# Associations of blood mercury and fatty acid concentrations with blood mitochondrial DNA copy number in the Seychelles Child Development Nutrition Study

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## Abstract

**Background:** Fish containsmethylmercury (MeHg) which can cause oxidative stress and neurodevelopmental toxicity at sufficiently high doses. Fish also contains polyunsaturated fatty acids (PUFA) which have both antioxidant (n-3) and oxidant (n-6) properties. Mitochondrial DNA (mtDNA) is sensitive to oxidative stress but has not been previously studied in relation to MeHg exposure or PUFA status.

**Objective:** To investigate the associations between MeHg exposure and PUFA status during pregnancy with relative mitochondrial DNA copy number (RmtDNAcn) in mothers and their newborns.

**Methods:** In total, 1488 mother-child pairs from the Seychelles Child Development Study Nutrition Cohort 2 were included in this study. Total Hg was measured in maternal blood collected at 28 weeks’ gestation, maternal hair at delivery, and in fetal cord blood. PUFA (n-3 and n-6) were measured only in maternal blood. RmtDNAcn was measured by qPCR in both maternal and cord blood.

**Results:** Increasing maternal blood Hg (β=0.001, 95%CI: 0.000, 0.002) and n-3 PUFA concentrations (β=0.183, 95%CI: 0.048, 0.317) were associated with higher maternal RmtDNAcn. Increasing maternal n-6 PUFA (β=-0.103, 95%CI: -0.145, -0.062) and n-6/n-3 ratio (β=-0.011, 95%CI: -0.017, -0.004) were associated with lower maternal RmtDNAcn. Increasing fetal cord blood Hg was associated with lower fetal RmtDNAcn (β=-0.002, 95%CI: -0.004, -0.000). Neither maternal blood Hg nor PUFA status was associated with fetal RmtDNAcn.

**Conclusions:** Our findings suggest that MeHg and PUFA may influence mitochondrial homeostasis although the magnitude of these associations are small. Future studies should confirm the findings and explore the underlying mechanisms.

***Key words*:** mitochondrial DNA, methylmercury, mercury, polyunsaturated fatty acids

## Introduction

Methylmercury (MeHg) is present in all fish to a varying extent and is a well-known developmental neurotoxicant in sufficiently high doses (Hong et al. 2012). However, the associations between low to moderate levels of MeHg exposure from consuming fish with naturally-occurring MeHg levels and neurodevelopmental outcomes have been contradictory in different epidemiological studies (Barbone et al. 2018; Daniels et al. 2004; Davidson et al. 1998; Grandjean et al. 1997; Llop et al. 2012; Strain et al. 2015b; Vejrup et al. 2016). A better understanding of the mechanisms behind MeHg toxicity, as well as the identification of factors that modify the potential toxicity are necessary for accurate risk assessment of MeHg exposure.

MeHg is a mitochondrial toxicant that can bind to proteins, and in experimental animals results in a loss of mitochondrial membrane potential (Zalups 2000). MeHg can also induce Ca2+ uptake and generation of reactive oxygen species (ROS) (Fonnum and Lock 2004; Jia et al. 2015), which indirectly impair mitochondrial DNA (mtDNA). Mitochondria have multiple copies of small circular DNA (mtDNA), whose copy number (cn) is regulated through the processes of biogenesis, i.e. production of new mitochondria, and mitophagy (elimination of damaged mitochondria) to appropriately satisfy the metabolic and energy demands of tissues and cells (Byun and Baccarelli 2014; Carelli et al. 2015). Since the main function of mitochondria is to provide energy to cells, disruption of mitochondria may affect a variety of organs, particularly those with the highest energetic demands, such as muscle, heart, liver, and brain (Magner et al. 2015). Thus, we hypothesized that mitochondrial function may be involved in the mechanisms of MeHg neurotoxicity.

mtDNA is sensitive to ROS insults due to the absence of protecting histones and DNA repair mechanisms, and resulting DNA alterations can affect mitochondrial biogenesis (Lee and Wei 2005). The content of mtDNA per cell is mainly measured in copy number and it has been suggested as a surrogate marker of mitochondrial biogenesis (Cho et al. 2007; Malik and Czajka 2013). MtDNA damage has been linked to environmental exposures (Roubicek and Souza-Pinto 2017), including metals (Zhang et al. 2011). To date, there are no studies in human populations assessing mtDNAcn in relation to MeHg exposure and toxicity. An *in vitro* study of human neural progenitor cell cultures showed increased mtDNAcn when the cells where exposed to a low dose of MeHg (Wang et al. 2016). An *in vivo* study of bats exposed to MeHg showed reduced mtDNAcn (Karouna-Renier et al. 2014). The reason for these contrasting findings is not known.

Fish is rich in polyunsaturated fatty acids (PUFA), particularly the n-3 PUFA (Gribble et al. 2016). N-3 PUFA can act as antioxidants and may, therefore, counteract toxicity associated with MeHg-induced oxidative stress (Strain et al. 2015b). In contrast, the n-6 PUFA, mainly found in vegetable oil but also present in fish, have pro-oxidative properties (Russo 2009). Studies addressing the influence of PUFA status on mtDNA in humans to date have been limited to cell culture studies. These have reported that n-3 PUFA can increase mtDNAcn in a dose and time dependent manner in human skeletal muscle cells (Vaughan et al. 2012), as well as in mouse muscle myoblasts (C2C12 cells) (Lee et al. 2016). This suggests a direct role for n-3 in mtDNA replication. In contrast, n-6 PUFA may have the opposite effect. Experimental evidence in male wistar rats showed increased oxidative damage to mtDNA following an n-6 PUFA rich diet (Ghosh et al. 2006).

To date, there have been no *in vivo* human studies of early-life exposure to MeHg and its effects on the mitochondrion. In this study, we used a large mother-child cohort from the Seychelles characterized by a fish-rich diet. We analyzed associations between maternal and fetal MeHg biomarkers, maternal n-3 and n-6 PUFA status, and mtDNAcn in maternal and fetal (cord) blood. We hypothesized that increasing exposure to MeHg and n-6 PUFA could impair mitochondrial function and biogenesis via increased pro-oxidative effects with reduced mtDNAcn as a consequence, whereas increasing exposure to n-3 PUFA would have a protective effect which may show as an increased mtDNAcn.

## Materials and methods

### 2.1 Study population

The Nutrition Cohort 2 (NC2) of the Seychelles Child Development Study (SCDS) includes mother-child pairs of mixed African, European and East Asian origin. The aim of the SCDS is to evaluate whether MeHg exposure from maternal fish consumption during pregnancy is associated with child developmental outcomes and if the relationship is influenced by nutrition and genetics. Between 2008 and 2011 a total of 1535 mothers were recruited at their first antenatal visit at eight health centers across Mahé, the main island of the Seychelles (Strain et al. 2015a). Inclusion criteria included being native Seychellois, being ≥16 y of age, having a singleton pregnancy, and having no obvious health concerns. Biological sampling included maternal blood samples collected at 28 weeks of gestation, fetal blood collected from cord at delivery and maternal hair collected at delivery. The following exclusions were made for this analysis: being a twin or lacking all of maternal hair, maternal blood, and cord blood for measurement of Hg and mtDNAcn, leaving 1488 eligible mother-child pairs. Maternal hair, maternal blood and cord blood were collected at different visits, leading to different numbers of responses for each measurement; there are 1447 maternal and 1055 cord blood mtDNAcn measurements (see counts in Table 1). The study was conducted according to guidelines laid down in the Declaration of Helsinki and all study procedures involving participants were reviewed and approved by the Seychelles Ethics Board, the Research Subjects Review Board at the University of Rochester, and the Regional Ethics Committee at Lund University, Sweden.

### 2.2 Measurements of biomarkers of methylmercury exposure

We measured total Hg (in maternal blood and in hair) as a proxy of MeHg exposure in the mother as well as in cord blood as a proxy of fetal exposure to MeHg, since more than 80% of total Hg in hair and blood is MeHg (Cernichiari et al. 1995a; National Research Council 2000). Maternal hair was collected at delivery to reflect exposure throughout pregnancy with an assumed growth rate of 1.1 cm/month. Total Hg content in the hair was measured as previously described (Cernichiari et al. 1995b) and reported in parts per million (ppm). Total Hg was measured in whole blood from mothers at 28 weeks’ gestation and in cord blood by atomic fluorescence spectrometry using a PSA Millennium Merlin System (PS Analytical, Kent, UK) with a limit of detection of approximately 0.01 ng/mL (McSorley et al. 2018).

### 2.3 Measurements of fatty acid

Maternal non-fasting blood samples (28 weeks’ gestation) were processed at the Public Health Laboratory of the Ministry of Health at Mahé, Seychelles and serum samples were shipped at −80 °C to the Ulster University for fatty acid measurements. Following total lipid extraction from serum, fatty acid methyl esters were prepared by boron trifluroide methanol according to an adaptation of the Folch method (Folch et al. 1957) and quantified by Gas Chromatography–Mass Spectrometry (Agilent 7890A-5975C, UK) as previously described (Strain et al. 2008).

### 2.4 Measurements of mitochondrial DNA copy number

DNA was isolated from maternal blood (28 weeks’ gestation) and cord blood samples by Qiagen DNA Blood Mini kit (Qiagen, Hilden, Germany) and diluted to 4 ng/μL. As previously described (Xu et al. 2017), mtDNAcn and single copy hemoglobin beta (*HBB*) gene were measured based on real-time quantitative polymerase chain reaction (PCR) and SYBR Green technology. In short, master mixes for mtDNA copy number and *HBB* were prepared with KAPA SYBR FAST qPCR Kit Master Mix (2X) ABI Prism (Kapa Biosystems, Woburn, MA, USA) and corresponding primers reported by Hou et al (Hou et al. 2010). A real-time PCR machine (7900HT, Applied Biosystems, Foster City, CA, USA) was used to perform PCR and each run included a standard curve, a control sample and a blank. For the standard curve, one reference DNA sample (from three persons) was diluted to 5 concentrations: 1, 2, 4, 8 and 16 ng/μL. The control sample was to monitor the variance between runs, and the coefficient of variation (CV) of the control sample was 5.3% based on 16 runs. All samples and standard curve points were run in triplicates. R2 for each standard curve was >0.99. The relative mtDNAcn (RmtDNAcn) was calculated by SDS 2.4.1 software (Life Technologies) and indicates average mtDNAcn in each cell.

### 2.5 Statistical analyses

Linear regression models were fit to investigate the pre-specified associations between RmtDNAcn and MeHg exposure, as well as between RmtDNAcn and maternal n-3 and n-6 PUFA status. The associations with PUFA were analyzed alone or together with MeHg, and all the association analyses were performed in mothers and newborns separately. In the maternal analyses, eight models examined associations between the maternal RmtDNAcn and concentrations. First, we evaluated the association of MeHg and PUFA status in separate models: 1) maternal hair Hg; 2) maternal blood Hg; 3) maternal blood n-3 and n-6 PUFA; 4) n-6/n-3 ratio. Subsequently, we ran models that included both MeHg and PUFA status: 5) hair Hg together with n-3 and n-6 PUFA; 6) hair Hg together with the n-6/n-3 ratio; 7) blood Hg together with n-3 and n-6 PUFA; and 8) blood Hg together with n-6/n-3 ratio. In the fetal analyses, 11 models were performed to analyze the associations with fetal RmtDNAcn. Among them, 8 models use the same predictors as in the maternal analyses as described above, and 3 additional models used the following predictors: 1) the concentration of cord blood Hg; 2) cord blood Hg together with maternal n-3 and n-6 PUFA status; and 3) cord blood Hg together with the maternal n-6/n-3 ratio. Covariates in the adjusted models were chosen *a priori* based on the literature and their known or suspected association with mtDNAcn (Brunstet al. 2017; Hosnijehet al. 2014; Kaamanet al. 2007; Liuet al. 2003). In the maternal analyses they included age at enrollment, BMI at 20 months (as a surrogate for pre-pregnancy BMI), and Hollingshead Socioeconomic Status (SES). In the fetal analyses they included maternal age at delivery, BMI at 20 months, and Hollingshead SES and child’s sex, birth weight and gestational age. Smoking and alcohol use during pregnancy were not included in the models due to their very low prevalence in our study population.

Model assumptions were checked through the distribution of the residuals and the residuals from each linear regression showed symmetric distributions. All the statistical analyses were performed using the software R (version 3.3.2; The R Foundation for Statistical Computing). A P-value of 0.05 (2-tailed) was chosen as the criterion for statistical significance in all analysis.

## Results

### 3.1 Characteristics of the mothers and newborns

Summary statistics for the biomarkers of MeHg exposure and PUFA status, as well as RmtDNAcn for pregnant women and their newborns are presented in Table 1. In this cohort of 1488 mother-child pairs, the mothers were on average 27.1 years old at childbirth. Their children were 51.7% male, had a mean gestational age of 38.9 weeks, and their mean weight was 3165 g at birth. Total Hg in cord blood (34.76 ng/mL) was 1.89 times higher than that in maternal blood (18.36 ng/mL). Fetal RmtDNAcn in cord blood (0.91 ng/mL) was 2.53 times higher than maternal blood RmtDNAcn (0.36 ng/mL).

Table 1. Summary statistics for relative mitochondrial DNA copy number (RmtDNAcn), MeHg biomarkers and PUFA status among pregnant women and their newborns (SD, standard deviation).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Counts** | **Mean** | **SD** | **Min** | **Median** | **Max** |
| Maternal RmtDNAcn | 1447 | 0.36 | 0.21 | 0.034 | 0.32 | 2.79 |
| Fetal RmtDNAcn | 1055 | 0.91 | 0.55 | 0.21 | 0.78 | 5.71 |
| Maternal hair Hg (ng/mL) | 1339 | 3.95 | 3.49 | 0.010 | 2.93 | 31.66 |
| Maternal blood Hg (ng/mL) | 1385 | 18.37 | 10.82 | 1.87 | 15.97 | 84.15 |
| Cord blood Hg (ng/mL) | 1036 | 34.79 | 20.89 | 1.91 | 30.25 | 181.27 |
| Maternal n-3 (mg/mL) | 1450 | 0.27 | 0.09 | 0.12 | 0.27 | 0.64 |
| Maternal n-6 (mg/mL) | 1450 | 1.10 | 0.29 | 0.43 | 1.09 | 2.71 |
| Maternal age (year) | 1482 | 27.12 | 6.30 | 16.27 | 26.15 | 46.76 |
| Maternal BMI at 20 month | 1364 | 26.95 | 6.57 | 14.67 | 26.15 | 49.65 |
| Hollingshead SES at 20 month | 1427 | 31.99 | 10.37 | 11 | 31.5 | 63 |
| Gestational age at birth (week) | 1474 | 38.94 | 1.68 | 28 | 39 | 41 |
| Weight at birth (kg) | 1482 | 3.17 | 0.51 | 1.10 | 3.19 | 5.20 |

Maternal blood Hg was significantly positively correlated with hair Hg and cord blood Hg (Table 2). N-3 PUFA was positively correlated with n-6 and weakly with Hg in maternal hair and blood; and Hg in cord blood. N-6 PUFA had a weak, negative correlation with cord blood Hg. There was no correlation between maternal and fetal RmtDNAcn. Maternal RmtDNAcn was positively correlated with maternal blood Hg and negatively with n-6 PUFA. Fetal RmtDNAcn was negatively correlated with cord blood Hg.

Table 2. Correlations between relative mitochondrial DNA copy number (RmtDNAcn), MeHg biomarkers, and n-3, n-6 PUFAs among pregnant women and their newborns. \* for p<0.05 and \*\* for p<0.01

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Maternal RmtDNAcn** | **Fetal RmtDNAcn** | **Maternal** **hair Hg** | **Maternal** **blood Hg** | **Cord** **blood Hg** | **Maternal n-3** | **Maternal n-6** |
| Maternal RmtDNAcn | 1.00 |  |  |  |  |  |  |
| Fetal RmtDNAcn | 0.049 | 1.00 |  |  |  |  |  |
| Maternal hair Hg | 0.021 | 0.0037 | 1.00 |  |  |  |  |
| Maternal blood Hg | 0.069\* | -0.045 | 0.43\*\* | 1.00 |  |  |  |
| Cord blood Hg | 0.073\* | -0.084\*\* | 0.36\*\* | 0.66\*\* | 1.00 |  |  |
| Maternal n-3 | 0.016 | -0.048 | 0.10\*\* | 0.13\*\* | 0.11\*\* | 1.00 |  |
| Maternal n-6 | -0.11\*\* | 0.015 | 0.031 | -0.016 | -0.063\* | 0.44\*\* | 1.00 |

### 3.2 RmtDNAcn and biomarkers of MeHg exposure and PUFA status

In mothers, RmtDNAcn in blood was positively associated with blood Hg. The effect estimate for hair Hg was similar to that of blood Hg, but the association was not significant and not changed by adjustment for n-3 and n-6 PUFA (Table 3). Moreover, a positive association was found for maternal RmtDNAcn with n-3, while negative associations were found with n-6 PUFA and the n-6/n-3 ratio. The associations between RmtDNAcn and Hg exposure or PUFA status were largely independent of each other. For example, adjustment for maternal hair or blood Hg caused little change in effect estimates and p-values for the associations between RmtDNAcn and PUFA status in the mothers (Table 3).

Table 3. Associations between maternal relative mitochondrial DNA copy number (RmtDNAcn) and MeHg biomarkers and PUFA status. Effect estimates from linear regression analyses are presented as β along with 95% confidence intervals (CI). Significant associations are marked in bold.

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **Unadjusted model** | **Adjusted model b** |
| **Model no. a** | **Predictors** | **Counts c** | **β** | **95% CI** | **Counts** | **β** | **95% CI** |
| 1) | Maternal hair Hg | 1308 | 0.001 | (-0.002, 0.005) | 1096 | 0.002 | (-0.001, 0.006) |
| 2) | Maternal blood Hg | 1377 | 0.001 | **(0.000, 0.002)** | 1136 | 0.001 | **(0.000, 0.002)** |
| 3) | Maternal n-3 | 1432 | 0.183 | **(0.048, 0.317)** | 1177 | 0.20 | **(0.049, 0.354)** |
| Maternal n-6 |  | -0.103 | **(-0.145, -0.062)** |  | -0.104 | **(-0.150, -0.058)** |
| 4) | Maternal n-6/n-3  | 1432 | -0.011 | **(-0.017, -0.004)** | 1177 | -0.012 | **(-0.020, -0.005)** |
| 5) | Maternal hair Hg | 1296 | 0.001 | (-0.002, 0.005) | 1085 | 0.002 | (-0.002, 0.006) |
| Maternal n-3 |  | 0.14 | (-0.004, 0.284) |  | 0.14 | (-0.020, 0.298) |
| Maternal n-6 |  | -0.095 | **(-0.139, -0.051)** |  | -0.094 | **(-0.142, -0.046)** |
| 6) | Maternal hair Hg | 1296 | 0.001 | (-0.002, 0.004) | 1085 | 0.002 | (-0.002, 0.006) |
| Maternal n-6/n-3  |  | -0.008 | **(-0.015, -0.001)** |  | -0.009 | **(-0.017, -0.001)** |
| 7) | Maternal blood Hg | 1366 | 0.001 | **(0.000, 0.002)** | 1127 | 0.001 | (-0.000, 0.002) |
| Maternal n-3 |  | 0.18 | **(0.035, 0.315)** |  | 0.198 | **(0.040, 0.355)** |
| Maternal n-6 |  | -0.101 | **(-0.144, -0.058)** |  | -0.103 | **(-0.150, -0.056)** |
| 8) | Maternal blood Hg | 1366 | 0.001 | **(0.000, 0.002)** | 1127 | 0.001 | (-0.000, 0.002) |
| Maternal n-6/n-3  |  | -0.011 | **(-0.017, -0.004)** |  | -0.012 | **(-0.020, -0.005)** |

1. Model 1): Maternal RmtDNAcn ~ Maternal hair Hg

Model 2): Maternal RmtDNAcn ~ Maternal 28 week blood Hg

Model 3): Maternal RmtDNAcn ~ Maternal 28 week blood n-3 + Maternal 28 week blood n-6

Model 4): Maternal RmtDNAcn ~ Maternal 28 week blood n-6/n-3 ratio

Model 5): Maternal RmtDNAcn ~ Maternal hair Hg + Maternal blood n-3 + Maternal blood n-6

Model 6): Maternal RmtDNAcn ~ Maternal hair Hg + Maternal blood n-6/n-3 ratio

Model 7): Maternal RmtDNAcn ~ Maternal blood Hg + Maternal blood n-3 + Maternal blood n-6

Model 8): Maternal RmtDNAcn ~ Maternal blood Hg + Maternal blood n-6/n-3 ratio

1. Adjusted for maternal age at enrollment, BMI at 20 months (as a surrogate for pre-pregnancy BMI and Hollingshead Socioeconomic Status (SES)
2. Counts of records in each regression models were different due to missing data on the measurements of total Hg, n-3 and n-6 PUFA and availability of the information related to potential confounders.

In the newborns, RmtDNAcn in cord blood was weakly negatively associated with cord blood Hg, even after including maternal PUFA and further adjustments. However, no associations were observed with maternal Hg concentrations (hair or blood) or PUFA status (Table 4).

Table 4. Associations between fetal (cord blood) relative mitochondrial DNA copy number (RmtDNAcn) and MeHg biomarkers and PUFA status. Effect estimates from linear regression analyses are presented as β along with 95% confidence intervals (CI). Significant associations are marked in bold.

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **Unadjusted model** | **Adjusted model b** |
| **Model no. a** | **Predictors** | **Counts c** | **β** | **95% CI** | **Counts** | **β** | **95% CI** |
| 1 | Cord blood Hg | 956 | -0.002 | **(-0.004, -0.001)** | 889 | -0.002 | **(-0.004, -0.001)** |
| 2 | Maternal hair Hg | 961 | 0.001 | (-0.009, 0.010) | 902 | -0.002 | (-0.012, 0.009) |
| 3 | Maternal blood Hg | 974 | -0.002 | (-0.006, 0.001) | 907 | -0.003 | (-0.006, 0.000) |
| 4 | Maternal n-3 | 1025 | -0.41 | (-0.827, 0.008) | 951 | -0.37 | (-0.802, 0.067) |
| Maternal n-6 |  | 0.084 | (-0.046, 0.214) |  | 0.063 | (-0.071, 0.196) |
| 5 | Maternal n-6/n-3  | 1025 | 0.016 | (-0.005, 0.036) | 951 | 0.011 | (-0.010, 0.033) |
| 6 | Cord blood Hg | 930 | -0.002 | **(-0.004, -0.000)** | 864 | -0.002 | **(-0.004, -0.001)** |
| Maternal n-3 |  | -0.43 | (-0.877, 0.018) |  | -0.42 | (-0.885, 0.048) |
| Maternal n-6 |  | 0.037 | (-0.102, 0.175) |  | 0.035 | (-0.108, 0.177) |
| 7 | Cord blood Hg | 930 | -0.002 | **(-0.004, -0.001)** | 864 | -0.003 | **(-0.004, -0.001)** |
| Maternal n-6/n-3  |  | 0.016 | (-0.007, 0.038) |  | 0.013 | (-0.010, 0.036) |
| 8 | Maternal hair Hg | 939 | 0.0001 | (-0.010, 0.010) | 881 | -0.002 | (-0.012, 0.008) |
| Maternal n-3 |  | -0.38 | (-0.823, 0.055) |  | -0.41 | (-0.859, 0.050) |
| Maternal n-6 |  | 0.075 | (-0.061, 0.210) |  | 0.076 | (-0.062, 0.215) |
| 9 | Maternal hair Hg | 939 | -0.0002 | (-0.010, 0.010) | 881 | -0.002 | (-0.013, 0.008) |
| Maternal n-6/n-3  |  | 0.014 | (-0.008, 0.035) |  | 0.013 | (-0.009, 0.036) |
| 10 | Maternal blood Hg | 968 | -0.002 | (-0.005, 0.001) | 901 | -0.003 | (-0.006, 0.001) |
| Maternal n-3 |  | -0.39 | (-0.820, 0.032) |  | -0.34 | (-0.783, 0.100) |
| Maternal n-6 |  | 0.067 | (-0.065, 0.198) |  | 0.046 | (-0.089, 0.181) |
| 11 | Maternal blood Hg | 968 | -0.002 | (-0.006, 0.001) | 901 | -0.003 | (-0.006, 0.000) |
| Maternal n-6/n-3  |  | 0.014 | (-0.008, 0.035) |  | 0.009 | (-0.013, 0.031) |

1. Model 1): Fetal RmtDNAcn ~ Cord blood Hg

Model 2): Fetal RmtDNAcn ~ Maternal hair Hg

Model 3): Fetal RmtDNAcn ~ Maternal 28 week blood Hg

Model 4): Fetal RmtDNAcn ~ Maternal 28 week blood n-3 + Maternal 28 week blood n-6

Model 5): Fetal RmtDNAcn ~ Maternal 28 week blood n-6/n-3 ratio

Model 6): Fetal RmtDNAcn ~ Cord blood Hg + Maternal blood n-3 + Maternal blood n-6

Model 7): Fetal RmtDNAcn ~ Cord blood Hg + Maternal blood n-6/n-3 ratio

Model 8): Fetal RmtDNAcn ~ Maternal hair Hg + Maternal blood n-3 + Maternal blood n-6

Model 9): Fetal RmtDNAcn ~ Maternal hair Hg + Maternal blood n-6/n-3 ratio

Model 10): Fetal RmtDNAcn ~ Maternal blood Hg + Maternal blood n-3 + Maternal blood n-6

Model 11): Fetal RmtDNAcn ~ Maternal blood Hg + Maternal blood n-6/n-3 ratio

1. Adjusted for maternal at delivery, BMI at 20 months, Hollingshead SES and childs sex, birth weight and gestational age.
2. Counts of records in each regression models were different due to missing data on the measurements of total Hg, n-3 and n-6 PUFA and availability of the information related to potential confounders.

## Discussion

In this study, we report for the first time associations between exposure to MeHg and mitochondrial DNA status in newborns and their mothers. Consistent with our hypothesis, we observed that in cord blood, increasing Hg concentration was associated with lower RmtDNAcn. In contrast, increasing Hg in maternal blood was associated with higher RmtDNAcn which was the opposite direction of our hypothesis. However, the results should be interpreted very cautiously since the effect estimates for the associations between Hg and RmtDNAcn were overall quite small. We also evaluated the associations of RmtDNAcn with n-3 and n-6 PUFA which are also present in fish. In line with our hypothesis we found that the anti-oxidative n-3 PUFA were positively associated with RmtDNAcn in the mothers suggesting a protective effect on mitochondria prior to other oxidative stress impacting mtDNA, whereas the pro-oxidative n-6 PUFA showed the opposite association. Together these findings suggest that dietary intake of fish may influence mitochondria homeostasis both via MeHg and PUFA. To note, the timing of assessment of exposure seems important as concurrent blood PUFA and Hg were associated with RmtDNAcn, but not the non-concurrent blood PUFA and hair Hg (e.g. maternal hair Hg or maternal blood Hg vs child RmtDNAcn).

In the newborns lower RmtDNAcn correlated with higher exposure to MeHg, whereas in the mothers the opposite was found. This result may stem from the timing of exposure as newborns and young children are often more susceptible to toxic insult induced oxidative stress due to organs structural and functional immaturity, rapidly growing energy demand for aerobic tissues metabolism, and lack of antioxidant systems (Dobbing 1990). As an adult, the human body is better able to regulate the redox balance tightly, partly through mtDNA replication to compensate for mitochondrial dysfunction and generate sufficient ATP. However, exposure occurring in the early stages of life has been hypothesized to lead to a condition of overloading of mitochondria and subsequent adverse health outcomes, including neurodevelopmental disorders (Perrone et al. 2010). Another, and more speculative explanation for the contrasting findings is that the difference in maternal and child RmtDNAcn effects stem from the different levels of MeHg exposure. For example, we observed that the cord blood contained nearly double the concentration of Hg compared to maternal blood, indicating that the fetus is at higher exposure than the mothers. A biphasic effect of oxidative stress on the mitochondrial proliferation has been suggested whereby low to moderate oxidative stress can stimulate mtDNA synthesis, while higher levels lead to a decompensation stage resulting in depletion of mtDNA and loss of mitochondrial function (Lee et al. 2005; Hou et al. 2010). Such a biphasic response may be present in the mothers and children, respectively. However, this hypothesis needs further experimental support.

Independently of MeHg exposure, n-3 and n-6 PUFA were also found to be associated with maternal RmtDNAcn, but in opposite directions. A mechanism for the influence of PUFA on mtDNA is not fully understood, but may involve n-3 or n-6 specific lipid metabolites, which have different activities towards inflammation and oxidation (Yeates et al. 2017). N-3 PUFA have been reported to regulate mitochondrial biogenesis partially through increased mtDNA replication in skeletal muscle cells and C2C12 myoblast cells (Lee et al. 2016; Vaughan et al. 2012). N-6 PUFA, on the other hand, have been reported to promote oxidative damage on mtDNA in the male wistar rats (Ghosh et al. 2006), which could result in decreasing in mtDNAcn. The negative association we found between n-6/n-3 ratio to the maternal RmtDNAcn suggests that n-6 PUFA may attenuate the positive effects on n-3 PUFA. This relationship is consistent with evidence that a high n-6/n-3 PUFA ratio in the diet can promote the pathogenesis of many chronic disease (Simopoulos 2006).

Strengths of this study include the large cohort of NC2 mother-child dyads in a high fish consuming population (meaning a high intake of both MeHg and n-3, n-6 PUFA), thus a better opportunity to detect subtle associations with mtDNA in both mothers and newborns. Additionally, we were able to account for important relevant covariates. Limitations of this study include the analyses of RmtDNAcn in blood cell populations may not accurately reflect mitochondrial homeostasis in the relevant MeHg target tissues, such as the brain, where energy demands are greater. Additionally, the study lacked information on PUFA levels in the newborns and additional measures of mitochondrial function or oxidative stress are not currently available.

## Conclusion

In conclusion, our findings indicate that both MeHg and PUFA may alter mitochondria homeostasis as measured by RmtDNAcn. Future studies are needed to confirm these findings and if confirmed, explore the potential causal mechanisms.

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## Competing interests

The authors declare that they have no competing financial interests.

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