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Iron status as a covariate in methylmercury-associated neurotoxicity risk ☆,☆☆

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HIGHLIGHTS

- Iron stores and methymercury exposure probably occur independently in the Amazon.
- Iron status is unassociated with hair-Hg concentration among high fish eaters.
- Iron status is not a confounder of methylmercury among Amazonian riparians.
- Methylmercury exposure is unrelated with iron stores among young Amazonian females.

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ABSTRACT

Intrauterine methylmercury exposure and prenatal iron deficiency negatively affect offspring's brain development. Since fish is a major source of both methylmercury and iron, occurrence of negative confounding may affect the interpretation of studies concerning cognition. We assessed relationships between methylmercury exposure and iron-status in childbearing females from a population naturally exposed to methylmercury through fish intake (Amazon). We concluded a census (refuse <20%) collecting samples from 274 healthy females (12–49 years) for hair-mercury determination and assessed iron-status through red cell tests and determination of serum ferritin and iron. Reactive C protein and thyroid hormones was used for excluding inflammation and severe thyroid dysfunctions that could affect results. We assessed the association between iron-status and hair-mercury by bivariate correlation analysis and also by different multivariate models: linear regression (to check trends); hierarchical agglomerative clustering method (groups of variables correlated with each other); and factor analysis (to examine redundancy or duplication from a set of correlated variables). Hair-mercury correlated weakly with mean corpuscular volume ($r = .141$; $P = .020$) and corpuscular hemoglobin ($r = .132$; .029), but not with the best biomarker of iron-status, ferritin ($r = .037$; $P = .545$). In the linear regression analysis, methylmercury exposure showed weak association with age-adjusted ferritin; age had a significant coefficient (Beta = .015; 95% CI: .003–.027; $P = .016$) but ferritin did not (Beta = .034; 95% CI: −.147 to .216; $P = .711$). In the hierarchical agglomerative clustering method, hair-mercury and iron-status showed the smallest similarities. Regarding factor analysis, iron-status and hair-mercury loaded different uncorrelated components. We concluded that iron-status and methylmercury exposure probably occur in an independent way.

Abbreviations: RBC, red blood cells count (10^6 μL^{-1}); MCV, mean corpuscular volume (fL); MCH, mean corpuscular hemoglobin (pg); MCHC, mean corpuscular hemoglobin concentrations (g dL^{-1}); Hair-mercury, total mercury concentration in hair (ppm = $\mu g\ g^{-1}$); Resex, extractive reserve; IQR, interquartile range.

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1. Introduction

Fish is the main natural source of methylmercury (MeHg) for humans IPCS, 1990; U.S. Environmental Protection Agency, 2013, but also one of the main sources of iron (WHO, 2001) and other essential nutrients. Mercury and iron stores may antagonistically affect cognition, but most clinical studies that addressed effects of intrauterine MeHg exposure have ignored the potential negative confounding by maternal iron stores (and vice versa). Actually, a possible confounding role of maternal nutrition in studies examining associations between prenatal fish–MeHg exposures and developmental outcomes in children has been suggested (Davidson et al., 2008), whereas a substantial underestimation of the effects of MeHg toxicity and fish benefits may occur from the lack of confounder adjustment (Choi et al., 2008).

Unknown interactions between maternal iron stores and MeHg exposure may actually exist so that some confounding might have occurred and affected prior studies concerning neurodevelopment outcomes as consequence of intrauterine MeHg exposure. In addition to noise from many covariates, there is also a concern about the precision of the exposure assessment, which may cause significant bias from the underestimation of dose-related toxicity (Grandjean and Budtz-Jørgensen, 2010; Grandjean and Herz, 2011; Karagas et al., 2012). In fact, low iron stores increase animal gastrointestinal absorption of iron and of certain trace elements (Pollack et al., 1965) and a strong positive correlation between iron status and serum mercury concentrations was recently found in humans (Bárány et al., 2005).

In Amazon, neurotoxic effects of intrauterine methylmercury exposure have been a concern due to the typical strong nutritional dependence of fish intake (Hacon et al., 2000, 2008). Since both intrauterine MeHg exposure (Grandjean and Herz, 2011) and prenatal iron deficiency (Beard, 2003) negatively affect offspring's brain development, this study aimed to assess the relationship between MeHg exposure and iron status in childbearing females within the specific geographical context of the typical Amazonian riparians living in Madeira River Basin: a population naturally exposed to MeHg, iron and endemic parasitic diseases (Malm et al., 1995; Hacon et al., 2000, 2008; Fonseca, 2007; Passos and Mergler, 2008).

2. Subjects and methods

2.1. Study area and population

The study area is located in the municipality of Porto Velho (8°47′31″S and 63°57′7″W) around Madeira River, the second largest river of the Amazon Basin that transports one of the largest loads of fluvial sediment in the world (Latrubesse et al., 2005). This cross-sectional observational study was performed from May 2009 to April 2011 and included individuals living in regions extending from about 80 km upstream and 180 km downstream of the Santo Antônio rapids (Fig. 1). Villages around Santo Antônio rapids were grouped in 5 different areas in relation to the left and right banks of the river as well as spatial considerations regarding upstream and downstream the rapids. Clusters were chosen in order to consider the possibility of urban–rural differences in food habit (Neuman et al., 2013) and in MeHg exposure, since lowest fish consumption has been found near urban areas (Dutra et al., 2012) and geographical isolation may be a major variable in studies concerning MeHg effects on humans (Fonseca, 2008).

Area 1 (the closest to Porto Velho city) is located at the right bank downstream the falls with access by roads, 7–10 km away from the urban area at the right bank of Madeira River. Despite close to Madeira River, the main source of fish for inhabitants of Area 1 has been a creek (Belmont igarapé). Area 2 (left bank, downstream the falls) comprises villages close to the city of Porto Velho by boat (8–15 km away from the urban area). The main source of fish has been Madeira River channel. Area 3 (upstream the falls at the right bank) comprises relatively isolated villages with regular access to the large carnivorous fish species from Madeira River channel; local people have some access to a major highway and to services and goods available in the capital city of Porto Velho. Area 4 (upstream the falls at the left bank) is another relatively isolated group that included families living up to 80 km from Porto Velho city; specific availability of fish is quite similar to Area 3. Area 5 included the most geographically isolated community among all assessed village groups, with no means of regular transportation in the middle of the forest with no sewer system or safe drinking water supply. It takes place in the extractive reserve (Resex) of the Cuniã Lake (total area of 55 850 ha and total estimated population of 309 individuals), located in the municipality of Porto Velho, on the left side of Madeira River, about 180 km downstream the urban area. The Area 5 (Resex Cuniã) was chosen as a reference area since it reflects the typical life style of several isolated riparian populations. The main source of fish in Resex Cuniã has been the central lake, including small creeks that link it to the Madeira River channel. There is some local primary-health-care only in Areas 2 and 3.

All residents of the areas described were invited to participate in the study (a census); the inclusion criterion was females aging 12–49 years who had lived in these communities for at least one year.

All the adults of each household in the studied areas answered a semi-structured questionnaire (interview) with information on socioeconomic status, demographic, life style, occupational history and self-reported morbidity. The objective dietary question *How often do you eat fish weekly?* was asked for each of the family members. Based on estimated number of residents (previous family enrollment), acceptance rate was 90% in the areas 1–4 and 81% in Area 5 (Resex Cuniã), which was regarded as a good result.

2.2. Biological samples

On the day of the interview, a sample of hair was collected from the occipital area near to the scalp of each participant. Total mercury concentration in individual hair samples (Hair-mercury) was determined according to routine laboratory procedures at the Wolfgang Christian Pfeiffer Environmental Biogeochemical Laboratory (BIOGEOQ) in Federal University of Rondônia (UNIR) and at the Chemistry Department of Pontifical Catholic University of Rio de Janeiro (PUC-Rio). Briefly, after mineralization in acid-oxidant medium, total mercury determination was performed by cold vapor atomic absorption spectrometry in a FIMS-400® Perkin–Elmer (Bastos et al., 1998). All analytical runs included material certified by the International Atomic Energy Agency (IAEA-085 and IAEA-086) and National Research Council Canada (DORM-2). Recovery rates were >80% and detection limit <0.03 mg kg^{-1}.

Blood sampling and analysis were performed by the laboratory of the Nove de Julho Hospital (CEACLIN®), which is located in the city of Porto Velho. Samples were collected in campaigns (visits to the communities) with 10 mL syringe with 25 × 7 BD® disposable needles, transferred to respective collection tubes (BD® Vacutainer) and immediately taken to the laboratory under adequate conditions for avoiding hemolysis. Blood cell tests assessing blood hemoglobin concentration, hematocrit, red blood cells count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC)