Prenatal and recent methylmercury exposure and heart rate variability in young adults: the Seychelles Child Development Study

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Abstract

Epidemiologic evidence of an adverse association between exposure to methylmercury (MeHg) from consuming fish and heart rate variability (HRV) is inconclusive. We aimed to evaluate MeHg exposure in relation to HRV parameters in a large cohort of young adults from a high fish consuming population in the Republic of Seychelles. Main Cohort participants in the Seychelles Child Development Study were evaluated at a mean age of 19 years. Prenatal MeHg exposure was determined in maternal hair growing during pregnancy and recent exposure in participant's hair taken at the evaluation. The evaluation consisted of short (~2 hours) and long (overnight) Holter recordings obtained in 514 and 203 participants, respectively. Multivariable analyses examined the association of prenatal and recent MeHg exposure (in separate models) with time-domain and frequency-domain HRV parameters in different physiologic circumstances: supine position, standing position, mental stress when undergoing a mathematics test, sleep, and long recording. Prenatal MeHg exposure was not associated with any of the 23 HRV parameters studied after adjustment for multiplicity. The recent MeHg showed a trend toward significance only for few variables in the primary model. However, after additional adjustment for activity levels, polyunsaturated fatty acids, and multiplicity none were significant after a Bonferroni adjustment. In conclusion, prenatal and recent MeHg exposure had no consistent pattern of associations to support the hypothesis that they are adversely associated with heart rate variability in this study population that consumes large amounts of fish.

Keywords	mercury, methylmercury, heart rate variability, autonomic nervous system, fish consumption
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Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request

Highlights

- High fish consumption might be associated with exposure to methylmercury.
- The effect of methylmercury on heart rate variability remains controversial.
- This is the most comprehensive assessment of HRV parameters to our knowledge among published studies focused on methylmercury toxicity
- In this study, methylmercury exposure from open ocean fish consumption did not adversely impact heart rate variability in young adults.

Prenatal and Recent Methylmercury Exposure and Heart Rate Variability in Young Adults: the Seychelles Child Development Study

Short Title: Methylmercury and HRV in Young Adults

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ABSTRACT

Epidemiologic evidence of an adverse association between exposure to methylmercury (MeHg) from consuming fish and heart rate variability (HRV) is inconclusive. We aimed to evaluate MeHg exposure in relation to HRV parameters in a large cohort of young adults from a high fish consuming population in the Republic of Seychelles. Main Cohort participants in the Seychelles Child Development Study were evaluated at a mean age of 19 years. Prenatal MeHg exposure was determined in maternal hair growing during pregnancy and recent exposure in participant's hair taken at the evaluation. The evaluation consisted of short (~2 hours) and long (overnight) Holter recordings obtained in 514 and 203 participants, respectively. Multivariable analyses examined the association of prenatal and recent MeHg exposure (in separate models) with time-domain and frequency-domain HRV parameters in different physiologic circumstances: supine position, standing position, mental stress when undergoing a mathematics test, sleep, and long recording. Prenatal MeHg exposure was not associated with any of the 23 HRV parameters studied after adjustment for multiplicity. The recent MeHg showed a trend toward significance only for few variables in the primary model. However, after additional adjustment for activity levels, polyunsaturated fatty acids, and multiplicity none were significant after a Bonferroni adjustment. In conclusion, prenatal and recent MeHg exposure had no consistent pattern of associations to support the hypothesis that they are adversely associated with heart rate variability in this study population that consumes large amounts of fish.

Fish is an important source of nutrition worldwide with about 3.1 billion people depending daily on fish as their primary source of protein (FAO 2016). However, all fish also contain naturally occurring methylmercury (MeHg) which is a known neurotoxicant in high dosages. Maternal consumption during pregnancy of industrially polluted fish (after MeHg was discharged in waste water from a chemical plant and seed grain treated with a MeHg fungicide) have been associated with severe neurological deficits in children (WHO 2007). Since neurotoxicity was observed after large exposure to MeHg, several studies have evaluated heart rate variability (HRV) to investigate the association between prenatal or recent MeHg exposure (Sorensen et al. 1999; Oka et al. 2002; Grandjean et al. 2004; Murata et al. 2004; Choi et al. 2009; Lim, Chung, and Paek 2010).

Heart rate variability (HRV) is an accepted method to assess changes in the autonomic nervous system during parasympathetic and sympathetic modulation of the cardiovascular system (HRV 1996). Heart rate is under constant influence of an interplay between parasympathetic and sympathetic modulation leading to slower heart rate at rest or during sleep, and faster heart rate with exercise or stress. Beat-to-beat variation in heart rate, or in duration of beat-to-beat intervals, could be measured reflecting changes in the autonomic control of the heart (Kleiger, Stein, and Bigger 2005). In healthy subjects, it is expected that there is a physiologic variation of on NN intervals (defined as normal beats with sinus rhythm, while excluding premature and ectopic beats) influenced by respiration, baroreflex sensitivity, in addition to the above mentioned parasympathetic and sympathetic modulation (Cygankiewicz and Zareba 2013). HRV might be altered in patients after myocardial infarction, patients with heart failure, or those with diabetes, obesity, frequently with a decrease in parasympathetic modulation and an increased sympathetic modulation (HRV 1996; El Agaty, Kirmani, and Labban 2017; Tsuji et al. 1996). These changes in HRV have been found as predictors of outcome (mortality, heart failure, ventricular tachyarrhythmias) in various patient populations (Kleiger et al. 1987; Bigger et al. 1992;

Stein et al. 1997; Kleiger, Stein, and Bigger 2005; Sherazi et al. 2015). There is profound evidence that in addition to environmental factors like exposure to air pollution or lead, pharmacological or electrical therapies might alter HRV parameters indicating changes in the autonomic control of the heart (Coumel et al. 1991; Malik et al. 1989; Simula et al. 2017; Jerling et al. 2018; Hoogerwaard et al. 2019; Petersen et al. 2018; Breitner et al. 2019; Gump et al. 2011). The guidelines regarding HRV analysis and its parameters recommended for research and clinical use were established in 1996 (HRV 1996). However, over the last 20 years there still have been inconsistencies regarding HRV methodology and interpretation (Shaffer and Ginsberg 2017).

Some epidemiologic studies have suggested that MeHg exposure may be associated with a decrease in HRV parameters but with general inconsistency of findings among studies, (Grandjean et al. 2004; Mozaffarian 2009; Oka et al. 2002; Sorensen et al. 1999) and some were not able to confirm it (Valera, Dewailly, and Poirier 2008; Miller et al. 2018; Gump et al. 2017; Choi et al. 2009) These studies have generally been based on short term HRV recordings at rest, and some were done in populations with co-exposures. Varying duration of recordings, diverse ECG sampling rate, different management of ectopic beats may influence results of HRV analyses (Shaffer and Ginsberg 2017; Karita et al. 2018; Stapelberg et al. 2018; Melo et al. 2018). These factors limit our understanding of whether there are systematic and direct cardiovascular effects of MeHg exposure (Karita et al. 2018). As recently summarized in two extensive reviews on the topic (Gribble et al. 2015; Karita et al. 2018) it is still uncertain whether MeHg exposure from repeated consumption of fish with naturally occurring background levels has any adverse and meaningful effect on HRV.

In a prospective study of 19-year-old healthy subjects, we assessed whether the prenatal and recent MeHg exposure from consuming fish adversely affects HRV in comprehensive analyses of 24-hour Holter recordings and short-term 5-minute recordings reflecting different

physiologic conditions associated with resting supine, standing, mental stress (mathematics test), and sleeping.

METHODS

Study Population and Design

The Republic of Seychelles is a 115-island archipelago in the middle of the Indian Ocean where citizens consume large amounts of ocean fish. The population is exposed to MeHg primarily from fish consumption and does not consume sea mammals (Cernichiari et al. 1995; Davidson et al. 1998). Fish consumed in Seychelles are contaminated only by natural background exposure to MeHg. This setting provides a unique opportunity to study the association of MeHg with health outcomes. In 1989-90, 779 mother-infant pairs were recruited as part of the Seychelles Child Development Study (SCDS) main cohort (Marsh et al. 1995). The demographics and earlier evaluations of this cohort have been reported previously (Davidson et al. 1998; Shamlaye et al. 1995; Myers et al. 2003; van Wijngaarden et al. 2013).

For this study, a total of 518 cohort participants returned for evaluations at the Study Centre at a mean ± SD of 19.0 ± 0.3 years of age. Data from four of these participants were not included due to technical errors in ECG data acquisition. The evaluation consisted of a questionnaires collecting demographic, socioeconomic, medial history, and lifestyle data, collection of biological samples (hair and blood samples), and Holter ECG recordings providing time- and frequency-domain HRV parameters. The study protocol was approved by the Institutional Review Boards of the Republic of Seychelles and the University of Rochester.

MeHg and Polyunsaturated Fatty Acids Measurements

Prenatal MeHg exposure was determined by measurement of total maternal Hg levels in hair in samples collected closed to the scalp after pregnancy. It reflected mercury concentrations during pregnancy assuming a hair growth rate of 1 cm per month. Recent MeHg exposure was available for 451 (87.7%) of the participants; 68% of those missing this variable were males, many of whom had shaved heads. Recent MeHg was analyzed in the 1 cm hair samples collected closest to the scalp taken at the time of Holter testing (Myers et al. 2009). Hair analysis was performed using cold vapor atomic absorption spectroscopy (Magos and Clarkson 1972). The detailed description of hair sampling, analysis and quality control procedures was previously described (Cernichiari et al. 1995). It has been documented that mercury hair levels recapitulate mercury exposure since hair concentrations correlate well with blood mercury levels (Clarkson, Magos, and Myers 2003).

Recent status of n-3 and n-6 PUFA were determined from plasma samples taken at the time of evaluation. Samples were processed, aliquoted, and stored at -80°C in the Ministry of Health's Clinical Laboratories and transported to the nutrition biochemistry laboratory in Coleraine, Northern Ireland for fatty acid analysis. Plasma samples were subjected to lipid extraction using an adaptation of the method of Folch et al. (Folch, Lees, and Sloane Stanley 1957) and solid phase extraction columns (Merck Lichrolut, UK) were used to isolate phospholipids. Fatty acid methyl esters were prepared with boron trifluoride in methanol (Sigma-Aldrich Co. Ltd.) and analyzed using GC-MS technology (5975C series, Agilent Technologies Ltd., Stockport, UK). Fatty acids were identified by their retention time with reference to commercially obtained fatty acid standards and were quantified by use of an internal standard C17:0 (Sigma Aldrich Co. Ltd.). The GC column used was a BPX 70 capillary column (Phenomenex, Cheshire, UK) with a length of 100 m, internal diameter of 0.25 mm and a capillary thickness of 0.25 μm.

Holter ECG Recordings

All 514 participants underwent 12-lead high-resolution Holter ECG recordings which were initiated between 8-10 am using digital (1000 Hz) recorders (H-Scribe, Mortara Instruments Inc., Milwaukee, WI). ECG recordings started with a 10-minute supine position followed by a 10-minute standing and subsequently a 10-minute ECG while the participant was completing mathematics (math) test. The supine recording served to analyze HRV parameters in the resting non-active state (parasympathetic dominance), the standing recording provided insight into the response of the heart rate and HRV parameters to activation of the autonomic system by changing body position (sympathetic activation), and the recording during a mathematical test reflected the effect of mental stress on the studied ECG parameters (Tsuji et al. 1996; Umetani et al. 1998). The Holter recordings were finished after 1-2 hours in 312 subjects, but in the remaining 202 subjects recordings were continued to obtain long-term overnight data to further characterize the normal physiologic predominance of parasympathetic modulation of the autonomic nervous system during sleep and provide insight into the overall long-term values of time-domain HRV parameters.

All recordings were sent to and analyzed at the University of Rochester Medical Center's ECG Core Laboratory by technicians blinded to participants' demographic and methylmercury exposure data. Routine beat annotation was performed identifying normal sinus beats and abnormal beats (atrial and ventricular ectopic beats, noise) and only normal sinus beats were included in the analyses. In the case of ectopic beats, the sinus normal-to normal beat (NN) interval following the ectopic beat was not included. All recordings were verified by a cardiologist (WZ).

Heart Rate Variability

From short-term recordings obtained in supine and standing positions and during the math test, 5-minute ECG recordings were extracted starting 3 minutes from the beginning of each segment to allow for adaptation to the changed conditions. A 5-minute segment from the overnight Holter recording was also analyzed at approximately 2 am to represent HRV during sleep.

Annotated NN intervals were used to compute HRV parameters for a given segment of data. The time-domain HRV parameters (Kleiger, Stein, and Bigger 2005) that were obtained from both the short (~5-minute) and long recordings included: SDNN (standard deviation of normal-to-normal sinus beat intervals) reflecting overall HRV and rMSSD intervals (root mean square of successive differences in NN intervals). We also computed: pNN50 (percentage of NN intervals differing more than 50 ms from the preceding beat) reflecting parasympathetic modulation, SDANN (standard deviation of averaged 5-min normal-to-normal RR intervals), and SDNIX (mean of the SDs of all normal-to-normal RR intervals) for all 5-min segments of the entire recording (SDNNIX). These reflect overall HRV and are similar to SDNN, which is why we elected to focus on SDNN and rMSSD as representative HRV parameters in time-domain analysis.

The frequency-domain HRV methods (Bigger et al. 1992) allow for identifying the relative contribution of specific frequency bands reflecting oscillatory behavior of the heart rate. The total power (TP) of the entire spectrum was measured from 0 to 0.40 Hz. Other components included high frequency (HF: 0.15-0.40 Hz), low frequency (LF: 0.04-0.15 Hz), and very low frequency (VLF: 0.003-0.04 Hz) bands. The HF power (expressed in normalized units as HF/[TP-VLF]) represents parasympathetic (respiratory) modulation of the heart rate whereas LF power (expressed in normalized units as LF/[TP-VLF]) reflects modulation of the heart rate by both sympathetic and parasympathetic tones, but with strong dominance of sympathetic influence and

baroreflexes. We also evaluated LF/HF ratio but elected to focus on LF and HF separately to illustrate effects of MeHg on individual components of these variables.

Our primary statistical analysis focused on 23 of the most commonly used HRV parameters reflecting autonomic activity in different physiologic conditions: 5 in supine position, 5 in standing position, 5 in response to a mathematics test, 5 during sleep, and 3 for the entire recording. Five outcomes measured at baseline while supine at rest included: NN, SDNN, rMSSD, LF, and HF. We also analyzed the five outcomes measured at baseline minus their values while standing, taking a math test, and sleeping. Unless otherwise noted, response variables are defined as differences from baseline values. Three primary outcomes measured on the long recordings included: NN, SDNN, rMSSD. Additionally, we computed three more HRV parameters (pNN50, SDANN, and SDNNIX) for secondary analysis by MeHg categories.

Statistical Analyses

We used linear regression to examine the association of each exposure (prenatal and recent MeHg, in separate models) and each of the 23 HR variables. Models first examined the association with a sex by exposure interaction term since significant differences in HRV are known to be present between males and females, and additionally, exposure differed by sex (Tsuji et al. 1996; Umetani et al. 1998). If the interaction was statistically significant ($p < 0.05$) then those results are reported. If the interaction was not significant ($p \geq 0.05$) then the analysis was rerun without the interaction term and those results are reported. Primary models were fit with and without covariate adjustment for sex, birth weight, and current BMI. We adjusted for birth weight due to its potential impact on childhood development and for BMI due to evidence of an association between ECG variables and obesity (Antelmi et al. 2004). For each analysis, we used

a two-sided test with alpha = 0.05 to assess significance. For the association of recent MeHg exposure with HRV we fit secondary models adjusting for recent n-3 and n-6 PUFA and for the participant's activity level. Physical activity level is a main predictor of HRV (Fisher, Young, and Fadel 2015). The Global Physical Activity Questionnaire (GPAQ) was developed by WHO for physical activity surveillance. The GPAQ standardizes level of total physical activity measured in MET-minutes/week categorizing the value <600 as low sedentary activity, 600-<3000 as moderate activity, and ≥3000 as high activity. The activity measurement was based on the participants self-report of their minutes of physical activity per week using three domains (work, walking, and leisure) and was treated as a categorical variable with three levels (<600, 600-<3,000, and > 3,000 minutes) following the WHO recommendation. We adjusted for n-3 and n-6 PUFA because of their reported cardiac effects (Christensen et al. 1999). Model assumptions were checked using standard methods, including checking for constant variance, nonlinearity, and normally distributed residuals (Weisberg 2005). In some cases, there was evidence of assumption violations which necessitated a log transformation of the outcome. For variables that required a logarithmic transformation, differences from baseline values were calculated after the log transform of both variables (e.g. the outcome is the logarithm of the ratio of values, rather than the logarithm of the difference from baseline). After transformations when required, there was no evidence of extreme outliers or highly influential observations. We report the individual associations and corresponding betas and p values from the primary analysis. Additionally, we applied the Bonferroni correction to account for multiple comparisons. Since we focused on 23 primary HRV parameters measured (5 in supine position, 5 in standing position, 5 in response to math test, 5 during sleep and 3 for the entire recording), we considered a p value of 0.05/23 = 0.0022 as significant after this correction.

We also performed a univariate comparison of studied HRV parameters by MeHg levels categorized as 0 to <5 ppm, 5 to <10 ppm, and ≥10 ppm in prenatal and recent exposure. Median value of MeHg level in hair of US females 16-49 years of age in the NHANES 1999-2000 was 0.19 μg/g with 95th percentile of 1.73 μg/g, reaching 2.75 μg/g in individuals eating fish ≥3 times in the past 30 days (McDowell et al. 2004). Prenatal assessments of women from the Seychelles high fish consumption population reported mean ± SD total hair MeHg of 6.85 ± 4.5 ppm and a median value of 5.94 ppm (Myers et al. 2003). Therefore, the cutoff of 0-5ppm was chosen as reflecting low levels of MeHg. The median total hair Hg level of women in the Faroes birth cohort study was 4.5 μg/g; 12% had levels > 10 μg/g, considered as elevated (Grandjean et al. 2004). Therefore, we proposed the above arbitrary cutoffs reflecting low, medium, and high levels of MeHg in the Seychelles fish eating populations.

RESULTS

Descriptive Analysis: Characteristics of Participants

There were 514 eligible participants (273 females and 241 males) who had short Holter recordings and 203 participants (117 women and 86 men) who had long Holter recordings. Table 1 shows the participants clinical characteristics, MeHg levels, and n-3 and n-6 PUFA levels compared by sex. The mean prenatal MeHg exposures were similar in males and females (6.77 vs 7.06 ppm, respectively), but recent MeHg exposures at 19 years of age were significantly higher in males than females (12.16 vs. 8.67 ppm, respectively). Males were physically more active and had significantly lower BMI values than females. The n-3 and n-6 PUFA levels did not differ by sex. Correlation between prenatal Hg and recent Hg (obtained at the age of 19 years) was weak: the correlation coefficient was 0.13.

As expected, time-domain SDNN and rMSSD parameters from long-term recordings and short-term supine recordings were significantly ($p<0.0022$) different between males and females (Supplementary Table S1). In supine resting position, males had significantly longer NN interval (lower heart rate) than females. Males also had significantly higher levels of long-term and baseline supine time-domain SDNN and rMSSD parameters, but response to standing, math test, and sleep did not significantly differ by sex. Differences in frequency-domain HRV parameters between males and females were less pronounced and did not reach $p<0.0022$ level.

Heart Rate Variability Parameters by Categorized MeHg Exposure Levels

Table 2 shows a univariate comparison of studied HRV parameters by MeHg levels categorized as 0 to <5 ppm (low), 5 to <10 ppm (medium), and ≥10 ppm (high) in prenatal and recent exposure (Table 2). For prenatal MeHg exposure this comparison did not show any significant associations indicating level-related changes in HRV parameters in long term recordings, baseline supine or response to standing, math and sleep. For recent MeHg categories there were few statistically borderline associations (p value between 0.0022 and 0.05) which were driven by lower values in medium than low and high MeHg exposure: NN in response to standing and math test, LF in response to sleep, and HF at baseline, in response to math and sleeps. Among these associations the direction was inconsistent and there was no evidence of a dose response. Since we observed sex-related differences in HRV parameters we reanalyzed 24-hour HRV data for males and females separately using the primary time domain parameters (NN, SDNN, rMSSD) and 3 additional ones (pNN50, SDANN, & SDNNIX). No significant associations were present in these HRV parameters among subject with low, medium, and high MeHg exposure (Supplementary Table S2).

Regression Analysis: Prenatal MeHg Exposure and Heart Rate Variability

The sex by prenatal exposure interaction was significant for the NN interval long recording and the NN in response to sleep (Table 3). After adjusting for sex, birth weight, and BMI, there were two borderline significant associations at p value between 0.0022 and 0.05. Both were in males; and one was beneficial and the other adverse. The NN interval in the long recording was predicted to increase in males by 5.5 ms per 1 ppm increase in MeHg (p=0.006). This beneficial association reflected a 0.5 beat per minute decrease in heart rate per 1 ppm increase in MeHg, or a 3.4 beat per minute decrease per IQR increase in MeHg. The NN interval in response to sleep decreased only in males by 10.49 ms per 1 ppm increase in MeHg (p = 0.0055), reflecting about 0.5 a beat per minute increase in heart rate, physiologically an adverse association. When the Bonferroni correction was applied, none of the associations reached statistical significance (p<0.0022). There were no other significant interactions or associations of prenatal MeHg with either the time- or frequency-domain HRV parameters computed from the long or short recordings.

Regression Analysis: Recent MeHg Exposure at 19 years and Heart Rate Variability

Recent MeHg exposure was associated with four outcomes in models that adjusted for sex, BMI, and birth weight (Supplementary Table S3). Only one sex by MeHg interaction was borderline significant at p between 0.0022 and 0.05, the NN interval in response to standing (p = 0.015). That association was only in females and was adverse (p = 0.0172). For the NN interval in response to standing, females had a 3 ms increase in the difference in the NN interval per 1 ppm increase of MeHg (p = 0.017), or a 20.8 ms increase in the difference in the NN interval for an interquartile range (IQR). The associations with the two most classical time-domain parameters: SDNN baseline supine and on the long recording were not significant (p=0.600 and 0.128 respectively). Similarly, the associations with rMSSD resting supine and on the long recording were not significant (p=0.197 and 0.489, respectively). For the difference in log

316 (rMSSD) in response to a math test, there was a 0.009 ms increase for each 1 ppm increase in
317 MeHg, a beneficial response. Stated differently, a 1 ppm increase in exposure was associated
318 with a 1% increase in the ratio of rMSSD at baseline to the rMSSD during the math test, or an
319 IQR increase in exposure was associated with a 6.4% increase in this ratio. For HF in response
320 to standing both sexes together had a 0.40% increase in difference from baseline (p=0.029), and
321 for HF in response to the math test, they had a 0.375% increase in difference from baseline
322 (p=0.032), indicating lower parasympathetic parameters during these tests. These associations
323 were inconsistent in direction, of small magnitude, nonsignificant after Bonferroni correction, and
324 of uncertain meaning.

325 After additional adjustment for activity levels and n-3 and n-6 PUFA, recent MeHg
326 exposure had borderline significant sex by recent MeHg interactions in two models: the NN
327 interval in response to standing and the LF in response to the math test (Table 4). The association
328 between MeHg and NN interval in response to standing was borderline at p=0.0499 only in
329 females who had on average a 2.6 ms increase per 1 ppm increase in recent MeHg. There was
330 also a borderline (p=0.0210) association between MeHg and LF in response to the math test for
331 males only. Each 1 ppm increase in exposure was associated with a 0.56 ms decrease in the
332 difference in LF (a beneficial effect).

333 Of the six significant borderline significant associations (at P between 0.0022 and 0.05)
334 associations with the combined sexes, three were also present in the primary analysis (the
335 difference in rMSSD on the logarithmic scale in response to the math test and the difference in
336 HF in response to standing and to the math test). The association with the difference in rMSSD
337 in response to the math test was virtually unchanged, and the associations with HF responses to
338 standing and the math test were both slightly attenuated. The three additional outcomes with
339 borderline significant MeHg associations were the NN interval for the long recording, the baseline

supine NN, and the LF in response to sleep. For the baseline supine NN, each 1 ppm increase in recent MeHg was associated with on average a decrease of 3.05 ms, an adverse association. For the LF in response to sleep, each 1 ppm increase in recent MeHg was associated with a 0.69 decrease (beneficial effect). The inconsistency of these associations and their small magnitude (milliseconds) make them challenging to interpret. After applying the Bonferroni correction for multiplicity, there were no statistically significant MeHg associations (all p values >0.0022).

DISCUSSION

We studied whether HRV parameters are associated with prenatal or recent MeHg exposure. We measured a comprehensive set of HRV parameters obtained from short-term (5 minutes) and long-term (24 hours) Holter recordings in a cohort of young adults exposed to MeHg from frequent consumption of open ocean fish. Their prenatal MeHg exposures were approximately 10 times more than those present in the United States. We focused on 23 measured HRV variables and found none of them significant at prespecified after Bonferroni correction with $p<0.0022$. There were two borderline significant (at p between 0.0022 and 0.05) associations with prenatal and eight borderline significant associations with recent MeHg exposure in secondary models. The two prenatal associations were present only in males and were in opposite directions suggesting random variation. The eight recent MeHg exposure significant associations were all of small magnitude, and there was no consistent pattern that would suggest they are related to exposure. Following Bonferroni correction for multiplicity, there were no statistically significant (at $p<0.0022$) associations with either prenatal or recent MeHg exposure.

We observed the expected sex differences in HRV parameters that have been previously reported in the literature. Males showed significantly higher values of HRV parameters than females (Agelink et al. 2001; Umetani et al. 1998), and the responses to standing and other conditions were as expected (Srinivasan, Sucharita, and Vaz 2002). These findings in a large cohort provide reassurance that there was sufficient statistical power to detect meaningful associations of exposures and HRV if they were present. The borderline significant findings were of very small magnitude without clear physiological or clinical meaning. Previously, we measured beat to beat blood pressure in 95 participants from this cohort, using the Finapres to record the baroreflex, another measure of autonomic nervous system integrity (Periard et al. 2015). That study found no association of blood pressure variability with either prenatal or recent MeHg levels. These data do not support the hypothesis that prenatal or recent exposure to MeHg from fish consumption influences HRV parameters.

The categorical analyses (Table 2 and Supplemental Table S2), although not adjusted for covariates, further corroborate the absence of associations between prenatal and recent MeHg exposure and the NN interval and other HRV parameters among the three exposure groups. If there was an association, one would expect some MeHg level-dependent changes in HRV parameters, but this categorical approach showed no trend toward any association, i.e. no decrease or increase in studied parameters across incremental levels of MeHg. At the same time, we were able to detect significant differences as we documented by detecting expected sex-related differences in HRV parameters (positive control test).

Prenatal MeHg exposure was associated with about a 0.5 beat per minute slower heart rate in males per 1 ppm increase in MeHg. This change does not have clinical meaning considering that exposure to MeHg in Western societies is generally less than 1 ppm. Even in high fish-consuming societies like Japan, MeHg exposure is generally less than 2 ppm which

386 would be associated with a potential variation in heart rate of approximately 1 beat per minute.
387 Neither time-domain nor frequency-domain HRV parameters were associated with prenatal MeHg
388 levels. Prenatal MeHg does not appear to be influencing parameters or autonomic nervous
389 system activity reflected by HRV at the age of 19 years in this cohort.

390 Several HRV parameters showed borderline significant associations after adjustment for
391 birth weight, BMI, activity level, and n-3 and n-6 PUFA levels. These associations were all of small
392 magnitude, inconsistent across parameters, and there was no indication of a dose response
393 curve. No associations were significant after Bonferroni adjustment. Additionally, it seems most
394 likely that if recent exposure to low levels of MeHg does impact the nervous system, it would be
395 associated with prolonged cumulative dosage rather than the one month value that our measure
396 determined. Considering all of these factors, we do not believe these data provide support for
397 the hypothesis that HRV parameters are associated with recent MeHg exposure.

398 In analyses adjusted for PUFA and activity level, heart rate assessed during supine,
399 standing, and math test periods increased with increasing MeHg levels with borderline
400 significance. A 3-milisecond decrease in NN interval translates to about 0.2- 0.3 beats per minute
401 increase in heart rate, and has no clinical relevance. Similarly rMSSD and HF decreased with
402 increasing recent MeHg during the math test and LF/HF with standing. These changes were very
403 small and not significant clinically or statistically after Bonferroni correction. In response to a math
404 test, there was a 0.010 ms decrease in rMSSD per 1 ppm increase in MeHg, which despite
405 borderline p=0.006 (after Bonferroni correction) is of no clinical or physiologic meaning.

406 Roman and colleagues reviewed the literature on associations of prenatal and recent
407 MeHg with cardiovascular outcomes (Roman et al. 2011). They noted that evidence of impact at
408 dose levels of interest was lacking and that the "…specific measures of HRV in each study vary."

However, based on the consistency of the epidemiological literature, they classified the evidence supporting the association of HRV with MeHg exposure as “strong” compared to other cardiovascular measures. This reasoning is tenuous when one considers the known bias against publishing negative studies. The European Food Safety Autority ((CONTAM) 2012) reviewed reports examining MeHg and HRV in 2012 with subsequent update in 2018 and the panel concluded that, although some studies suggest an autonomic effect of MeHg, the results were inconsistent among studies and the implication for health were unclear.

Recently, Japanese researchers (Karita et al. 2018) reviewed 13 studies examining the association between MeHg and HRV parameters and 8 of them showed the significant association with MeHg and 5 failed to demonstrate any significant association. Earlier studies that reported an association between prenatal MeHg exposure and HRV parameters varied from the current study in a number of ways. They were conducted in a variety of populations and measured HRV variables using different methods and recording times. Also, some populations studied had significant exposures to other toxicants that might also influence HRV.

Discussing studies supporting the MeHg and HRV associations, the Faroe Islands investigators reported a decrease in the coefficient of variation of RR (a HRV parameter similar to SDNN) and in LF and HF levels as prenatal MeHg increased (Sorensen et al. 1999; Grandjean et al. 2004). Their studies differed in that Hg exposure was primarily from whale consumption and were thus co-exposed to polychlorinated biphenyls. In addition, the ECG recordings were short. The largest cohort studied to date evaluated 1,589 Koreans living near an industrial complex. Participants ranged in age from 5 to 83 years with 389 below age 20 years, had mean hair Hg levels that were low (0.83 ug/gm), and had 5-minute ECG recordings obtained in sitting position, which affect results (Lim, Chung, and Paek 2010). When categorized by decades of life, the 104 subjects below age 10 years had an 8.4% decrease in the HF parameter with each 1 ppm

433 increase in hair mercury concentration (95% confidence interval: 2.2-15.1). In a study of French
434 Polynesians aged 12–17 years (Valera et al. 2011), significant differences were observed in
435 LF/HF ratio, LF, and HF between the second (7.9–10.0 g/L) and third (11.0–26.0 g/L) tertiles of
436 blood mercury concentration. That study reported no associations between HRV parameters and
437 MeHg exposures in the 180 adults studied. In the only intervention study (Yaginuma-Sakurai et
438 al. 2010), a significant difference in LF was observed between the experimental group (mean
439 mercury levels of 8.76 μg/g in hair and 26.9 μg/L in blood) and control group, but one cannot
440 exclude the effect of concomitant changes in levels of n-3 PUFA influenced these results.

441 Discussing studies with no clear evidence of the MeHg-HRV association, analysis of
442 Faroese whaling men showed that blood mercury level was associated with increased coefficient
443 of variation of RR intervals, and coefficient of variation of LF, but latent mercury level, estimated
444 from mercury levels in blood, toe nail, and hair was not significantly associated with any HRV
445 parameter (Choi et al. 2009). In Inuit adults (Valera, Dewailly, and Poirier 2008), blood mercury
446 was significantly correlated with coefficient of variation of RR interval and LF in univariate
447 analyses, but these associations were not significant after adjusting for confounders. In fish
448 consumers from Long Island (Miller et al. 2018), HRV parameter in a multiple regression analysis
449 were not significant after adjustment for serum docosahexaenoic acid and eicosapentaenoic acid
450 levels. In the study of 203 children aged 9-11 years (Gump et al. 2017), LF, HF, and LF/HF ratio
451 at rest and during stress were not significantly associated with blood mercury levels.

452 As summarized above the effect of MeHg on HRV parameters does not seem consistent
453 and remains uncertain. Some studies indicate changes in the sympathetic (LF) component, some
454 in parasympathetic (HF, rMSSD), and some in both. Recording time and conditions varied among
455 the studies as did MeHg assessments. Competing environmental pollutions such as persistent
456 organic pollutants from consuming ocean mammals (Sorensen et al. 1999; Valera et al. 2011),

457 and underlying comorbidities such as in the Korean study (Lim, Chung, and Paek 2010), might
458 also have influenced the findings. If there is a causal association of prenatal or recent MeHg
459 exposure with changes in the autonomic nervous system, one would expect consistency in the
460 directionality of the associations for both classical time-domain and frequency-domain HRV
461 parameters. No study has demonstrated this. Our study with a large cohort and a wide range of
462 prenatal and recent MeHg exposures does not confirm the earlier reports.

463 We want to stress than none of the studies published to date in the literature on MeHg
464 association with HRV parameters has used 24-hour recording, the most standard approach to
465 evaluate HRV in cardiovascular literature. The strengths of this study consist of having accurate
466 prenatal and recent MeHg exposure measures at 19 years of age, a large cohort whose MeHg
467 exposure is significantly higher than other reported cohorts, a comprehensive battery of HRV
468 parameters completed under controlled conditions over a prolonged time period, blinding of
469 clinicians to exposure, and *a priori* specified analysis plans. Additionally, the analyses identified
470 known associations of HRV parameters indicating that if MeHg did influence autonomic control of
471 HRV the study could have detected it. As with all epidemiological studies, there are also
472 limitations. Complete data were not available on all subjects, conditions during the overnight
473 recordings were not monitored, and we were not able to complete long recordings on all
474 participants. Furthermore, our measure of recent exposure was limited to the month prior to
475 evaluation.

476 In conclusion, in a large cohort of 19 year old participants with MeHg exposures
477 significantly above those in previous reports, we found variations in HRV parameters related to
478 sex that have been previously reported, but found no association of them with prenatal MeHg
479 exposure. We found some trends toward associations of recent (at the age of 19 years) MeHg
480 exposure with heart rate and HRV parameters when subjects were challenged with math test that

481 could suggest that concurrent MeHg exposure might influence autonomic response, but the
482 magnitude of these findings is so miniscule that it could not be considered meaningful from a
483 clinical standpoint. In addition, we are cautious with this interpretation because of the multiple
484 comparisons and small magnitude of the associations. We do not believe that these data support
485 the hypothesis that either prenatal or recent MeHg exposure from consuming naturally
486 contaminated ocean fish adversely influences heart rate variability in young adults.

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646

Table 1: Characteristics of 19-year old subjects with comparison of males and females.

	All		Females		Males		P value
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	
BMI (kg/m^2)	507	22.25 ± 4.83	270	22.93 ± 5.60	237	21.47 ± 3.6	0.06
Child's Birth Weight (Kg)	514	3.18 ± 0.5	273	3.12 ± 0.48	241	3.25 ± 0.51	**0.01**
Prenatal MeHg (ppm)	514	6.92 ± 4.54	273	7.06 ± 4.68	241	6.77 ± 4.37	0.65
Recent MeHg (ppm)	451	10.21 ± 5.79	253	8.67 ± 4.76	198	12.16 ± 6.37	**<0.01**
n-3 LCPUFA (mg/ml)	482	0.05 ± 0.02	256	0.05 ± 0.02	226	0.04 ± 0.02	0.48
n-6 LCPUFA (mg/ml)	482	0.15 ± 0.04	256	0.15 ± 0.04	226	0.15 ± 0.05	0.85
Activity Level (total MET):		**N (%)**		**N (%)**		**N (%)**	
Sedentary (< 600)		171 (33.6%)		137 (50.6%)		34 (14.3%)	**<0.01**
Moderate (600 - < 3,000)		139 (27.3%)		87 (32.1%)		52 (21.9%)	**0.01**
High (3,000+)		199 (39.1%)		47 (17.3%)		152 (63.9%)	**<0.01**

Activity level: categorized from participants reports of their weekly walking, working, and recreational activities, in minutes estimated time per week (MET).
P values are from the nonparametric Wilcoxon rank-sum test
n-3 = sum of DHA, EPA, and ALA. n-6 = sum of AA and LA

Table 2. Average heart rate variability measurements by categories of prenatal and recent (at 19 years of age) MeHg (ppm) exposure^

Outcome	Prenatal MeHg				Recent MeHg			
	0-<5 ppm (n=211) Mean±SD	5-<10 ppm (n=184) Mean±SD	10+ ppm (n=119) Mean±SD	P value	0-<5 ppm (n=63) mean±SD	5-<10 ppm (n=201) mean±SD	10+ ppm (n=187) mean±SD	P value*
NN (ms)								
Long-term Recording	794±84	795±95	825±98	0.19	768±75	806±90	806±102	0.12
Baseline Supine	928±167	915±162	922±154	0.76	890±156	907±160	936±168	0.08
Response to Standing Δ	157±99	149±94	160±92	0.43	152±99	141±93	168±96	**0.02**
Response to Math Test Δ	65±72	67±80	81±87	0.28	78±84	59±71	80±87	**0.04**
Response to Sleep Δ	-101±153	-110±146	-140±114	0.44	-112±130	-109±150	-118±142	0.98
SDNN (ms)								
Long-term Recording	181±40	181±421	182±39	0.97	173±44	180±41	184±40	0.31
Baseline Supine	83±39	80±37	82±36	0.74	83±39	78±34	86±41	0.39
Response to Standing Δ	5±33	8±32	3±27	0.73	12±32	3±27	8±37	0.36
Response to Math Test Δ	9±24	8±27	6±28	0.74	10±28	5±23	11±28	0.20
Response to Sleep Δ	-11±40	-17±43	-17±38	0.64	-4±34	-12±38	-18±46	0.29
rMSSD (ms)								
Long-term Recording	66±28	63±33	64±24	0.52	64±25	63±25	69±35	0.88
Baseline Supine	77±42	73±44	74±42	0.46	76±44	71±41	79±46	0.37
Response to Standing Δ	31±32	31±35	31±32	0.70	35±38	27±29	35±35	0.13
Response to Math Test Δ	11±27	12±30	11±34	0.49	14±35	8±24	15±36	0.25
Response to Sleep Δ	-10±36	-11±40	-18±32	0.60	-8±36	-15±36	-9±41	0.44
LF								
Baseline Supine	39±16	41±17	40±16	0.72	35±15	42±17	39±15	0.03
Response to Standing Δ	-15±25	-15±23	-13±26	0.72	-17±24	-12±25	-17±24	0.23
Response to Math Test Δ	-7±19	-4±22	-6±20	0.51	-8±19	-3±22	-8±18	0.23
Response to Sleep Δ	3±22	8±21	8±22	0.33	0±18	10±22	3±23	**0.03**
HF								
Baseline Supine	50±19	49±20	40±16	0.72	55±18	47±19	51±19	**0.01**
Response to Standing Δ	29±21	26±21	-13±26	0.72	30±24	23±21	30±20	**0.01**
Response to Math Test Δ	7±19	7±22	-6±20	0.51	10±20	4±20	10±20	0.10
Response to Sleep Δ	-4±26	-3±27	8±22	0.33	0±27	-10±27	1±24	**0.02**

^P = value is from 2-df nonparametric Kruskal Wallis ANOVA test

Δ = Difference between baseline values and values obtained at standing, math test, or sleeping, respectively

* Please note that significant differences were observed when comparing middle MeHg dose group with lower and higher dose groups without dose-dependent effect.

Table 3. Prenatal MeHg (ppm) exposure association with heart rate variability measurements, adjusting for sex, birth weight and BMI

Outcome	N	P value for sex interaction	β	P value	95% CI
NN (ms)					
Long-term Recording	202	0.0230	1.883	0.1160	(-0.4710, 4.2360)
female			-0.1729	0.9073	(-3.0974, 2.7516)
male			**5.494**	**0.0058**	**(1.6105, 9.3775)**
Baseline Supine	507	0.3050	-1.303	0.3470	(-4.0210, 1.4150)
Response to Standing Δ	497	0.2717	-0.185	0.8420	(-2.0120, 1.6410)
Response to Math Test Δ	442	0.5480	1.534	0.0620	(-0.0760, 3.1440)
Response to Sleep Δ	202	0.0120	-2.915	0.2000	(-7.3900, 1.5600)
female			1.3949	0.6203	(-4.1492, 6.9390)
male			**-10.4873**	**0.0055**	**(-17.8493, -3.1253)**
SDNN (ms)					
Long-term Recording +	200	0.1820	-0.001	0.7160	(-0.0080, 0.0050)
Baseline Supine +	507	0.4077	-0.004	0.4120	(-0.0120, 0.0050)
Response to Standing *	497	0.8190	-0.001	0.7080	(-0.0090, 0.0060)
Response to Math Test *	442	0.9039	0.000	0.9360	(-0.0070, 0.0060)
Response to Sleep *	202	0.7592	-0.003	0.7310	(-0.0180, 0.0130)
rMSSD (ms)					
Long-term Recording +	202	0.5522	-0.006	0.3470	(-0.0180, 0.0060)
Baseline Supine +	499	0.2371	-0.008	0.1730	(-0.0190, 0.0030)
Response to Standing *	490	0.3928	0.002	0.6740	(-0.0070, 0.0110)
Response to Math Test *	434	0.3294	0.003	0.3760	(-0.0040, 0.0110)
Response to Sleep *	199	0.6891	-0.001	0.9150	(-0.0170, 0.0150)
LF					
Baseline Supine	505	0.9939	0.07	0.6610	(-0.2440, 0.3850)
Response to Standing Δ	496	0.3730	0.037	0.8800	(-0.4390, 0.5120)
Response to Math Test Δ	441	0.7007	-0.05	0.8130	(-0.4620, 0.3630)
Response to Sleep Δ	201	0.6549	0.246	0.4730	(-0.4280, 0.9200)
HF					
Baseline Supine	505	0.4400	-0.064	0.7310	(-0.4300, 0.3020)
Response to Standing Δ	496	0.3806	-0.12	0.5650	(-0.5310, 0.2910)
Response to Math Test Δ	441	0.2875	0.131	0.5230	(-0.2700, 0.5320)
Response to Sleep Δ	201	0.1498	-0.107	0.8010	(-0.9380, 0.7250)

+ = Model uses the natural logarithm of the outcome, and slopes are multiplicative effects
Δ = Difference between baseline values and values obtained at standing, math test, or sleeping respectively
* = Difference in natural logarithms of baseline values and values obtained at standing, math test, or sleeping, respectively
If Male*MeHg interaction is NOT significant (p > .05), mercury coefficient reported for entire sample
If Male*MeHg interaction IS significant (p <= .05), mercury coefficient reported by sex

Table 4. Recent MeHg (ppm) exposure and n-3 and n-6 PUFA status association with heart rate variability measurements, adjusting for birth weight, BMI, and activity level

Outcome	N	P value for sex interaction	β	P value	95% CI
NN (ms)					
Long-term Recording	167	0.3404	**-2.755**	**0.0270**	**(-5.191, -0.319)**
Baseline Supine	418	0.0717	**-3.048**	**0.0160**	**(-5.525, -0.571)**
Response to Standing Δ	411	***0.0185***	0.167	0.8420	(-1.485, 1.82)
female			**2.617**	**0.0499**	**(0.0011, 5.2323)**
male			-1.426	0.1847	(-3.5366, 0.6838)
Response to Math Test Δ	365	0.1291	0.578	0.4420	(-0.9, 2.056)
Response to Sleep Δ	167	0.4733	-0.095	0.9680	(-4.77, 4.581)
SDNN (ms)					
Long-term Recording +	165	0.4254	-0.006	0.0950	(-0.012, 0.001)
Baseline Supine +	418	0.6280	-0.005	0.1870	(-0.013, 0.003)
Response to Standing Δ *	411	0.5215	0	0.9400	(-0.007, 0.007)
Response to Math Test Δ *	365	0.7105	0.004	0.1480	(-0.002, 0.01)
Response to Sleep Δ *	167	0.4633	-0.010	0.2050	(-0.026, 0.006)
rMSSD (ms)					
Long-term Recording +	167	0.1793	-0.010	0.1300	(-0.022, 0.003)
Baseline Supine +	411	0.5038	-0.008	0.1280	(-0.018, 0.002)
Response to Standing Δ *	405	0.1855	0.004	0.3360	(-0.004, 0.012)
Response to Math Test Δ *	358	0.4383	**0.010**	**0.0060**	**(0.003, 0.016)**
Response to Sleep Δ *	165	0.4679	-0.007	0.4200	(-0.024, 0.01)
LF					
Baseline Supine	416	0.3200	-0.128	0.3860	(-0.417, 0.161)
Response to Standing Δ	410	0.2926	-0.361	0.1060	(-0.798, 0.077)
Response to Math Test Δ	364	***0.0425***	-0.245	0.1910	(-0.612, 0.122)
female			0.209	0.4720	(-0.3615, 0.7791)
male			**-0.563**	**0.0210**	**(-1.0401, -0.0855)**
Response to Sleep Δ	166	0.2318	-0.688	0.0500	(-1.377, 0.001)
HF					
Baseline Supine	416	0.6151	0.226	0.1890	(-0.112, 0.564)
Response to Standing Δ	410	0.8996	**0.490**	**0.0110**	**(0.114, 0.866)**
Response to Math Test Δ	364	0.7939	**0.544**	**0.0030**	**(0.188, 0.901)**
Response to Sleep Δ	166	0.8533	0.814	0.0550	(-0.018, 1.646)

+ = Model uses the natural logarithm of the outcome and slopes assume multiplicative effects
Δ = Difference between baseline values and values obtained at standing, math test, or sleeping respectively
* = Difference in natural logarithms of baseline values and values obtained at standing, math test, or sleeping respectively
If Male*MeHg interaction is not significant (p > .05), mercury coefficient reported for entire sample
If Male*MeHg interaction is significant (p <= .05), the mercury coefficient is reported by sex

Supplementary Tables

For the manuscript entitled: "Prenatal and Recent Methylmercury Exposure and Heart Rate Variability in Young Adults: the Seychelles Child Development Study" by Zareba et al.

Table S1: Heart rate variability parameters (n=23) from Holter recordings comparing males and females.

	All		Female		Male		P value
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	
NN (ms)							
Long-term Recording	203	802 ± 92	117	757 ± 68	86	863 ± 85	**<0.0001**
Baseline Supine	514	922 ± 162	273	847 ± 122	241	1007 ±161	**<0.0001**
Response to Standing Δ	504	155 ± 95	267	133 ± 85	237	179 ± 101	**<0.0001**
Response to Math Test Δ	448	69 ± 79	240	69 ± 79	208	70 ± 80	0.9731
Response to Sleep Δ	203	-113 ± 142	117	-102 ± 114	86	-128 ± 173	0.0414
SDNN (ms)							
Long-term Recording	201	181 ± 40	117	166 ± 35	84	203 ± 36	**<0.0001**
Baseline Supine	514	82 ± 38	273	74 ± 37	241	90 ± 36	**<0.0001**
Response to Standing Δ	504	6 ± 32	267	8 ± 31	237	3 ± 33	0.0951
Response to Math Test Δ	448	8 ± 26	240	9 ± 24	208	6 ± 28	0.2266
Response to Sleep Δ	203	-14 ± 40	117	-11 ± 32	86	-19 ± 50	0.3927
rMSSD (ms)							
Long-term Recording	203	65 ± 28	117	59 ± 25	86	72 ± 30	**0.0002**
Baseline Supine	506	75 ± 43	267	67 ± 42	239	83 ± 42	**<0.0001**
Response to Standing Δ	497	31± 33	262	30 ± 33	235	33 ± 33	0.0217
Response to Math Test Δ	440	11 ± 30	234	14 ± 28	206	8 ± 32	0.0149
Response to Sleep Δ	200	-12 ± 36	116	-13 ± 34	84	-11 ± 40	0.9733
LF							
Baseline Supine	512	40 ± 16	272	38 ± 16	240	42 ± 16	0.0119
Response to Standing Δ	503	-15 ± 24	266	-13 ± 25	237	-17 ± 24	0.1223
Response to Math Test Δ	447	-6 ± 20	239	-5 ± 21	208	-6 ± 20	0.8824
Response to Sleep Δ	202	6 ± 22	117	3 ± 22	85	10 ± 21	0.0131
HF							
Baseline Supine	512	49 ± 19	272	51 ± 20	240	48 ± 18	0.0774
Response to Standing Δ	503	27 ± 21	266	29 ± 22	237	25 ± 20	0.0415
Response to Math Test Δ	447	7 ± 20	239	8 ± 21	208	6 ± 19	0.0911
Response to Sleep Δ	202	-4.7 ± 27	117	-3 ± 28	85	-7 ± 25	0.2488

Δ = Difference between baseline values and values obtained at standing, math test, or sleeping, respectively.
P values are from the nonparametric Wilcoxon rank-sum test

Table S2. Time-domain HRV parameters from long-term ECG recordings according to recent MeHg level categories at 19 years of age in males and females separately.

Recent MeHg Levels:	**0-5 ppm**	**5-10 ppm**	**10+ppm**	**P value**
Males	**N=14**	**N=77**	**n=109**	
NN	899 ± 147	866 ± 121	871 ± 122	0.66
SDNN	187 ± 50	181 ± 49	183 ± 45	0.88
rMSSD	84 ± 37	77 ± 29	74 ± 37	0.31
pNN50	16.9 ± 6.7	13.8 ± 6.9	13.8 ± 6.1	0.24
SDANN	148 ± 43	153 ± 54	155 ± 48	0.90
SDNNIX	108 ± 36	93 ± 26	95 ± 27	0.19
Females	**n=49**	**n=127**	**n=79**	
NN	752 ± 91	759 ± 82	758 ± 82	0.87
SDNN	150 ± 44	144 ± 45	143 ± 41	0.71
rMSSD	60 ± 27	57 ± 29	64 ± 38	0.32
pNN50	11.2 ± 6.2	11.0 ± 6.7	11.2 ± 6.9	0.96
SDANN	126 ± 46	119 ± 42	116 ± 40	0.45
SDNNIX	75 ± 22	74 ± 23	77 ± 27	0.65

The same findings of no significant difference among three groups were observed for all other tested HRV parameters (data not shown - could be provided if needed).

Table S3. Recent MeHg (ppm) exposure association with heart rate variability measurements after adjusting for sex, birth weight and BMI (Primary Models)

Outcome	N	P value for sex interaction	β	P value	95% CI
NN (ms)					
Long-term Recording	174	0.3674	-1.821	0.1110	(-4.0650, 0.4230)
Baseline Supine	446	0.0975	-1.448	0.2370	(-3.8540, 0.9570)
Response to Standing Δ	438	0.0149	0.645	0.4250	(-0.9420, 2.2330)
AC.RR:female			**2.9957**	**0.0172**	**(0.5341, 5.4573)**
AC.RR:male			-0.9796	0.3479	(-3.0282, 1.069)
Response to Math Test Δ	391	0.0547	0.646	0.3720	(-0.7760, 2.0680)
Response to Sleep Δ	174	0.4293	0.512	0.8120	(-3.7330, 4.7570)
SDNN (ms)					
Long-term Recording +	172	0.2179	-0.005	0.1280	(-0.0110, 0.0010)
Baseline Supine +	446	0.5166	-0.002	0.6000	(-0.0100, 0.0060)
Response to Standing *	438	0.4297	0.001	0.8650	(-0.0060, 0.0070)
Response to Math Test *	391	0.8776	0.005	0.1150	(-0.0010, 0.0100)
Response to Sleep *	174	0.7759	-0.002	0.8050	(-0.0170, 0.0130)
rMSSD (ms)					
Long-term Recording +	174	0.1906	-0.008	0.1970	(-0.0190, 0.0040)
Baseline Supine +	438	0.4000	-0.003	0.4890	(-0.0130, 0.0060)
Response to Standing *	431	0.0783	0.005	0.2250	(-0.0030, 0.0130)
Response to Math Test *	383	0.1257	**0.009**	**0.0070**	**(0.0020, 0.0160)**
Response to Sleep *	171	0.9234	0.003	0.7520	(-0.0130, 0.0180)
LF					
Baseline Supine	444	0.4601	-0.065	0.6390	(-0.3400, 0.2090)
Response to Standing Δ	437	0.5816	-0.323	0.1260	(-0.7360, 0.0910)
Response to Math Test Δ	390	0.0940	-0.162	0.3660	(-0.5130, 0.1900)
Response to Sleep Δ	173	0.0929	-0.58	0.0730	(-1.2140, 0.0540)
HF					
Baseline Supine	444	0.7396	0.188	0.2510	(-0.1340, 0.5100)
Response to Standing Δ	437	0.9000	**0.403**	**0.0290**	**(0.0420, 0.7650)**
Response to Math Test Δ	390	0.9269	**0.375**	**0.0320**	**(0.0330, 0.7160)**
Response to Sleep Δ	173	0.5599	0.597	0.1270	(-0.1710, 1.3650)

+ = Model uses the natural logarithm of the outcome and slopes are multiplicative effects
Δ = Difference between baseline values and values obtained at standing, math test, or sleeping, respectively
* = Difference in natural logarithms of baseline values and values obtained at standing, math test, or sleeping, respectively. If Male*MeHg interaction is NOT significant (p > .05), mercury coefficient reported for entire sample
If Male*MeHg interaction IS significant (p <= .05), mercury coefficient reported by sex