Science of the Total Environment 409 (2011) 1399–1405

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Total blood mercury, plasma homocysteine, methylmalonic acid and folate in US children aged 3–5 years, NHANES 1999–2004

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ARTICLE INFO

Article history:
Received 23 November 2010
Received in revised form 11 January 2011
Accepted 11 January 2011
Available online 3 February 2011

Keywords:
Homocysteine
Methylmalonic acid
Folate
Vitamin B-12
Mercury
Children

ABSTRACT

Background: Mercury is a known neurotoxicant; however, the relationship between childhood exposures and neurodevelopmental outcomes is uncertain, and may be modified by nutrition-related susceptibilities. In vitro studies found that mercury inhibited methionine synthase, an enzyme that interacts with vitamin B-12 and folate to regenerate the amino acid methionine from homocysteine, and inhibition of methionine synthase diverted homocysteine to cysteine and glutathione synthesis. The relationships between mercury, homocysteine, B-12, and folate have not been examined in children.

Objective: This study aimed to evaluate associations between Hg and homocysteine in male and female children differentiated by higher and lower methylmalonic acid (MMA, an indicator of vitamin B-12 deficiency) and folate status.

Design: Cross-sectional data on total blood mercury (Hg), plasma homocysteine, MMA, and serum folate were obtained from the 1999–2004 National Health and Nutrition Examination Surveys for children aged 3–5 years ($n = 1005$). We used multiple linear regression to evaluate relationships between homocysteine and Hg quartiles, stratified by sex, MMA ≥ and folate < sample medians, adjusted for demographic, anthropometric, and environmental factors.

Results: In boys with higher MMA and lower folate ($n = 135$), but not in other children, we observed inverse associations between homocysteine and Hg. Children with Hg > 3.49 μmol/L showed 1.14 μmol/L lower homocysteine ($p < 0.001$) relative to the lowest quartile (≤0.70 μmol/L) {p-value for trend < 0.001}. Compared to other subsamples, this subsample had significantly higher homocysteine levels.

Conclusion: Hg was inversely correlated with plasma homocysteine in young boys, but not girls, with higher MMA and lower folate. Additional studies are merited to evaluate Hg and amino acid metabolism in susceptible children.

1. Introduction

Mercury is a known neurotoxicant (ATSDR, 1999; NRC, 2000; EFSA, 2004); however, the relationship between childhood exposures and neurodevelopmental outcomes is uncertain (Myers and Davidson, 2000; Aschner and Ceccatelli, 2010), and may be modified by nutrition-related susceptibilities (US EPA, 1997; Myers and Davidson, 2000; Dufault et al., 2009a). People are exposed to different forms of mercury, including organic (methyl- or ethylmercury), inorganic, or metallic; neurologic damage is evident at sufficient levels of exposure to metallic mercury vapors and some organic mercury compounds (ATSDR, 1999). Although a general mechanism of toxicity is the binding of mercury to exposed cysteine residues on proteins (Wang and Horisberger, 1996; Li et al., 2007; Klaassen, 2008), its precise mechanism of toxicity has not yet been elucidated, especially at low levels of exposure.

One possible mechanism of mercury's actions involves alteration in metabolic processes critical to human neuronal cell function by inhibiting methionine synthase (Waly et al., 2004), an enzyme that interacts with vitamin B-12 and folate to regenerate the amino acid methionine from homocysteine (Deth et al., 2008; James, 2010). The vitamin B-12 and folate-dependent methionine cycle supports the transsulfuration pathway for the metabolism of the amino acids homocysteine and cysteine to glutathione, an anti-oxidant that protects cells against oxidative stress (Ercal et al., 2001). Experimental studies have also shown that mercury inhibits cysteine transport to and uptake in brain cells (Simmons-Willis et al., 2002; Shanker et al., 2001) and decreases neuronal glutathione levels (James et al., 2005). Therefore, mercury may interact with nutritional factors to alter metabolic processes of the methionine cycle and transsulfuration pathway that influence neuronal cell function. Because homocysteine bridges the methionine cycle and transsulfuration pathway, changes

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doi:10.1016/j.scitotenv.2011.01.021

in homocysteine levels may provide a biochemical indication of impaired amino acid metabolism. Lee et al. (2009) hypothesized that low dose chemical exposures increase the demand for glutathione, and so, divert homocysteine from methionine remethylation to glutathione synthesis, with resultant reductions in homocysteine availability for metabolic homeostasis. Inhibition of methionine synthase activity has been shown to divert homocysteine to cysteine and glutathione production in human lens epithelial cells (Persa et al., 2004); therefore, findings that mercury inhibited methionine synthase function in human neuronal cells (Waly et al., 2004) suggest the potential for an inverse relationship between mercury and homocysteine (Deth et al., 2008).

Gender differences have been shown with regard to metal toxicity and homocysteine levels. For example, boys may be more susceptible to the neurotoxic effects of mercury (McKeown-Eyssen et al., 1983; Marsh et al., 1987; Grandjean et al., 1998; Sakamoto et al., 2001; Cordier et al., 2002). Increased homocysteine levels in children have been associated with male gender (Osganian et al., 1999), decreased vitamin B-12 and folate levels (Bates et al., 2002), obesity (Gallistl et al., 2000), increasing age, and maternal smoking (Bates et al., 2002).

The objective of the present study was to evaluate the relationships between total blood mercury (Hg) and homocysteine in subsets of male and female children aged 3 to 5 years, differentiated by higher and lower vitamin B-12 and folate status. Considering [1] prior evidence of greater susceptibility to mercury toxicity among males (McKeown-Eyssen et al., 1983; Marsh et al., 1987; Grandjean et al., 1998; Sakamoto et al., 2001; Cordier et al., 2002), [2] in vitro findings that mercury inhibits methionine synthase (Waly et al., 2004), [3] evidence that inhibition of methionine synthase diverts homocysteine to the production of other amino acids (Persa et al., 2004), and [4] findings that vitamin B-12 and folate supplementation increased homocysteine levels in children with prior evidence of altered amino acid metabolism (James et al., 2009), we hypothesized that an inverse association between Hg and homocysteine would most likely be detected in boys with lower B-12 and folate levels.

2. Methods

Cross-sectional data on total blood mercury (Hg), plasma homocysteine (Hcy), methylmalonic acid (MMA), and serum folate were obtained from the 1999–2004 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2009a) for children aged 3–5 years. NHANES uses a complex, multistage, probability sampling design to select participants representative of the civilian, non-institutionalized US population (CDC, 2009b). Each sample person is assigned a sample weight that reflects adjustments for complex survey design (including oversampling), survey nonresponses, and post-stratification, in order to ensure that calculated estimates are US population representative (CDC, 2009b).

Total blood mercury (Hg) is a measure of inorganic and organic mercury exposure over the past several days to months (ATSDR, 1999; EFSA, 2004), although it is primarily an indicator of organic mercury exposure, with detectable levels of inorganic mercury only among the 90–95th percentile of NHANES participants (CDC, 2010). Increased MMA levels in serum are considered direct measures of vitamin B-12 tissue stores and the first indication of B-12 deficiency (Moelby et al., 1990). Blood samples from children were non-fasting samples (CDC, 2004). The following covariates were selected for examination based on existing literature regarding their relationships with homocysteine: age (Bates et al., 2002), obesity (Gallistl et al., 2000), serum cotinine (Bates et al., 2002), lead, cadmium (Guallar et al., 2006), and fish high in omega-3 fatty acids (Oken et al., 2005; Mahaffey et al., 2008). Additionally, poverty status was evaluated. The analytic sample domain was restricted to survey participants without missing values for these measures ($n = 1005$). Continuous blood mercury (Hg), lead (Pb), cadmium (Cd) and cotinine measures were log-transformed. Based upon sample weighted frequency distributions, the following categorical variables were created for total blood mercury: quartile 1: ≤ 0.70 μmol/L, quartile 2: >0.70 and ≤ 1.50 μmol/L, quartile 3: >1.50 and ≤ 3.49 μmol/L, and quartile 4: >3.49 μmol/L. Methylmalonic acid values were used as an indicator of vitamin B-12 status, and were dichotomized into values below or equal to the sample median of 0.118 μmol/L (higher vitamin B-12) relative to values above the sample median (lower vitamin B-12), and serum folate values were dichotomized into values below the sample median of 17.1 ng/mL (lower folate) relative to values equal to or above the sample median (higher folate).We used two sets of multiple linear regression analyses to evaluate the relationships between homocysteine and Hg stratified by sex, MMA $\geq$ and folate $<$ sample medians, adjusted for age, race, poverty, obesity, categorical serum cotinine, Pb and Cd; the first set used continuous log-transformed measures of Hg and the second used categorical Hg quartile measures that compared children with higher Hg levels to children with the lowest Hg levels. In order to examine in a separate model the potential influence of beneficial omega-3 (n-3) fatty acids from fish consumption, a variable was created to proxy the effects of beneficial fish intake that compared no fish consumption to consumption of low Hg, high n-3 fish (salmon and sardines, but not shark and not swordfish) (Oken et al., 2005), and to consumption of all other fish, over the past 30 days; the correlation between this variable and Hg was evaluated using linear regression. All combinations of sex, MMA $\geq$ and folate $<$ sample medians were evaluated. Results from the following four subsets are presented: Boys with lower B-12 and lower folate; all other boys; girls with lower B-12 and folate; all other girls.

Age was defined as a categorical variable with age 3 years as the reference group, and age 4 years and 5 years as separate groups. A race/ethnicity variable was dichotomized into non-Hispanic white with non-white as the reference group, and a poverty variable was dichotomized into poverty income ratio (PIR) <1 relative to PIR ≥ 1. A dichotomized variable was created that defined obesity as a body mass index (BMI) ≥ 18.2 kg/m^2 for children aged 3 or 5 years or BMI ≥ 18 kg/m^2 for children aged 4 years, per the National Center for Health Statistics body mass index charts for girls and boys 2–20 years (NCHS, 2000). Statistical analysis was conducted using SAS version 9.2. Medical examination center sample weights, primary sampling units and strata were incorporated to calculate sample means and 95% confidence intervals, to determine statistically significant differences among subset sample means and distributions of sample characteristics, and to perform linear regression analysis using the Taylor Linearization procedure in accordance with complex survey design (CDC, 2009b). The Rao-Scott Chi-square test used to determine statistically significant differences in proportions for weighted sample characteristics among subsets, and 95% confidence intervals were calculated for the difference between weighted means to determine statistical significance. To convert Hg reported as μmol/L to μg/L, μmol/L was divided by 4.99 (CDC, 2004). Statistical significance was defined as an alpha level ≤ 0.05.

3. Results

Distributions of key parameters weighted for complex survey design are presented in Table 1 by sample subset. Race/ethnicity was the only variable that showed a significant difference in proportions among subsets with non-Hispanic white children comprising the greatest proportion of boys with lower B-12 and folate (69%). Table 2 presents weighted sample means for plasma homocysteine and total blood mercury. Boys with lower B-12 and lower folate levels had significantly higher homocysteine levels relative to all other boys and girls who did not have both lower B-12 and lower folate levels. Girls with lower B-12 and lower folate levels had significantly higher homocysteine levels relative to all other girls. Boys with lower B-12

Table 1
Weighted sample characteristics by sex and lower B12 and folate status, children age 3–5, NHANES 1999–2004.

	Boys with lower B12 and lower folate (n = 135)	All other boys (n = 393)	Girls with lower B12 and lower folate (n = 91)	All other girls (n = 386)
Blood mercury quartiles: # (%)				
Q1: ≤0.70 μmol/L	29 (27%)	76 (28%)	18 (21%)	64 (17%)
Q2: >0.70 and ≤1.50 μmol/L	46 (35%)	119 (29%)	22 (30%)	100 (27%)
Q3: >1.50 and ≤3.49 μmol/L	36 (23%)	105 (23%)	29 (23%)	103 (26%)
Q4: >3.49 μmol/L	24 (15%)	93 (20%)	22 (26%)	119 (30%)
Methylmalonic acid>median (0.118 μmol/L): # (%)	135 (100%)	97 (32%)	91 (100%)	130 (36%)
Serum folate<median (17.1 ng/mL) # (%)	135 (100%)	170 (35%)	91 (100%)	136 (30%)
# (%) by age:				
3 years	57 (38%)	126 (29%)	23 (21%)	109 (26%)
4 years	35 (23%)	141 (37%)	36 (45%)	131 (34%)
5 years	43 (40%)	126 (34%)	32 (34%)	146 (40%)
Non-Hispanic white[a] # (%)	50 (69%)	102 (53%)	24 (57%)	115 (58%)
Poverty index ratio<1: # (%)	50 (24%)	165 (31%)	36 (26%)	155 (31%)
Obese # (%)	21 (15%)	49 (11%)	8 (7%)	52 (12%)
Beneficial fish intake past 30 days:				
No fish eaten	59 (48%)	141 (38%)	34 (32%)	140 (33%)
Low Hg, High n-3[b]	16 (11%)	38 (10%)	11 (10%)	36 (11%)
All other fish	60 (41%)	214 (52%)	46 (59%)	210 (56%)
Total fish intake frequency past 30 days:				
No fish eaten	70 (52%)	216 (54%)	45 (40%)	206 (50%)
1-3×/month	39 (29%)	115 (31%)	31 (38%)	121 (31%)
4 or more/month	26 (19%)	62 (15%)	15 (22%)	59 (19%)

Note: Quartiles and tertiles approximated based upon weighted sample frequency distributions.
[a] Statistically significant difference among subgroups at $\alpha \leq 0.05$.
[b] Children with beneficial fish intake defined as those with parental report of past 30 day intake of fish low in Hg and high in omega-3 (n-3) fatty acids, i.e., salmon and/or sardines, but not swordfish and not shark.

and lower folate levels had significantly lower Hg levels compared to girls with lower B-12 and lower folate levels, as well as all other girls.

In regression analyses (Table 3), only the subsample of boys with lower vitamin B-12 and lower folate showed a significant and inverse association between continuous log-transformed Hg (μmol/L) and untransformed homocysteine levels (μmol/L) in unadjusted analyses (Model I) (ß = −0.52; p<0.001; $R^2 = 0.07$); adjusted, but without the beneficial fish covariate (Model II) (ß = −0.49; p = 0.003; $R^2 = 0.19$); and fully adjusted with the beneficial fish covariate (Model III) (ß = −0.52; p = 0.005; $R^2 = 0.25$). No relationship was detected between homocysteine and Hg in unadjusted or adjusted analyses in other groupings of boys and girls.

Using categorical Hg exposure measures in regression analysis limited to boys with lower vitamin B-12 and folate levels (Table 4), findings adjusted for covariates, but not for beneficial fish consumption (Model 2), showed that, relative to the lowest Hg quartile (≤0.70 μmol/L), boys with Hg levels in the 2nd quartile (>0.70 and ≤1.50 μmol/L) had 0.73 μmol/L lower homocysteine levels (p = 0.005); boys with Hg levels in the 3rd quartile (>1.50 and ≤3.49 μmol/L) had 0.65 μmol/L lower homocysteine levels (p = 0.043); and boys with Hg levels in the 4th quartile (>3.49 μmol/L) had 1.14 μmol/L lower homocysteine levels (p<0.001). The R^2 for model 2 was equal to 0.25 and the p-value for trend was statistically significant (<0.001). Results from unadjusted analyses (Model 1) were similar with $R^2 = 0.13$ and a significant p-value for trend (<0.001). The model that also adjusted for beneficial fish consumption (Model 3) showed similar results; however the 3rd quartile lost statistical significance and the 4th quartile showed an 11% greater decrease in homocysteine levels compared to Model 2 ($R^2 = 0.30$; p-value for trend = 0.002).

4. Discussion

To our knowledge, this is the first study to examine the relationships between Hg, MMA, folate and plasma homocysteine in children. Results provide some evidence in support of our hypothesis of an inverse association between total blood Hg and homocysteine levels in boys aged 3–5 years with lower vitamin B-12 and folate levels. This inverse relationship was evident at Hg exposures as low as the range of 0.70 to 1.50 μmol/L (0.14–0.30 μg/L); substantially lower than the 5.8 μg/L reference dose for maternal cord blood methylmercury exposure associated with adverse neurobehavioral endpoints (NRC, 2000; US EPA, 2010). Mean homocysteine, however, was significantly higher in this sample subset compared to the other subsets. Given the entirety of these findings, and suggestion of a mercury-homocysteine association at very low levels of mercury, these results require confirmation before drawing any strong conclusions.

We report lower B-12 and folate levels associated with higher plasma homocysteine in both boys and girls, consistent with findings of Bates et al. (2002), and male sex was associated with higher mean homocysteine, consistent with findings of Osganian et al. (1999). Our finding of an inverse relationship between Hg and homocysteine

Table 2
Sample means (and standard errors) for plasma homocysteine and total blood mercury (Hg), weighted for complex survey design, by sex and lower B12 and folate status, children aged 3–5 years, NHANES 1999–2004.

	Boys with lower B-12 and lower folate (n = 135)	All other boys (n = 393)	Girls with lower B-12 and lower folate (n = 91)	All other girls (n = 386)
Homocysteine, μmol/L: mean (SE)	4.78 (0.13)[a,b]	4.27 (0.09)	4.59 (0.13)[b]	4.18 (0.07)
Mean total blood Hg, μg/L: mean (SE)	0.45 (0.04)[b,c]	0.56 (0.06)	0.79 (0.13)	0.79 (0.09)

[a] Significantly different compared to all other boys, $\propto = 0.05$.
[b] Significantly different compared to all other girls, $\propto = 0.05$.
[c] Significantly different compared to girls with lower B-12 and lower folate, $\propto = 0.05$.

Table 3
Unadjusted and adjusted regression coefficients for the relationship between log-transformed total blood mercury (μmol/L) and plasma homocysteine (μmol/L), by sex and lower B12 and folate status, children aged 3–5 years, NHANES 1999–2004.

Sample subset	Model I: unadjusted regression coefficient (p-value) R^2	Model II (without beneficial fish intake proxy): adjusted[a] regression coefficient (p-value) R^2	Model III (with beneficial fish intake proxy): adjusted[b] regression coefficient (p-value) R^2
Boys with lower B12 and lower folate (n = 135)	−0.52 (<0.001) $R^2 = 0.07$	−0.49 (0.003) $R^2 = 0.19$	−0.52 (0.005) $R^2 = 0.25$
All other boys (n = 393)	0.08 (0.397) $R^2 < 0.01$	0.11 (0.323) $R^2 = 0.06$	0.09 (0.326) $R^2 = 0.06$
Girls with lower B12 and lower folate (n = 91)	0.13 (0.313) $R^2 < 0.01$	0.13 (0.277) $R^2 = 0.13$	0.13 (0.369) $R^2 = 0.13$
All other girls (n = 386)	−0.05 (0.386) $R^2 < 0.01$	−0.06 (0.361) $R^2 = 0.04$	−0.05 (0.464) $R^2 = 0.04$

[a] Model II adjusted for continuous log-transformed cadmium, lead, and serum cotinine; non-Hispanic white race/ethnicity; ages 4 and 5 years relative to 3 years; poverty income ratio (PIR) < 1 relative to PIR ≥ 1; and obesity.
[b] Model III adjusted for same as Model II plus proxy variable for beneficial fish intake (relative to children with no reported fish intake over the past 30 days, children with 30 day fish intake that included low Hg, hi omega-3 fatty acid fish, i.e., salmon or sardine, but not high Hg fish, i.e., not swordfish and not shark; and children with all other fish intake).

among boys with higher homocysteine levels relative to other children raises questions regarding possible biological mechanisms in this subset of children. Although we cannot rule out the possibility of chance findings, there are several biologically plausible mechanisms pertinent to compromised nutrient status, higher homocysteine levels, and mercury toxicity that, in concert, may have contributed to this finding. First, certain children may be genetically susceptible to inadequate folate metabolism and cobalamin deficiency, as well as higher homocysteine levels (Brattstrom et al., 1990; Jacques et al., 1996). In addition, homocysteine has been shown to inhibit function of metallothionein (Barbato et al., 2007), a cysteine-rich protein that binds with inorganic mercury to reduce its bioavailability for cytotoxicity (Aschner, 1997; ATSDR, 2006). Metallothionein expression in neonatal rat primary astrocyte cultures and astrocytomas has also been shown to protect against methylmercury-induced cytotoxicity (Rising et al., 1995; Vitarella et al., 1996; Yao et al., 1999), and ethyl mercury has been shown to induce metallothionein protein in mouse cerebellum (Minami et al., 2010). Therefore, mercury, a sulfhydryl metal, may be more bioavailable to bind with sulfur-containing amino acids in children with higher homocysteine levels, and this subset of children may be more susceptible to both inorganic and organic mercury-induced toxicities. Further, Waly et al. (2004) showed that both inorganic mercury and ethylmercury thiosalicylate (thimerosal) inhibit methionine synthase activity. Data are not yet available in support of or in opposition to these hypothesized mechanisms of action.

The cross-sectional nature of the current study precludes discernment of the dynamics of the relationship between mercury and homocysteine and how other factors, e.g., genetic, metallothionein, nutrients, might influence this relationship. Another unmeasured cofactor is the metabolism of mercury from metallic or organic forms, able to penetrate the blood-brain barrier, to inorganic mercury, which may accumulate in brain tissue (Holmes et al., 2009). Biotransformation of organic to inorganic mercury (Havarinasab and Hultman, 2005), a longer half-life for methylmercury in the blood compared to ethylmercury, and variable blood to tissue distributions of methyl- compared to ethyl-mercury (Burbacher et al., 2005) add to the challenges in using Hg as a biomarker of exposure. The current study

Table 4
Multiple linear regression results for the relationship between categorical blood mercury[a] and homocysteine (μmol/L), boys with lower B12 and lower folate, aged 3–5 years (n = 135), NHANES 1999–2004.

	Regression coefficient	95% CI	p-value
Model 1—unadjusted			
Blood mercury quartiles			
Q1: ≤0.70 μmol/L	Reference	–	–
Q2: >0.70 and ≤1.50 μmol/L	−0.75	−1.43, −0.08	0.030
Q3: >1.50 and ≤3.49 μmol/L	−0.59	−1.13, −0.04	0.035
Q4: >3.49 μmol/L	−1.22	−1.77, −0.67	<0.001
	$R^2 = 0.13$	p-value for trend: <0.001	
Model 2—adjusted[b] model (without beneficial fish proxy)			
Blood mercury quartiles:			
Q1: ≤0.70 μmol/L	Reference	–	–
Q2: >0.70 and ≤1.50 μmol/L	−0.73	−1.23, −0.24	0.005
Q3: >1.50 and ≤3.49 μmol/L	−0.65	−1.27, −0.02	0.043
Q4: >3.49 μmol/L	−1.14	−1.68, −0.60	<0.001
	$R^2 = .25$	p-value for trend: <0.001	
Model 3—adjusted for all variables in model 2 plus beneficial fish intake[c]			
Blood mercury quartiles			
Q1: ≤0.70 μmol/L	Reference	–	–
Q2: >0.70 and ≤1.50 μmol/L	−0.61	−1.06, −0.16	0.009
Q3: >1.50 and ≤3.49 μmol/L	−0.51	−1.16, 0.15	0.127
Q4: >3.49 μmol/L	−1.27	−1.91, −0.63	<0.001
	$R^2 = .30$	p-value for trend: 0.002	

[a] quartiles approximated based upon weighted frequency distributions.
[b] Model 2 adjusted for continuous log-transformed cadmium, lead, and serum cotinine; age (4 and 5 years relative to 3 years old); non-Hispanic white relative to non-white; poverty income ratio (PIR) < 1 relative to PIR ≥ 1; obesity = child age 3 or age 5 and BMI ≥ 18.2 kg/m^2; child age 4 and BMI ≥ 18 kg/m^2.
[c] Model 3 adjusted for same variables as Model 2 plus proxy variable for beneficial fish intake (relative to children with no reported fish intake over the past 30 days, children with 30 day fish intake that included low Hg, hi omega-3 fatty acid fish, i.e., salmon or sardine, but not high Hg fish, i.e., not swordfish and not shark; and children with all other fish intake).

also did not have information on historical exposures, and this may be important, as Holmes et al. (2009) suggested mechanistic studies be conducted to discern the effects of timing of neonatal exposures. Further examination of the temporal relationships between Hg, nutrient status, and homocysteine may shed important insights.

Findings of a relationship between mercury and homocysteine at very low levels of mercury are surprising but not impossible. Short term Hg exposures during critical periods are of particular concern in very young children, as animal studies have shown that adverse effects on the nervous system occur at lower doses relative to other body systems (ATSDR, 1999). Exposure to methylmercury was estimated at 0.28 μg/kg body weight/day for children aged 3–6 years who were in the 50th percentile for fish consumption (US EPA, 1997). The US Environmental Protection Agency- (EPA) exposure limit for dietary methylmercury intake is 0.1 μg/kg body weight/day (Ball et al., 2001; US EPA, 1997), based on a study of children born to mothers exposed to methylmercury during gestation (Marsh et al., 1987), and represents chronic in utero methylmercury exposures associated with late walking, late talking and neurological effects per standardized test scores (US EPA, 1997). An uncertainty factor of 10 was applied to cover lack of data from a two-generation reproductive assay, human population and biological half-life variability; however, because the developing fetus is considered a sensitive human group, there was no additional factor applied for sensitive subpopulations influenced by effect modifiers such as sex and nutrition (USA EPA, 1997). Further study is merited to evaluate the relationships between postnatal exposures, amino acid metabolism and neurodevelopmental disabilities in susceptible subgroups. By investigating sensitive endpoints, e.g., homocysteine and essential amino acids, in susceptible subpopulations we may learn more about risks from low-level exposure.

Deth et al. (2008) hypothesized that environmental factors such as heavy metals cause oxidative stress and methionine synthase inhibition, perhaps mediated via homocysteine, that disrupt neurodevelopment, although weight of evidence does not yet show a clear relationship between amino acid metabolism and neurodevelopment. In a recent review, Dufault et al. (2009a) concluded that mercury exposure and nutritional deficiencies have been shown to impair neuronal function and increase oxidative stress in children with autism, and suggested that the combination of mercury exposure, nutritional deficiencies and impaired amino acid metabolism may interfere with behavior and learning in children. While associations between low level methylmercury exposure and developmental disability are inconsistent (Myers and Davidson, 2000), and a link between thimerosal exposure and autism is largely discounted (Aschner and Ceccatelli, 2010), there remains the possibility of a relationship between mercury and neurodevelopment, especially among susceptible subgroups.

Some have raised concerns that increased homocysteine levels may be a risk factor for cognitive disorders by causing synaptic dysfunction (Mattson and Shea, 2003). Others, however, have suggested that decreased homocysteine levels indicate metabolic disturbances that may underlie neurodevelopmental disorders (Deth et al., 2008; Lee et al., 2009). James et al. (2004) reported lower baseline levels of plasma methionine, S-adenosylmethionine (SAM), cysteine and total glutathione in children with autism compared to typically developing (TD) children, and that treatment with folate and methylcobalamin (B-12) increased levels of homocysteine and other amino acids of the methionine cycle (James et al., 2009). On the other hand, Pasca et al. (2005) found statistically significant higher levels of homocysteine in children with autism compared to TD children. Main et al. (2010) suggested that different methodologies for the measurement of homocysteine, heterogeneity of study participants and small sample sizes might have contributed to disparate findings, leaving open many questions about the relationships between homocysteine and neurodevelopmental outcomes.

Limitations of this study are lack of measures of the amino acids methionine, SAM, cysteine, and glutathione, as well as neurodevelopmental endpoints. Additionally, data were not available on sources and forms of mercury such as methylmercury from fish (Mahaffey et al., 2008) and rice (Dorea, 2010), mercury from chlor-alkali plants found in high fructose corn syrup (Dufault et al., 2009b), ethylmercury from thimerosal-containing vaccines (Ball et al., 2001), metallic mercury from dental amalgams (Counter and Buchanan, 2004), among other sources. In light of EPA estimated median methylmercury exposure from fish intake among children aged 3–6 years of age (0.28 μg/kg body weight/day) in excess of the EPA exposure limit (1 μg/kg body weight/day) (US EPA, 1997), exposures from fish consumption may particularly warrant closer scrutiny.

Other unmeasured potential confounders include serum measures of omega-3 fatty acids, which have been positively correlated with methymercury levels estimated from fish consumption in adults ($r = 0.66$) (Mahaffey et al., 2008). Higher infant cognition was associated with higher maternal consumption of fish with both higher beneficial omega-3 fatty acid and lower, but not higher, mercury content (Oken et al., 2005). Therefore, estimated effects of Hg may also reflect the influence of omega-3 fatty acids, which have been shown to decrease plasma homocysteine by regulating methionine metabolism in rats (Huang et al., 2010); however, we modeled the relationship between Hg and homocysteine with and without a surrogate measure for beneficial fish intake, and results were similar. Our finding of a stronger inverse relationship between continuous Hg and homocysteine in the model with the fish intake covariate compared to the model without this covariate is consistent with findings of Grotto et al. (2010) regarding a stronger inverse association between biomarkers of mercury exposure and oxidative stress, e.g., glutathione and glutathione peroxidase, when fish consumption was included in the multiple regression model. While every effort was made to account for potential confounding variables, the presence of additional unmeasured confounders is a possibility in any epidemiologic study.

Study strengths include strict quality control of blood sample collection and measurement of analytes at the same Medical Examination Center visit (CDC, 2004). Concurrent measurement of Hg, homocysteine, MMA and folate represent recent, and thus, temporally relevant measures in this cross-sectional study. Levels of plasma homocysteine have been shown to exhibit minimal intraindividual variation (Poirier et al., 2001); MMA is an established indicator of B12 deficiency (21); and serum folate correlates with dietary folate in children (Kerr et al., 2009). Because analysis was performed using a US probability sample, findings, are generalizeable to the US population of children aged 3–5 years.

In summary, our findings suggest that boys with lower vitamin B-12 and folate levels may represent a subpopulation uniquely susceptible to mercury-induced alterations in amino acid metabolism at levels of Hg exposure below the US EPA (2010) methylmercury reference dose for adverse neurobehavioral endpoints. The finding of a significantly inverse relationship between Hg and homocysteine only among the subset with lower mean Hg suggests potential susceptibility to Hg-altered amino acid metabolism among boys with lower Hg exposures. It is unknown why an analogous susceptibility was not evident in girls, although previous mercury studies similarly support sex difference (McKeown-Eyssen et al., 1983; Marsh et al., 1987; Grandjean et al., 1998; Sakamoto et al., 2001; Cordier et al., 2002), and further research is warranted. Similar to historical precedent of increasingly more stringent guidelines for blood lead levels due to evidence of low-level effects (Canfield et al., 2003), it is possible that some children might be susceptible to Hg exposures below levels currently considered safe. Additional studies are merited to expand and confirm findings of the present study, and to evaluate associations of amino acid profiles by B-12 and folate status with specific Hg exposures and neurodevelopmental outcomes.

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