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Review

The role of mercury, selenium and the Se-Hg antagonism on cognitive neurodevelopment: A 40-month follow-up of the Italian mother-child PHIME cohort

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ABSTRACT

Despite a 15-year long effort to define the "safety" of fish intake during pregnancy, there remains still uncertainty on this important public health issue. The evaluation of the toxic effects of contaminants, particularly mercury (Hg) in fish-eating populations is complicated by the fact that sea-food is also rich in beneficial nutrients, such as selenium (Se). There is toxicological plausibility of an antagonistic effects between Se and Hg, and some theoretical support for the inclusion of the Se–Hg interaction to better assess the risk linked with fish intake. To assess the effects of exposure to low-level Hg through fish consumption on the developing brain and the interaction between Hg and Se, we conducted an analysis at age 40 months in Italian children, enrolled in a prospective mother-child cohort, comparing additive and multiplicative models.

Participant subjects were the 470 children born within the Northern Adriatic Cohort II (NAC-II) cohort who were tested by using the Bayley Scales of Infant and Toddler Development third edition (Bayley-III) (BSID-III) at age 40. Family demographic and socioeconomic information, pregnancy and delivery history, parental and child medical history and food consumption were assessed through questionnaires. Maternal blood samples were collected during pregnancy, cord blood at birth and maternal milk 1 month after delivery. As other exposures of interest, we considered the level of Se in maternal and cord blood and in breast milk and the potential Se–Hg antagonism. Se and inverse of THg (1:THg) concentrations were categorized according to the tertiles of their distributions, in low, medium and high levels of exposure. The lower end of the composite cognitive score distribution closest to 20% was defined as suboptimal development. Multiple logistic regression were applied to assess the association between the dichotomized composite cognitive score and the categorized exposure to Se and 1:THg, and the antagonism between Se and 1:THg.

In the recruiting period, 900 pregnant women were enrolled in the cohort; 767 of these remained in the study at delivery and 470 children at 40 months. After excluding preterm births, 456 children were used in the final analyses. The larger difference in risk for suboptimal neurodevelopment was observed for the category with High THg and Low Se with OR = 2.55 (90% CI 1.02; 6.41) under the multiplicative and OR = 1.33 (90% CI 0.80; 1.87) under the additive model. The category High THg and High Se showed a very slightly better fit of the additive model (OR = 1.07, 90% CI 0.65; 1.50) versus the multiplicative (OR = 1.66, 90% CI 0.73; 1.77). A negative – antagonistic – interaction term for this category was estimated under the multiplicative model giving an OR = 1.17 (90% CI 0.42; 3.28).

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Although this evidence of the effects of the Se–Hg antagonism on the children neuro-development needs to be confirmed, if Se can counterbalance Hg toxicity, the evaluation of the effect on human health of fish consumption, should also consider the diverse ratios between Se and Hg concentration in different fish species.

1. Introduction

Despite a 15-year long effort (JECFA, 2004; FDA, 2014) to define the "safety" of fish intake during pregnancy, there remains still uncertainty on this important public health issue.

The evaluation of the toxic effects of contaminants, particularly mercury (Hg) (Bose-O'Reilly et al., 2010; Barbone et al., 2019), in fish-eating populations is complicated by the fact that sea-food is also rich in beneficial nutrients, including selenium (Se) (Flores-Mateo et al., 2006; Greenwald et al., 2007; Park and Mozaffarian, 2010).

Selenium is present in fish and other foods (Brazilian nuts, whole-wheat bread, sunflower seeds, turkey and chicken meat etc.), it acts as a growth factor, contributes to antioxidant systems and supports thyroid hormone synthesis (Greenwald et al., 2007; Raymond and Ralston, 2004; Flores-Mateo et al., 2006; Ventura et al., 2017). Two amino acids actually contains selenium: selenomethionine and selenocysteine; however, while selenomethionine seems to represent a reserve for selenium, selenocysteine is incorporated into several proteins with important biological functions. Despite its important role, it has been noted that the association between selenium and health effects is characterized by a U-shaped relationship; both selenium deficiency and excess have showed adverse health effects (Chiang, 2009; Rayman, 2012, 2020. Vinceti, 2017). Long-term toxic effects of a chronically high intake of selenium are probably less known since this condition is rarely observed. However, it has been associated with several diseases such as selenosis, alopecia, dermatitis, non-melanoma skin cancer, types 2 diabetes and prostate cancer. As mentioned, even selenium deficiency has been associated with poor health conditions and diseases as Keshan disease, Kashin-Beck disease, altered immune function, thyroid autoimmune disease, cognitive decline and dementia, prostate and colorectal cancer, type 2 diabetes (Chiang, 2009. Vinceti, 2017. Rayman, 2020).

Methyl-mercury, which can cross the blood-brain barrier, shows high affinity for selenium leading to mercury-selenide precipitates that seems to be metabolically inert. This may reduce selenium bioavailability impairing selenoprotein synthesis with a reduced formation of essential Se-dependent enzymes (Raymond and Ralston, 2004). The actual evidence suggests that the protective effect of selenium against mercury (Park and Mozaffarian, 2010) may be related to the amount of Selenium available for the selenoprotein synthesis. Since the neurological development is directly associated to the normal thyroid function, a reduced availability of free selenium may leads to changes in thyroid hormone homeostasis that during susceptible periods, can lead to neurological damages (Ventura et al., 2017; Raymond and Ralston, 2004). It seems reasonable, therefore, that to correctly evaluate the health benefit of sea-food and fish consumption particularly during pregnancy and childhood, the amount of mercury present must be considered together with the concentration of selenium.

Evidence from three studies suggests that adjusting statistical analysis also for the intake of selenium can reveal associations with contaminants that were not evident or otherwise weaker (Budtz-Jørgensen et al., 2007; Lederman et al., 2008; Strain et al., 2008). On the other hand, Golding et al., in 2016 studied the association between prenatal Hg exposure and early child development and found that the addition of pre-natal Se exposure to the regression models generally determined only a small difference to the strength of the association between mercury and the outcomes. In addition, Oken et al. between 1999 and 2002 enrolled a cohort of 1068 pregnant women to study the association between maternal prenatal fish consumption and cognition in childhood and found no evidence of an association between prenatal Hg and cognitive outcomes, nor any change in the effect estimate for Hg after adjusting for Se (Oken et al., 2016).

Because of the toxicological plausibility of an antagonistic effects between Se and Hg, and some theoretical support (Burger et al., 2011) for the inclusion of the Hg and Se interaction as a useful tool to better assess the risk linked with fish intake, it may be informative to estimate the Hg and Se interaction in models which assess child neurodevelopment.

To assess the effects of exposure to low-level Hg through food (in particular fish consumption) on the developing brain, we conducted an analysis at age 40 months in Italian children enrolled in the Northern Adriatic Cohort II (NAC-II) accounting also for the beneficial effect of fish intake by considering the pre- and postnatal exposure to Se and the Se–Hg antagonism. Italian NAC-II is a prospective cohort residing in the coastal area of north east of Italy and is part of the Mediterranean cohort involved in the "Public health impact of long-term, low-level, mixed element exposure in susceptible population strata" project (PHIME) (PHIME, 2011).

2. Materials and methods

2.1. Study population

Participant subjects were the 470 children born within the NAC-II cohort who were tested by the Bayley Scales of Infant and Toddler Development third edition (Bayley-III) - (BSID-III) (Bayley, 2006) at age 40 months and their mothers. A detailed description of the study protocol, inclusion and exclusion criteria, and power's calculation, has been published elsewhere (Valent et al., 2013a).

2.2. Ethics

The protocol was approved by the Ethics Committees of the University of Udine and the Institute for Maternal and Child Health IRCCS Burlo Garofolo, Trieste. Recruitment and follow-up took place at the Institute for Maternal and Child Health. The follow-up at 40 months of age of children born within the cohort was approved by the ethics committee of the Institute for Maternal and Child Health IRCCS Burlo Garofolo (CE/V-109-12/04/2010).

During the follow-up, participants and their parents were free to withdraw from participating in the study at any time upon request.

2.3. Questionnaires

At recruitment (20–22 gestational weeks), a short questionnaire was administered to mothers to provide some brief information on family and lifestyles during pregnancy and to identify any excluding conditions (Valent et al., 2013a).

At 20 to 32 gestational weeks, mothers enrolled in the study were tested with the Standard Progressive Matrices (SPM), a version of the Raven's Progressive Matrices, to estimate the maternal intelligence quotient. (Raven et al., 1998).

At 1 month after delivery, a detailed questionnaire was administered to mothers to collect family demographic and socioeconomic information, pregnancy and delivery history, parental and child medical history, residential and occupational history, mother's lifestyles and general dietary habits. A detailed food frequency section of 138 food item was used to estimate food components (macro- and micro-nutrients, amino acids, fatty acids, vitamins, minerals, caloric intake) and fish consumption during pregnancy. A conversion from categories of consumption into continuous intakes of fish servings was done for each item

of the questionnaire by assigning a consumption level equal to the median value for that category (e.g., 2–4 times/week became 3 times/week). Overall fish intake was calculated by summing the estimated weekly intake of all fish types.

At 18 and 40 months after delivery, supplementary questionnaires were completed to detect changes in sociodemographic information, socio-economic status and fish consumption by the child.

2.4. Exposure definition and sample collection

The primary exposure of interest was the level of total mercury (THg) (in ng/g). THg was measured in maternal blood collected during pregnancy and cord blood collected at birth (reflecting prenatal exposure), and in maternal milk (reflecting postnatal exposure). As other exposures of interest, we considered the level of Se (in ng/g) in maternal and cord blood (reflecting prenatal exposure) and in breast milk (reflecting postnatal exposure) and the potential Se–Hg antagonism.

A venous blood sample was collected during weeks 20–22 (whenever possible) or during week 32 (if the woman refused blood withdrawal during weeks 20–22 due to lack of time or due to other personal reasons). A cord blood sample was collected few minutes after delivery and before clamping the umbilical cord. Both venous and cord samples were collected in Vacutainer Blue Cup NaH tubes, specific for heavy metals determination and divided, after processing, in three aliquots of 1500 μL.

At approximately 1 month after delivery, a sample of about 50 mL of 24-h breast milk was collected in Sarsted polypropylene containers. In most cases, breast milk from lactating women was collected by trained research personnel at the participants' homes; in a small number of cases, mothers preferred to hand in the samples to research staff at the study hospital.

Aliquots of venous blood, cord blood and breast milk samples were stored in freezers (temperature −80 °C) and then transported on dry ice to the Jozef Stefan Institute (JSI) in Ljubljana for THg and Se determination.

2.5. Chemical analysis

All analyses were performed at the JSI laboratory. THg in venous and cord blood have been measured by cold vapor atomic absorption spectrometry (CVAAS) (Akagi, 1997) as described in details earlier (Valent et al., 2013b; Miklavčič et al., 2013; Barbone et al., 2019). Se was measured in venous and cord blood samples with an Octapole Reaction System (ORS) Inductively Coupled Plasma Mass Spectrometer (7500ce, Agilent) equipped with an ASX-510 Autosampler (Cetac). The analytical procedure is described in detail elsewhere (Miklavčič et al., 2011, 2013). Instrumental conditions were as follows: Babington nebulizer, Scott-type spray chamber, spray chamber temperature 5 °C, plasma gas flow rate 15 L/min, carrier gas flow rate 0.8 L/min, make-up gas flow rate 0.1 L/min, sample solution uptake flow rate 1 mL/min, RF power 1500 W, reaction cell gas helium 4 mL/min, isotopes monitored 77Se and 78Se. Tuning of the instrument was made daily using a solution containing Li, Mg, Y, Ce, Tl, and Co. Quantification on all isotopes was performed using one central point of the spectral peaks and three repetitions. The reference material Seronorm trace elements whole blood L-1 (Sero) was used to check the accuracy of the results and the values found were in good agreement with the reference values.

The LOD for Se calculated as three times the standard deviations of the blank sample was 5 ng/g blood.

For determination of Se in breast milk samples were used two samples preparation procedures: microwave digestion and dilution as described in detail in Miklavčič et al. (2013). Measurements of prepared solutions were determined by inductively coupled plasma mass spectrometry (ICP-MS) as described for venous and cord blood samples. The accuracy of the results was checked by analyzing the certified reference material Whole Milk Powder NIST 8435 from the National Institute of Standards and Technology (Gaithersburg) and the measured values (microwave: Se:121 ± 16 ng/g; dilution: Se: 128 ± 15 ng/g) were in good agreement with each other and with the certified reference value of 131 ± 14 ng/g.

An internal milk control material in liquid form was also used for accuracy checking. The values found by microwave digestion was 14.2 ± 2.7 ng/g which was comparable with the values obtained by dilution 14.0 ± 1.7 ng/g. The LOD of microwave digestion and dilution procedures, calculated as three times the standard deviation of the blanks, was 1.5 ng/g and 0.9 ng/g blood respectively.

2.6. Outcome definition and measurement

The primary outcome of interest was the evaluation of children neurodevelopment at 40 months of age (range, 38–42 months) assessed through the BSID-III by calculating the cognitive, language, motor, social-emotional, and adaptive behavior composite scores. The BSID-III is designed to evaluate the neurodevelopment of children aged 1–42 months. It includes growth scores to monitor the individual's progress over time (Bayley, 2006). Here, we report only results of the cognitive composite score as study outcome. The tests were performed by trained psychologists.

Composite scores have an average of 100, a standard deviation of 15 and a range from 40 to 160. The cognitive composite was considered both as continuous and dichotomous score. As a continuous variable, the higher was the composite score and the better the child's performance. In addition, as a dichotomous variable, children who scored under the 20th percentile of the cognitive composite score were considered as having suboptimal cognitive development and were compared with children who scored above that cut-off. (Hibbeln et al., 2007).

2.7. Covariates

The following list of covariates was considered for estimating the total effect of THg, Se and Se–Hg antagonism on child neurodevelopment: BMI before pregnancy, maternal intake of fish during pregnancy, birth weight, maternal intelligence quotient (IQ), mother's occupational status, size of the home, smoking habits and alcohol intake during pregnancy, breastfeeding history (duration up to 18 months), child intake of fish until age 18 months and fish consumption at 40 months of age.

2.8. Statistical analysis plan

Consistently with previous analyses of the Italian cohort (Valent et al., 2013b), only data from children, born at term (≥37 gestational week), who had at least 1 measure of THg and Se exposure, and completed the BSID-III testing at 40 months were included in the analysis. Distribution of the reason of withdrawal, of the lost to follow-up at 40 months of age (compared with the mother-child pairs remained in the study at delivery) and of the general characteristics of mothers and their children were described as frequency and percentage or as mean ± standard deviation and median, respectively for discrete or continuous variables. The distribution of composite cognitive score, fish intake and concentrations of THg and Se was represented by arithmetic means (mean) and standard deviation (SD), quintiles, median and minimum-maximum ranges. Differences between groups were assessed by the Wilcoxon's or Kruskal Wallis test (for continuous variables) and the $\chi 2$ test (for categorical variables); the associations of cognitive composite score (continuous) with continuous covariates were measured by Spearman correlation.

Se and inverse of THg (1:THg) concentrations, in each biological sample, were categorized according to the tertiles of their distributions, and low, medium and high levels of exposure were determined.

An exploratory analysis for the non linear bivariate relationship between metals concentrations in biological samples (maternal blood,

cord blood and breast milk) and cognitive score was performed. Specifically, bivariate thinplate splines, with 80% confidence bound, of Se and inverse THg concentrations for each biological sample to the composite cognitive score were fitted. The splines show the non linear relationship between inverse THg concentrations and cognitive score separately for the three levels of Se (low, medium, high). Medium Se was labelled as reference for consistency with the subsequent analysis.

The relation between cognitive composite score and categorized inverse THg and Se co-exposure was explored with logistic regression analysis. Logistic regression was conducted to evaluate the hypothesis that the relative risk of suboptimal development (Golding et al., 2016), measured as a dichotomous variable derived from the observed distribution of the composite cognitive score, depended on the different levels of Se and inverse THg concentrations. The interaction between Se and the inverse of THg was also included in the logistic regression model. The lower end of the observed distribution of the composite cognitive score closest to 20% was defined as suboptimal development (Golding et al., 2016), and considered as a cut-off to dichotomize it. Separate models were built for each biological sample. In the multiple logistic regression, the covariates were automatically selected through the VanderWeele's selection method (VanderWeele et al., 2011), while the categorized Se, inverse THg and the interaction between Se and the inverse THg were forced into models. Odds ratios (ORs) and 90% confidence interval (90% CI) were estimated.

The multiple multiplicative and additive main effects models (without the interaction term) were estimated through the Odds Power transformation family which include as special cases the multiplicative and the additive models. Using this extended model we were able to compare the fit of the multiplicative model to the fit of the additive model. Finally, we choose this family to mantain the Odds Ratio as effect measure.

The ORs obtained by the multiple logistic regression (with the interaction term) and those obtained by the multiple multiplicative and additive main effects models were compared to determine whether the interaction was multiplicative or additive. In the literature synergism/antagonism of action refers to departure from a biological model for the independent action of two determinants. When the joint effect is greater/lesser than expected from the separate effects we speak of synergism/antagonism. Of course this depends on the particular biological model chosen. When not otherwise specified, synergism/antagonism is generally measured as a departure from an additive model. With binary responses we specified logistic regression models with Odds Power transformation family to fit additive models, with continuous response we specified linear (additive) regression models.

The same analysis were performed for Se and inverse THg concentrations measured in maternal blood and breast milk. These results are shown in the supplementary material.

2.9. Software for the statistical analysis

STATA 14 and SAS 9.4 are the statistical software used for the analysis.

3. Results

In the recruiting period, 900 pregnant women were enrolled in the cohort; 767 (85%) of these remained in the study at delivery, 632 children underwent BSID-III testing at 18 months and 470 children at 40 months (respectively 82% and 61% of those born within cohort).

The most frequent causes of discontinuation from delivery to 40 months of age of children; were parents' lack of time (n = 134; 45.1%) and loss of contact with parents including missing appointments (n = 86; 29.0%). Only in 6 cases withdrawal was due to child's disease.

Consistently with the loss-to-follow-up analysis at 18 months, the mothers of children withdrawn/lost to follow-up at 40 months had a lower IQ (median 122 vs 125, p = 0.003), were less likely to be married (87.5% vs 91.4%) and more likely to be separated (6.1% vs 2.6%, p = 0.05), lived less likely in owned house (72.0% vs 80.3%, p = 0.008) than those who were followed-up; on the other hand educational level, employment status, age at delivery, the fish consumption during pregnancy were similar as well as, the concentrations of THg and Se in maternal blood, cord blood and breast milk. (Valent et al., 2013b).

After excluding preterm births, 456 children were used in the final analyses. The sociodemographic characteristics of the Italian cohort are shown in Table 1. A large proportion of mothers had at least a college degree and were on maternity leave during pregnancy. Mothers' nonverbal intelligence score (Raven) was rather high (median = 125).

The distribution of cognitive composite score at 40 months, and mother's and child's fish consumption are shown in Table 2. Mean

Table 1
General characteristics of mothers and their children included in the 40-month follow-up (n = 456).

At delivery:	
Mother's age (year) at delivery, mean±SD[a] (median)	33.4 ± 4.3 (33.0)
Maternal BMI before pregnancy, mean±SD[a] (median)	22.8 ± 3.8 (22.2)
Weight gain (kg) during pregnancy, mean±SD[a] (median)	19.9 ± 19.5 (14.0)
Mother's occupation, n (%):	
Employed on maternity leave	349 (77.4)
Employed worker	37 (8.2)
Housewife	36 (8.0)
Other condition	29 (6.4)
Father's occupation, n (%):	
Employed on paternity leave	39 (8.7)
Employed worker	390 (87.3)
Househusband	3 (0.7)
Other condition	15 (3.4)
Mother's marital status, n (%):	
Married/Living together	410 (91.3)
Widow/single/never married/Separated/divorcing	39 (8.7)
Mother's educational level, n (%):	
Elementary and middle school	82 (18.1)
High school	207 (45.6)
University degree	165 (36.3)
Home size, n (%):	
< 50 m^2	34 (7.5)
50–100 m^2	307 (67.6)
>100 m^2	113 (24.9)
Number of cigarettes smoked, by mother, during pregnancy, mean±SD[a] (median)	128 ± 478 (0)
Alcoholic drinks per week during pregnancy, mean±SD[a] (median)	1.5 ± 3.4 (0.4)
Raven's Progressive Matrices score, mean±SD[a] (median)	119.0 ± 11.4 (125)
Fish consumption of mother per week during pregnancy, mean±SD[a] (median)	2.2 ± 1.6 (2.0)
Children's sex, n (%):	
Boys	238 (52.2)
Girls	218 (47.8)
Birth weight (g), mean±SD[a] (median)	3426.4 ± 441.2 (3400.0)
Breastfeeding months, mean±SD[a] (median)	10.0 ± 5.9 (10.0)
Number of children living in home (excluding the newborn), n (%):	
0	265 (58.1)
1 or more	191 (41.9)
At 18 months of age:	
Child's consumption of homogenized fish (number of months with at least one portion of fish per week), mean±SD[a] (median)	2.9 ± 4.6 (0)
Child's consumption of fresh fish (number of months with at least one portion of fish per week), mean±SD[a] (median)	9.2 ± 3.9 (10.0)
Day care attendance during the week, n (%):	
Member of the family or other people not included in the family	281 (61.6)
Kindergarden	175 (38.4)
At 40 months of age:	
Child's consumption of fish per week, mean±SD[a] (median)	1.5 ± 0.8 (1.0)
Day care attendance during the week, n (%):	
Member of the family or other people not included in the family	64 (14.9)
Kindergarden	365 (85.1)

[a] Standard Deviation.

cognitive composite score at 40 months was 113 (SD 14); mean fish consumption of mother per week, during pregnancy, was 2.2 servings/week (SD 1.6). At 18 months of age, child's consumption of homogenized fish (number of months with at least one portion of fish per week), was 2.9 (SD 4.6) while child's consumption of fresh fish (number of months with at least one portion of fish per week) was 9.2 (SD 3.9). At age of 40 months, child's consumption of fish per week was 1.5 servings/week (SD 0.8).

The distributions of metals measured in maternal blood, cord blood and breast milk are displayed in Table 3. The means of THg in maternal blood, cord blood and breast milk were 3.4 ng/g (SD 3.8), 5.6 ng/g (SD 4.9), 0.36 ng/g (SD 1.49) respectively. Means of Se in maternal blood, cord blood and breast milk were 122.1 ng/g (SD 26.5), 117.4 ng/g (SD 27.1) and 18.7 ng/g (SD 6.2), respectively.

Detailed comparisons of the cognitive composite score at 40 months of age -as continuous and dichotomous outcome-by covariates are presented in Supplemental Tables A1 to A4. In brief, smaller home size (p-value: 0.01) and living in a home where the subject was the only child (p-value: 0.06) were associated with lower cognitive composite score.

3.1. THg - cognitive development

In the simple model for high THg levels, the OR of the children falling in the first quintile of the cognitive composite score is 1.12 (90% CI: 0.67–1.88) while in the adjusted model it is 0.96 (90% CI: 0.56–1.66). (Table 4).

3.2. Se - cognitive development

In both the simple and multiple models, the children with low and high Se exposure levels had a higher OR of falling in the first quintile of the cognitive composite score (Table 5).

3.3. THg and Se - cognitive development

Table 6 reportes the results of the simple and multiple logistic regression of Se and THg (1:THg) exposure levels in cord blood on the dichotomized cognitive composite score.

3.4. THg-Se interaction - cognitive development

Using continuous data we fitted a linear additive model with a bivariate thinplate spline to model the not linear interaction between THg and Se. The dose-response curve for THg when Se is medium-low (105–125), is quadratic but it changes for High Se (LR test for interaction p = 0.0715). As shown in Fig. 1 composite cognitive score for High and Low Se with Low THg subjects is on average 10 points below the reference category of Medium Se, while only composite cognitive score for Low Se with High THg subjects is on average 10 points below the reference category of Medium Se. A mild effect of THg is evident for Low and Medium Se. In Table 7 the frequency distributions of dichotomized cognitive composite score at 40 months of age by Se and inverse THg levels are shown. The results from the multiple logistic regression (Table 8) show no evident effects of increasing levels of THg on the cognitive composite score, in the various categories of exposure to Se. Usually the empirical information to compare the fit of multiplicative and additive models is low (we set the parameter in the Odds Power transformation family to 0.8 instead of 1 for fitting the additive model to achieve stability in the estimate). Both models provide acceptable description of the data, as shown in Table 9 (the log-likelihoods are very close each other). The larger difference is observed for the category with High THg and Low Se with OR = 2.55 (90% CI 1.02; 6.41) under the multiplicative and OR = 1.33 (90% CI 0.80; 1.87) under the additive model. The interesting – for evidence of an antagonism of action - category High THg and High Se shows a very slightly better fit of the additive model (OR = 1.07, 90% CI 0.65; 1.50) versus the multiplicative (OR = 1.66, 90% CI 0.73; 1.77). A negative – antagonistic – interaction term for this category is estimated under the multiplicative model giving an OR = 1.17 (90% CI 0.42; 3.28) (see Table 8; LR test comparing the multiplicative model to the model with the interaction terms p = 0.0822). This is not the case for the additive model because under this model there is no effect of THg and a modest effect of Low Se (LR test p = 0.120). Moreover, the main effects models (Table 9), especially the multiplicative model, show the U-shaped tendency of the detrimental effect as a result of increasing exposure to Se.

Results regarding the association of THg and Se, measured in maternal blood and breast milk, and the children composite cognitive score are available as supplementary material.

4. Discussion

In our cohort, the reported consumption of maternal fish during pregnancy was moderate, considerably lower than consumption reported in the Seychelles (12 fish meals a week) (Myers et al., 2007), but comparable to that of a US cohort of pregnant women (1.5 servings/-week; SD 1.4) (Oken et al., 2008). THg and Se concentrations in biological samples from mothers and children were consistently low; in this scenario of relatively low exposures, no evidence of a clear, positive

Table 2
Distributions of cognitive composite score at 40 months of age and fish consumption.

	N	Mean	SD[a]	Min[b]	20th P[c]	40th P[c]	Median	60th P[c]	80th P[c]	Max[d]
Scores at 40 months of age:										
Cognitive composite score	454	113	14	70	100	105	110	115	120	145
Mother's fish consumption:										
Fish consumption per week, during pregnancy	455	2.2	1.6	0.0	1.0	1.5	2.0	2.3	3.0	22.5
Child's fish consumption:										
Child's consumption of homogenized fish (number of months with at least one portion of fish per week), until 18 months	430	2.9	4.6	0.0	0.0	0.0	0.0	0.0	6.5	17.0
Child's consumption of fresh fish (number of months with at least one portion of fish per week), mean ± std (median), until 18 months	430	9.2	3.9	0.0	7.0	9.0	10.0	11.0	13.0	15.0
Child's consumption of fish per week, at 40 months	416	1.5	0.8	0.0	1.0	1.0	1.0	2.0	2.0	5.0

[a] Standard Deviation.
[b] Minimum.
[c] Percentile.
[d] Maximum.

Table 3
Distributions of THg and Se concentrations.

Pre- and post-natal metal's concentrations	N	Mean	SD[a]	Min[b]	20th P[c]	40th P[c]	Median	60th P[c]	80th P[c]	Max[d]
In maternal blood:										
THg (ng/g)	456	3.4	3.8	0.1	1.0	2.00	2.4	3.0	4.6	39.6
Se (ng/g)	454	122.1	26.5	67.0	100.9	111.4	117.7	123.0	142.2	228.8
In cord blood:										
THg (ng/g)	347	5.6	4.9	0.1	2.2	3.3	4.0	5.2	7.9	29.9
Se (ng/g)	350	117.4	27.1	70.5	94.4	108.1	113.7	120.5	136.0	269.5
In breast milk:										
THg (ng/g)	373	0.36	1.49	0.01	0.09	0.15	0.18	0.22	0.34	28.30
Se (ng/g)	372	18.7	6.2	6.7	14.0	16.9	18.3	19.5	22.7	68.3

[a] Standard Deviation.
[b] Minimum.
[c] Percentile.
[d] Maximum.

Table 4
Simple and multiple logistic regression of THg (1:THg) exposure levels in cord blood on the dichotomized cognitive composite score. The table shows the odds ratios (OR) and the 90% confidence intervals (90% CI.

THg exposure's levels[a]	OR[b] (90%CI)	OR[c](90%CI)
Medium vs Low	0.65 (0.37–1.14)	0.67 (0.37–1.20)
High vs Low	1.12 (0.67–1.88)	0.96 (0.56–1.66)
	N = 349; Log Likelihood = −174.15	N = 323; Log Likelihood = −159.82

[a] THg (ng/g): low (1:THg>0.33); medium (0.17 < 1:THg≤0.33); high (1:THg ≤ 0.17).
[b] Model: cognitive composite score ~ $\alpha + \beta_1$ (1:THg).
[c] Adjusted model: cognitive composite score ~ $\alpha + \beta_1$ (1:THg) + β_2 (home size) + β_3 (children fish intake up to 18 months).

Table 5
Simple and multiple logistic regression of Se exposure levels in cord blood on the dichotomized cognitive composite score. The table shows the odds ratios (OR) and the 90% confidence intervals (90% CI).

Se exposure's levels[a]	OR[b] (90%CI)	OR[c](90%CI)
Low vs Medium	1.80 (1.04–3.14)	2.13 (1.18–3.85)
High vs Medium	1.37 (0.77–2.43)	1.41 (0.76; 2.62)
	N = 346; Log Likelihood = −176.06	N = 326; Log Likelihood = −159.13

[a] Se (ng/g): low (Se ≤ 105); medium (105 < Se ≤ 125); high (Se > 125).
[b] Model: cognitive composite score ~ $\alpha + \beta_1$(Se).
[c] Adjusted model: cognitive composite score ~ $\alpha + \beta_1$(Se) + β_2 (home size) + β_3 (children fish intake up to 18 months).

association between THg and adverse effects on children cognitive development emerged, even after adjustment for potential confounders and although different biological samples were considered. This results appear consistent with our previous assessment at 18 months of age (Valent et al., 2013b; Barbone et al., 2019). Our main results show the non linear relationship between inverse THg cord blood concentrations and cognitive composite score separately for levels of cord blood Se. While the dose-response curves of mercury were similar for low and medium cord blood selenium concentrations, with a decrease in cognitive score for higher mercury concentrations, the dose-response curve of mercury for high selenium concentrations did not show a decrease in cognitive score for higher mercury concentrations, suggesting an antagonistic effect of high selenium concentrations. As mentioned, Se is a trace element and by contributing to the antioxidant systems (Flores-Mateo et al., 2006; Greenwald et al., 2007), it is essential for early

Table 6
Simple and multiple logistic regression of Se and THg (1:THg) exposure levels in cord blood on the dichotomized cognitive composite score. The table shows the odds ratios (OR) and the 90% confidence intervals (90% CI).

Metal's exposure	OR[c] (90%CI)	OR[d](90%CI)
THg exposure's levels[a]:		
Medium vs Low	0.69 (0.39–1.22)	0.74 (0.41–1.34)
High vs Low	1.28 (0.74–2.21)	1.15 (0.65–2.05)
Se exposure's levels[b]:		
Low vs Medium	2.02 (1.14–3.59)	2.21 (1.21–4.06)
High vs Medium	1.43 (0.80–2.58)	1.44 (0.77–2.68)
	N = 345; Log Likelihood = −171.80	N = 323; Log Likelihood = −157.37

[a] THg (ng/g): low (1:THg>0.33); medium (0.17 < 1:THg≤0.33); high (1:THg ≤ 0.17).
[b] Se (ng/g): low (Se ≤ 105); medium (105 < Se ≤ 125); high (Se > 125).
[c] Model: cognitive composite score ~ $\alpha + \beta_1$ (1:THg) + β_2(Se).
[d] Adjusted model: cognitive composite score ~ $\alpha + \beta_1$ (1:THg) + β_2(Se)+ β_3 (home size) + β_4 (children fish intake up to 18 months).

development, counteracting the toxic effect of Hg exposure (Park and Mozaffarian, 2010). As we showed, for low mercury concentrations in cord blood, highest composite cognitive scores were achieved when Se concentration in cord blood was medium, while for high and low Se concentrations the cognitive performance worsened, suggesting a U-shaped dose-response curve for selenium, consistently with evidences reported in literature (Chiang, 2009; Rayman, 2012, 2020. Vinceti, 2017). For high mercury concentrations, the higher the concentration of Se the higher the composite cognitive score, although the confidence bands, which widely overlaps, make the interpretation uncertain.

The results from the multiple logistic regression (Table 8) show no evident effects of increasing levels of mercury on the cognitive composite score, in the various categories of exposure to Se. As mentioned, the main effects models (Table 9) show the larger difference for the category with High THg and Low Se and a weak evidence of interaction (antagonism) between selenium and mercury. Interestingly, category High THg and High Se shows a very slightly better fit of the additive model versus the multiplicative. The main effects models describe also the U-shaped curve of the effects due to increasing exposure to Se. The weak evidence of antagonism between selenium and mercury appears weaker when considering the association of Hg and Se, measured in maternal blood and breast milk, and the children composite cognitive score (results available in supplementary material). Although this weak evidence of the effects of the Se–Hg antagonism on the children neuro-

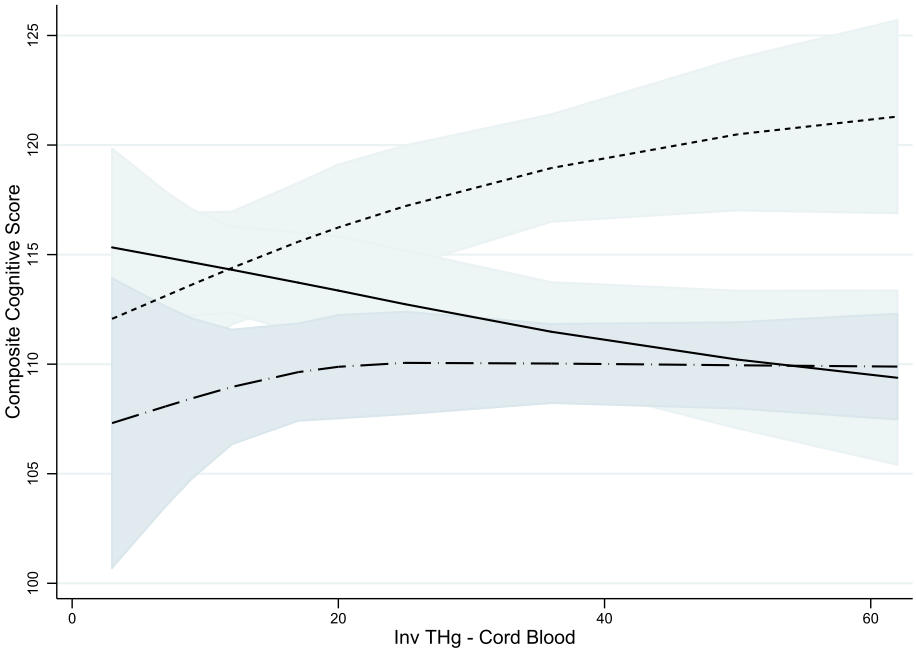

Fig. 1. Bivariate thinplate spline, with 80% confidence bands, of cognitive composite score and inverse THg concentrations in cord blood, for three levels (low, medium and high) of Se concentrations in cord blood. Continuous line (–) = High selenium; Short dash (- - -) = Reference Medium selenium; Long dash and dots (—) = Low selenium. 80% confidence bands.

Table 7
Frequency distributions of dichotomized cognitive composite score by Se and inverse THg levels.

Frequency distribution of Cognitive Composite score ≤100 (>100)			
Se exposure's levels[a]	THg exposure's levels[b]		
	Low	Medium	High
Low	**15** (45)	**9** (31)	**7** (15)
Medium	**5** (27)	**3** (35)	**8** (29)
High	**6** (19)	**6** (32)	**12** (41)

[a] Se (ng/g): low (Se ≤ 105); medium (105 < Se ≤ 125); high (Se > 125).
[b] THg (ng/g): low (1:THg>0.33); medium (0.17 < 1:THg≤0.33); high (1:THg ≤ 0.17).

Table 8
Multiple logistic regression of the interaction effect between Se and THg (1:THg) exposure levels in cord blood on the dichotomized cognitive composite score. The table shows the odds ratios (OR) and the 90% confidence intervals (90% CI) of the interaction between Se and THg exposure.

Se exposure's levels[b]	OR[a] (90% CI)		
	THg exposure's levels[c]		
	Low	Medium	High
Low	1.75 (0.67–4.60)	1.77 (0.62–5.05)	2.57 (0.83–8.00)
Medium	1	0.33 (0.08–1.42)	1.36 (0.47–3.93)
High	1.76 (0.56–5.56)	1.14 (0.37–3.46)	1.17 (0.42–3.28)
N = 323; Log Likelihood = −155.86			

[a] Adjusted model: cognitive composite score ~ $\alpha + \beta_1$ (Se) + β_2 (1:THg) + β_3 ((Se)*(1:THg)) + β_4 (home size) + β_5 (children fish intake up to 18 months).
[b] Se (ng/g): low (Se ≤ 105); medium (105 < Se ≤ 125); high (Se > 125).
[c] THg (ng/g): low (1:THg>0.33); medium (0.17 < 1:THg≤0.33); high (1:THg ≤ 0.17).

development needs to be confirmed, if Se can counterbalance Hg toxicity, the evaluation of the effect on human health of fish consumption, should also consider the diverse ratios between Se and Hg concentration in different fish species. Under this light, also sources of Se, other than fish, should be taken into account in the whole diet.

The results presented in this article are limited to the cognitive assessment and future analyses of the motor and language development could lead to different results. In addition, our analyses assumed that measured THg was entirely represented by MeHg, the Hg species derived from diet whereas inorganic Hg, derived from occupational and/or environmental exposure, was considered negligible end ineffective. However, MeHg was actually measured in approximately one third of the subjects and the Spearman correlation between MeHg and THg was very high (0.97 in cord blood). However, neurologic development is a multifactorial process hence our results might be affected also by unmeasured confounders (Stiles and Terry, 2010). Finally, this article focuses on a cognitive assessment conducted at 40 months of age. Therefore, the full effects of prenatal Hg exposure may not be measurable yet.

Despite the above-mentioned limitations, our study has several strengths. One of the strengths is the measurement of mercury in different biological samples; measuring THg concentration in different samples permitted us to evaluate better the effect of the exposure on cognitive development. Another strength is the measurement, in several samples, of other neurotoxic and beneficial elements essential to the developing nervous system. We also accounted for several covariates and assessed neurocognitive outcomes using validated instruments, administered by 2 trained psychologists that showed high levels of agreement.

5. Conclusions

No clear relation between prenatal Hg exposure and adverse effects on children neurodevelopment was found at 40 months of age, although the results pertaining to the Se-THg interaction may add some evidence on the role of THg in relative rather than absolute terms when antagonists agents are also considered. Further follow-up of the Mediterranean PHIME cohorts could elucidate the long-term cognitive effects of prenatal THg and Se exposure and Se-THg interaction.

Table 9
Multiple logistic regression (multiplicative and additive main effects models) of the effect of Se and THg (1:THg) exposure levels in cord blood on the dichotomized cognitive composite score. The table shows the odds ratios (ORs) and the 90% confidence intervals (90% CI) of Se and THg (1:THg) exposure levels.

Se exposure's levels[b]	Multiplicative model: ORs[a] (90% CI)			Additive model: ORs[a] (90% CI)		
	THg exposure's levels[c]			THg exposure's levels[c]		
	Low	Medium	High	Low	Medium	High
Low	**2.21 (1.21; 4.06)**	1.64 (0.67–4.00)	**2.55 (1.02–6.41)**	**1.41 (1.01–1.80)**	1.16 (0.77–1.54)	1.33 (0.80–1.87)
Medium	1	0.74 (0.41–1.34)	1.15 (0.65–2.05)	1	0.77 (0.53–1.01)	0.93 (0.66–1.20)
High	1.44 (0.77–2.68)	1.06 (0.45–2.49)	1.66 (0.73–3.77)	1.14 (0.87–1.42)	0.91 (0.60–1.22)	1.07 (0.65–1.50)
	N = 323; Log Likelihood = −157.37			N = 323; Log Likelihood = −157.07		

[a] The ORs (90% CI) were obtained setting opportunely (0 in the multiplicative model and 0.8 in the additive model) the opower link function in the following adjusted model: cognitive composite score ~ $\alpha + \beta_1$(Se) + β_2 (1:THg) + β_3 (home size) + β_4 (children fish intake up to 18 months).
[b] Se (ng/g): low (Se ≤ 105); medium (105 < Se ≤ 125); high (Se > 125).
[c] THg (ng/g): low (1:THg>0.33); medium (0.17 < 1:THg≤0.33); high (1:THg ≤ 0.17).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2020.113604.

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