19	2	228	79
Cruciferous Vegetables	0	21	8	360	14
Leafy Green Vegetables	0	18	2	179	7
Brazil Nuts	1090	160	3	659	376
Citrus Fruits	0	42	0	193	13

Appendix 4. Dietary Covariates

Table 3. Energy and Macronutrient values per 100 grams of edible food from the Norwegian Food Composition Table (NFCT)

Food in questionnaire	**Calorie (kcal)**	**Fat (g)**	**Saturated Fat (g)**	**Carbohydrate (g)**	**Fiber (g)**	**Protein (g)**	**Starch (g)**
Bushmeat/Wild Game	105	1.50	0.50	0.00	0.00	23.00	0.00
Beef	124	2.00	0.90	0.00	0.00	26.50	0.00
Pork	313	23.40	8.00	0.00	0.00	25.60	0.00
Chicken	165	10.30	3.70	0.00	0.00	18.30	0.00
Junk Food	273	13.60	5.30	22.70	0.80	14.60	20.00
French Fries/Chips	517	31.50	2.60	50.50	5.50	5.20	50.00
Soda	42	0.00	0.00	10.80	0.00	0.00	0.00
Candy/Sweets	458	22.40	14.30	56.70	1.90	6.50	2.70
Ice Cream	194	10.00	6.20	23.00	0.00	3.00	0.00
Quinoa	144	2.40	0.30	23.40	2.80	5.70	21.00
Cruciferous Vegetables	23	0.20	0.10	2.30	2.30	1.90	0.00
Leafy Green Vegetables	12	0.10	0.00	1.50	1.10	0.80	0.00
Brazil Nuts	680	66.40	15.10	2.60	7.50	14.30	0.20
Citrus Fruits	37	0.10	0.00	7.20	1.80	0.90	0.00

Appendix 4. Dietary Covariates

Table 4. Typical serving size in grams of food listed on food frequency questionnaire

Food in questionnaire	**Typical serving size (g)**
Bushmeat/Wild Game	100
Beef	100
Pork	85
Chicken	90
Junk Food	100
French Fries/Chips	78
Soda	500
Candy/Sweets	43
Ice Cream	150
Quinoa	28
Cruciferous Vegetables	128
Leafy Green Vegetables	87
Brazil Nuts	30
Citrus Fruits	131

Appendix 4. Dietary Covariates

Table 5. Calculated energy, macronutrient and micronutrient scores

Food in questionnaire	**Saturated Fat Score**	**Selenium Score**	**Magnesium Score**	**Sodium Score**	**Potassium Score**	**Calcium Score**	**Calorie Score**	**Starch Score**	**Protein Score**
Bushmeat/Wild Game	0.50	13.00	26.00	66.00	367.00	4.00	105.00	0.00	23.00
Beef	0.90	7.00	29.00	54.00	471.00	4.00	124.00	0.00	26.50
Pork	6.80	16.15	20.40	75.65	413.10	5.10	266.05	0.00	21.76
Chicken	3.33	9.90	21.60	68.40	270.90	7.20	148.50	0.00	16.47
Junk Food	5.30	3.00	17.00	536.00	59.00	102.00	273.00	20.00	14.60
French Fries/Chips	2.03	0.00	51.41	354.45	880.27	6.23	402.74	38.95	4.05
Soda	0.00	0.00	0.00	64.50	10.00	0.00	210.00	0.00	0.00
Candy/Sweets	6.15	0.00	23.65	36.12	156.95	75.25	196.94	1.16	2.80
Ice Cream	9.30	0.00	21.00	66.00	319.50	181.50	291.00	0.00	4.50
Quinoa	0.08	0.84	22.12	0.56	63.84	5.32	40.32	5.88	1.60
Cruciferous Vegetables	0.13	0.00	17.92	10.24	460.80	26.88	29.44	0.00	2.43
Leafy Green Vegetables	0.00	0.00	6.09	1.74	155.73	15.66	10.44	0.00	0.70
Brazil Nuts	4.53	327.00	112.80	0.90	197.70	48.00	204.00	0.06	4.29
Citrus Fruits	0.00	0.00	17.03	0.00	252.83	55.02	48.47	0.00	1.18

Appendix 5. Information table for fatty acids analyzed including association with blood pressure (BP)

Fatty Acid	Lipid Numbers [C:D]	Fatty Acid Type	Association with BP	Statistical association	Adjusted OR for hypertension*	Sources
Stearic	[18:0]	Saturated	-	Each SD increase (0.2%) was associated with a decrease of 1.4 (95% CI: -2.5 to -0.2) mmHg in diastolic pressure [Simon et al. 1996]		Simon et al. 1996
Palmitic	[16:0]	Saturated	+	2-SD increase was associated with 1.4 (95% CI: 0.5 to 2.3) mmHg increase in systolic BP [Grimsgaard et al. 1999]		Grimsgaard et al. 1999 Uusitupa et al. 1994
Myristic	[14:0]	Saturated	+			Uusitupa et al. 1994
Docosahexaenoic	[22:6n-3]	ω−3 Unsaturated	-			Bønaa et al. 1990
Alpha-linolenic	[18:3n-3]	ω−3 Unsaturated	-		0.26 (95%CI: 0.07 to 0.95; p-value = 0.042) at 0.43 mol % compared to 0.13 mol%	Tsukamoto and Sugawara 2018
Eicosapentaenoic	[20:5n-3]	ω−3 Unsaturated	+/-			Positive association: Grimsgaard et al. 1999 Inverse association: Miyajima et al. 2001; Bønaa et al. 1990

[C:D] C = number of carbon atoms in the fatty acid; D= number of double bonds in the fatty acid; Source: Rigaudy J and Klesney SP (1979) Nomenclature of Organic Chemistry. Pergamon. ISBN 0080223699. OCLC 5008199.
OR = Odds Ratio
CI = Confidence Interval
*Tsukamoto and Sugawara 2018: ORs adjusted for age, BMI, physical activity (yes or no), current smoking (yes or no), alcohol consumption (≥20 g/day or no), salt intake and serum levels of glucose and hemoglobin A1c

Appendix 5. Information table for fatty acids analyzed including association with blood pressure (BP), continued

Fatty Acid	Lipid Numbers [C:D]	Fatty Acid Type	Association with BP	Statistical association	Adjusted OR for hypertension*	Sources
Linoleic	[18:2n-6]	ω−6 Unsaturated	-	2-SD increase was associated with a 1.9 (95% CI: 1.0 to 2.8) mmHg decrease in SBP [Grimsgaard et al. 1999]	0.17 (95% CI: 0.05 to 0.61; p-value = 0.003) at 21.01 mol % compared to 15.04 mol%	Uusitupa et al. 1994 Tsukamoto and Sugawara 2018
Gamma-linolenic	[18:3n-6]	ω−6 Unsaturated	-			Engler et al. 1998
Arachidonic	[20:4n-6]	ω−6 Unsaturated	+		2.04 (95% CI: 0.71 to 5.85; p-value = 0.047) at 8.47 mol% compare to 5.68 mol%	Tsukamoto and Sugawara 2018
Dihomo-γ-linolenic	[20:3n-6]	ω−6 Unsaturated	+	Each SD increase (0.16%) was associated with an increase of 1.2 (95% CI: 0.1 to 2.4) mmHg in diastolic pressure [Simon et al. 1996]		Grimsgaard et al. 1999 Simon et al. 1996
Palmitoleic	[16:1]	ω−7 Unsaturated	+	Each SD increase (1.9%) was associated with a systolic BP increase of 3.3 (95% CI: 0.9 to 5.6) mmHg. [Simon et al. 1996]		Grimsgaard et al. 1999 Cambien et al. 1988 Simon et al. 1996 Uusitupa et al. 1994
Oleic	[18:1]	ω−9 Unsaturated	+			Grimsgaard et al. 1999
Elaidic	[trans-18:1]	ω−9 Unsaturated	No			Teres et al. 2008

[C:D] C = number of carbon atoms in the fatty acid; D= number of double bonds in the fatty acid; Source: Rigaudy J and Klesney SP (1979) Nomenclature of Organic Chemistry. Pergamon. ISBN 0080223699. OCLC 5008199.
OR = Odds Ratio
CI = Confidence Interval
*Tsukamoto and Sugawara 2018: ORs adjusted for age, BMI, physical activity (yes or no), current smoking (yes or no), alcohol consumption (≥20 g/day or no), salt intake and serum levels of glucose and hemoglobin A1c.

Appendix 6. Variable selection: potential confounding variables
Table 1. Potential confounding variables considered

Variable			
1	Body fat percentage	17	Serum arachidonic acid z-score
2	Dietary calcium intake z-score	18	Serum cholesterol (mg/dL)
3	Dietary calorie intake z-score	19	Serum dihomo-γ-linolenic acid z-score
4	Dietary magnesium intake z-score	20	Serum docosahexaenoic acid z-score
5	Dietary potassium intake z-score	21	Serum eicosapentaenoic acid z-score
6	Dietary protein intake z-score	22	Serum elaidic acid z-score
7	Dietary saturated fat intake z-score	23	Serum gamma-linolenic acid z-score
8	Dietary selenium intake z-score	24	Serum linoleic acid z-score
9	Dietary sodium intake z-score	25	Serum myristic acid z-score
10	Dietary starch intake z-score	26	Serum oleic acid z-score
11	Muscle mass percentage	27	Serum palmitic acid z-score
12	Native community	28	Serum palmitoleic acid z-score
13	Natural log-transformed serum triglycerides	29	Serum stearic acid z-score
14	Physical activity: always gets at least 30 minutes of moderately intense activity at least 5 times per week	30	Smoking status: current smoker
15	Physical activity: sometimes gets at least 30 minutes of moderately intense activity at least 5 times per week	31	Smoking status: smoker in the past
16	Serum alpha-linolenic acid z-score	32	Western diet z-score

Appendix 6. Variable selection: potential confounding variables

Table 2. Potential confounding variables whose point estimate p-value was less than 0.2 in the base models

Variable	P-value of point estimate	
	MAP model	**Hypertension model**
Dietary calcium intake z-score	0.188	-
Dietary magnesium intake z-score	0.112	-
Dietary potassium intake z-score	0.119	0.170
Dietary protein intake z-score	0.049*	0.054
Dietary saturated fat intake z-score	0.181	0.176
Native community	0.000*	0.004*
Natural log-transformed serum triglycerides	0.016*	-
Physical activity: always gets at least 30 minutes of moderately intense activity at least 5 times per week	0.037*	-
Serum alpha-linolenic acid z-score	-	0.150
Serum cholesterol (mg/dL)	0.004*	0.055
Serum dihomo-γ-linolenic acid z-score	0.055	-
Serum docosahexaenoic acid z-score	-	0.073
Serum elaidic acid z-score	0.015*	0.042*
Serum myristic acid z-score	0.063	-
Serum oleic acid z-score	0.027*	0.188
Serum palmitic acid z-score	0.033*	-
Serum palmitoleic acid z-score	0.028*	-
Serum stearic acid z-score	0.111	0.115
Smoking status: smoker in the past	0.052	0.126
Western diet z-score	-	0.147

*Significant at 5% significance level

Appendix 7. Figure 3 from Hu et al. 2018

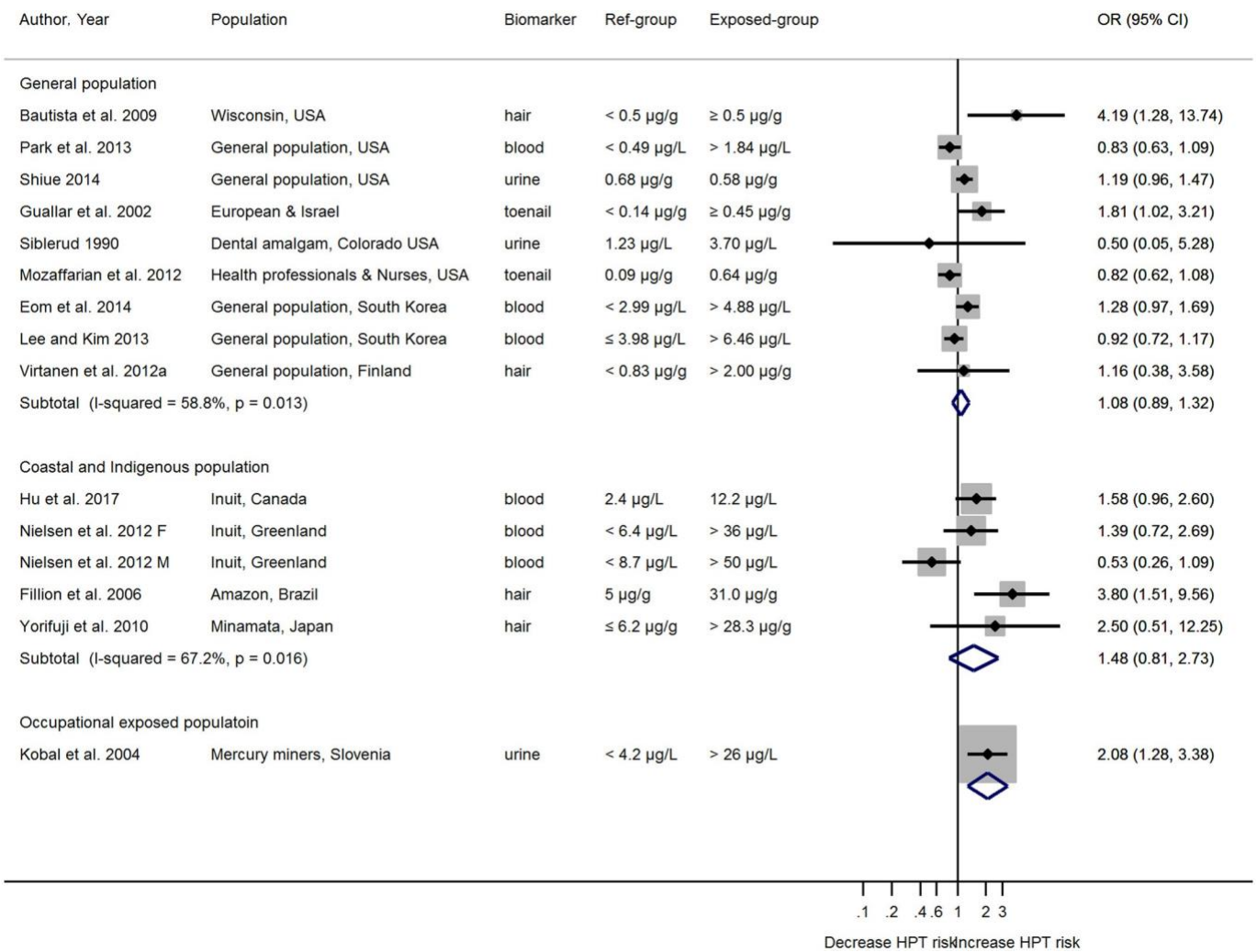


Figure 3. Odds ratios (ORs) of hypertension by mercury exposure groups. The area of each square is proportional to the inverse of the variance of the esti- mated log OR. Black diamonds represent point estimates of OR and horizontal lines represent 95% CIs. The open diamonds represent the combined OR for each subgroup. The solid line represents OR = 1. Note: CI, confidence interval; HPT, hypertension; OR, odds ratio.

Appendix 8. Table 2 from Fillion et al. 2006 summarizing the study population's socio-demographic characteristics

Table 2: Socio-demographic characteristics of the study population

	Women			Men		
Characteristics	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
Alcohol consumption						
Drinks	27		22.9	67		50.4
No longer drinks	15		12.7	25		18.8
Never drank	76		64.4	41		30.8
Smoking habits						
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

Source: Fillion M, Mergler D, Passos CJS, Larribe F, Lemire M, Guimarães JRD (2006) A preliminary study of mercury exposure and blood pressure in the Brazilian Amazon. Environ Health 5:29