



Copper and zinc status in cord blood and breast milk and child's neurodevelopment at 18 months: Results of the Italian PHIME cohort

Fabiano Barbiero^{a,*}, Valentina Rosolen^b, Dario Consonni^c, Marika Mariuz^b, Maria Parpinel^d, Luca Ronfani^e, Liza Vecchi Brumatti^e, Maura Bin^e, Luigi Castriotta^f, Francesca Valent^g, D'Anna Little^h, Janja Snoj Tratnikⁱ, Darja Mazejⁱ, Ingrid Falnogaⁱ, Milena Horvat^{i,j}, Fabio Barbone^k

^a UOC Unit of Occupational Medicine, Department of Medical Sciences, University of Trieste, Trieste, Italy

^b Central Directorate for Health, Social Policies and Disability, Friuli Venezia Giulia Region, Trieste, Italy

^c Epidemiology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

^d Department of Medicine (DMED), University of Udine, Udine, Italy

^e Institute for Maternal and Child Health, IRCCS 'Burlo Garofolo', Trieste, Italy

^f Institute of Hygiene and Evaluative Epidemiology, Friuli Centrale University Health Authority, Udine, Italy

^g Hygiene and Public Health, Friuli Centrale University Health Authority, Udine, Italy

^h Office of Chief Medical Officer, Western Friuli Health Authority, Pordenone, Italy

ⁱ Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia

^j Jožef Stefan International Postgraduate School, Ljubljana, Slovenia

^k Department of Medicine, Surgery and Health Sciences, University of Trieste, Trieste, Italy

ARTICLE INFO

Keywords:

Child neurodevelopment
Bayley scales of infant and toddler
Copper
Zinc
Trace element
Cohort study

ABSTRACT

Background: Trace elements, including zinc (Zn) and copper (Cu), although toxic at higher concentrations are known to play important roles in the maintenance of human health and neurodevelopment. Few epidemiological studies have investigated the association between prenatal or early postnatal Cu and Zn levels and child neurodevelopment. The aim of this research is to assess the association between child neurodevelopment at 18 months of age and cord blood and breast milk concentrations of Cu and Zn in Italian mother-child pairs enrolled in the Italian Northern Adriatic Cohort II (NAC-II), a part of the "Public health impact of long-term, low-level, mixed element exposure in susceptible population strata" project PHIME.

Methods: The study population consisted of 632 children, and their mothers, born within the NAC-II, who were tested with the Bayley Scales of Infant and Toddler Development third edition (BSID-III) at age 18 months. Cu and Zn concentrations were measured in cord blood and breast milk samples. Only children born at term (≥ 37 gestational week), who completed the BSID-III test and had at least 1 measure of Cu and Zn concentrations were included in the analysis. Information about socio-demographics and lifestyles were collected through questionnaires at different phases of follow-up. Cu and Zn concentrations were $\log_2$ transformed because of their skewed distribution. Multiple linear regression models were performed to study the association between each BSID-III composite score (cognitive, motor and language) and each metal concentration. Separate models were applied for each biological sample. The β coefficient (β) and its 95% confidence interval (95% CI) were estimated. Stratified analyses by child's sex were also conducted.

Results: The mean $\pm$ standard deviation (SD) of cognitive, motor and language composite scores were respectively: 106 ± 8 , 101 ± 5 and 97 ± 8 . The mean $\pm$ SD of Cu and Zn concentrations (ng/g) were respectively 699.2 ± 129.0 and 2538 ± 589 in cord blood and 607 ± 498 and 3226 ± 1428 in breast milk. No association between metal concentration and cognitive composite score was found. A higher motor composite score was associated with higher Cu concentrations in cord blood ($\beta = 4.31$ 95% CI 2.03; 6.59). No associations were found between language composite score and metal concentrations. The effect of Cu cord blood concentration on motor composite score was confirmed when stratified by sex: males ($\beta = 5.49$ 95% CI 2.15; 8.36) and females ($\beta = 3.11$; 95% CI 0.00; 6.22). A direct association, in females only, was found between language composite score and Cu

* Corresponding author.

E-mail address: fabiano.barbiero@units.it (F. Barbiero).

<https://doi.org/10.1016/j.ijheh.2024.114485>

Received 3 July 2024; Received in revised form 22 October 2024; Accepted 11 November 2024

Available online 16 November 2024

1438-4639/© 2025 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

concentration in cord blood ($\beta = 5.60$ 95% CI 0.63; 10.57) and in breast milk ($\beta = 3.04$ 95% CI 1.06; 5.03), respectively.

Conclusion: The results from this cohort study showed a strong direct association between prenatal Cu levels and child motor neurodevelopment at 18 months. However, for generalizability, future research on the effects of Zn and Cu on neurodevelopment should include a larger range of early-life concentration of trace elements.

1. Introduction

Trace elements, including zinc (Zn) and copper (Cu), although toxic at higher concentrations (Sharma and Agrawal, 2005; Wright and Baccarelli, 2007), are known to play important roles in the maintenance of human health and neurodevelopment, including synaptic transmission, myelination, and antioxidant defense mechanisms (Amorós et al., 2019). Diet is the main determinant of Cu and Zn status. However, due to anthropogenic activities, these elements can also be found in the environment, particularly in their inorganic forms (Amorós et al., 2019; Osredkar et al., 2022; Sharma and Agrawal, 2005). The maturation of the central nervous system requires nutrients, such as glucose and vitamins and minerals such as Cu, Zn, iron and selenium to maintain energy requirements for neurodevelopment (Georgieff et al., 2018; Krebs et al., 2017; Lockyer et al., 2021). Zn is involved in numerous aspects of cellular metabolism (Classen et al., 2011), in the nervous system and brain function and supports the process of neural differentiation (Adamo and Oteiza, 2010; Lockyer et al., 2021). The need for Zn is higher in stages of rapid growth such as infancy, adolescence and pregnancy (Grantham-McGregor and Ani, 1999). Zn deficiency may influence neurodevelopment by altering attention, neuropsychological behavior and motor development (Bhatnagar and Taneja, 2001). In many countries, such as Australia and the United States, Zn supplementation is recommended for pregnant women (Amorós et al., 2019; Osredkar et al., 2022) despite the fact that the benefits of Zn supplementation during pregnancy have not been proven, and strategies aimed at improving maternal nutrition in general could produce more tangible health benefits than the use of Zn supplements alone (Mousa et al., 2019). Although Zn toxicity is also possible, it is far more common to observe Zn deficiency and Cu toxicity (Harris, 2001). During pregnancy before reaching the fetus both Zn and Cu have to be transported from the maternal circulatory system, crossing two barriers: the placenta and the mammary gland (Osredkar et al., 2022; Rossipal et al., 2000). After passing the placenta, part of the copper, as well as zinc, accumulates in the fetal liver to ensure its availability in the first months of the baby's life (Amorós et al., 2019; Gambling et al., 2003). Although some studies found an inverse association between prenatal Cu exposure and child development (Liu et al., 2022), few epidemiological studies evaluated the association between prenatal or early postnatal Cu levels and child neuropsychological development (Amorós et al., 2019; Osredkar et al., 2022). Elevated Cu levels, especially when Zn levels were also low, were associated with some neurological conditions (Bjorklund, 2013; Skalny et al., 2020). On the other hand, a decreased Zn-to-Cu ratio in cord blood was associated with lower gestational age and lower birth weight suggesting an overall direct effects of Zn and negative effects of Cu on pregnancy outcomes (Osredkar et al., 2022).

The aim of the present research is to assess the association between child neurodevelopment at 18 months of age and the concentrations of Cu and Zn in cord blood and breastmilk in Italian mother-child pairs enrolled in the Northern Adriatic Cohort II (NAC-II). The Italian NAC-II is part of the Mediterranean cohort involved in the "Public health impact of long-term, low-level, mixed element exposure in susceptible population strata" (PHIME) project (Valent et al., 2013a).

2. Materials & methods

2.1. Study population

The study population consisted of the 632 children, and their mothers, born from April 2007 to April 2009 within the NAC-II cohort, who were tested by the Bayley Scales of Infant and Toddler Development third edition (BSID-III) (Bayley N, 2006) at age 18 ± 2 months. The study protocol with inclusion and exclusion criteria has been described in detail in Valent et al. (2013a). The study protocol was approved by the Ethics Committee of the University of Udine and the Institute for Maternal and Child Health IRCCS Burlo Garofolo, Trieste (CE/V-70-05/02/2007).

Informed consent was obtained from all participants' caregivers involved in the study. During follow-up, they were free to withdraw from participating in the study at any time upon request. The study was conducted according to the Declaration of Helsinki.

2.2. Questionnaires

At recruitment, eligible pregnant women between the 20th and 22nd week of gestation, filled in a short questionnaire to identify any excluding condition and to collect brief socio-demographic information about the family. At 20 and 32 gestational weeks, pregnant women enrolled in the study were tested with the Raven Progressive Matrices (Raven, J. et al., 1998), a non-verbal intelligence test that allowed evaluation of the level of general intelligence and through which it was possible to obtain the measure of the intelligence quotient (IQ) of the women examined. At 1 month after delivery, mothers filled in a detailed questionnaire to provide information on demographic, socioeconomic and health status, lifestyles and dietary habits, during pregnancy and/or at the time of delivery. At approximately 18 months after delivery, mothers completed a supplementary questionnaire to collect information on any changes in the demographic and socioeconomic status of the family, and children underwent the BSID-III testing to the neurodevelopment assessment.

2.3. Sample collection and exposure

A cord blood sample was collected a few minutes after delivery and before clamping the umbilical cord in Vacutainer Blue Cup NaH tubes, specific for metals determination and, at approximately 1 month after delivery a 24-h breast milk sample was collected in Sarsted polypropylene containers. Aliquots of cord blood and breast milk samples were stored in freezer at -80°C and then transported on dry ice to the Josef Stefan Institute (JSI) in Ljubljana for metals determination.

The analytical procedure of Cu and Zn determination in cord blood (whole blood samples) and breast milk samples has been described in Valent et al. (2013b) (Supplementary Materials) and Osredkar et al. (2022), respectively. A brief description of analytical methods is reported in Supplementary Materials.

2.4. Outcomes

The neurological and psychological development of the children was assessed through the BSID-III test, at 18 ± 2 months of age. The BSID-III test measured the major areas of development and consisted of 5 scales: (i) the cognitive scale; (ii) the language scale; (iii) the motor scale. The

cognitive, language and motor scale were administered to children by trained psychologists. Raw scores, the sum of the number of points earned for a subtest, were converted to scaled score. The scaled scores represented a child's performance on a subtest relative to his/her same age peer and were derived from the standardization of total raw score for child's age in days at test administration. For each composite score, the distribution of scaled score (or the sum of scaled score for language and motor scales) were used to derived corresponding percentiles which were converted to composite scores. Composite scores had an average of 100, a standard deviation of 15 and a range from 40 to 160. The higher was the composite score and the better the child's performance. The Italian validation of BSID-III test was described in [Ferri et al. \(2015\)](#).

In the NAC-II cohort, two trained psychologists administered the BSID-III test at age 18 months, each one assigning an independent score and serving as primary examiner for half of the sample and as observer for the other half. The sample consisted of 53 children consecutively enrolled in the Italian NAC-II cohort. The cognitive, language, and motor scales of the BSID-III test were assessed and the respective intraclass correlation coefficients (ICC) were calculated: the ICC was 0.98 (95% CI: 0.97–0.99) for the cognitive scale, 0.99 (95% CI: 0.99–1.00) for the language scale, and 0.93 (95% CI: 0.90–0.97) for the motor scale ([Deroma et al., 2013](#)).

The cognitive, language and motor composite scores, obtained by children in the BSID-III assessment, were considered in the analysis.

2.5. Covariates

To estimate the effect of Cu, Zn and Zn-to-Cu ratio on child neurodevelopment, the following covariates we considered, that, in accordance with existing literature, may act as confounders or effect modifiers ([Bhutta et al., 2002](#); [Cornelius and Day, 2009](#); [Hibbeln et al., 2007](#); [Oken et al., 2008](#); [Riley et al., 2011](#); [Rivera et al., 2015](#); [van Bakel and Riksen-Walraven, 2002](#)), of cognitive, motor and language outcomes: maternal age at delivery, maternal BMI before pregnancy, the number of cigarettes smoked during pregnancy by mother, the number of alcoholic drinks per week during pregnancy by mother, the maternal IQ, the number of months with at least one portion of fresh fish per week by child, the number of months with at least one portion of homogenized fish per week by child, the sex of child, the child's birth weight in grams and the socio-economic index of family (SES) adapted from Bennett and colleagues ([Bennett et al., 2007](#); [Ronfani et al., 2015](#)). Fish consumption is an important source of trace elements such as Zn and Cu ([Bilandzić et al., 2014](#); [ELLINGSEN et al., 2007](#); [Mai et al., 2024](#)) and it is also related to child neurodevelopment. Therefore, information on fresh fish intake and homogenized fish intake of child was obtained and considered in our analyses as potential confounding or intermediate covariates. The SES index was built taking into account the following parameters: parents' occupation (1 = yes vs 0 = no), type of employment (1 = white collar vs 0 = blue collar following the Hollingshead classification), educational level (0 = up to middle school, 0.5 = high school, 1 = university degree), homeownership (1 = yes). Finally, the scores were summed (for type of employment and educational level, the mean between parents was used).

2.6. Statistical analysis

Only children born at term (≥ 37 gestational week) who had at least one measure of Cu and Zn concentrations, and had completed the BSID-III test at 18 ± 2 months of age were included in the analysis, which is consistent with previous analyses of the Italian and Mediterranean cohort ([Barbone et al., 2019](#); [Castriotta et al., 2020](#); [Valent et al., 2013b](#)).

The distribution of the main characteristics of mother-child pairs was expressed for continuous data as mean, standard deviation (SD) and median, and for categorical data as absolute frequencies and percentages. For metal concentrations and its ratio (Zn-to-Cu) in cord blood and

breast milk, the 25th percentile and 75th percentile were also shown.

Simple and multiple linear regression models were performed to study the association between each composite score and each metal concentration. Separate models were applied for each biological sample. Among all the covariates, only those associated with the composite score ($p < 0.15$), were included in the final models, in a backward method. In final analysis Cu and Zn concentrations were $\log_2$ transformed because of their skewed distribution: for this reason, in linear regression models the β coefficient corresponded to the variation in BSID-III score associated with the doubling in the main predictor levels. β coefficient and 95% confidence interval (95% CI) were estimated. Stratified final analyses by sex were also conducted.

STATA (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LP) was used for the statistical analysis.

3. Results

In total, 900 pregnant women were enrolled in the cohort; 767 (85%) of these women remained in the study at delivery, and of these 632 children underwent BSID-III testing at age 18 months (82%). After excluding preterm births, data from 607 records were used in the final analyses. The socio-demographic characteristics of 607 children and their mothers are presented in [Table 1](#). In brief, the mean age of women at delivery was 33 years (SD = 4.3), the mean maternal BMI before pregnancy was 22.8 (SD = 3.8) and the mean maternal weight gain during pregnancy was 13.5 Kg. The majority of women (89.3%) declared to be married/living with partner and to be employed (84.0%). More than 48% of women had a high school degree and almost 35% of them had a university degree. The mean maternal IQ was 119 (SD = 11, median 125), the mean number of months with at least one portion of fresh fish per week by child was 9.1 (SD = 4.0, median 10.0). More than 58% of enrolled women were non-smokers (N 354) and more than 32% were former smoker (N 197) (data not shown): among smoker women, the mean number of cigarettes smoked during pregnancy was 144.2 (SD = 559.6). The mean alcoholic drinks per week consumed during pregnancy by mother was 1.6 (SD = 3.3; median 0.35) ([Table 1](#)). 50.4% of children were male (N 306) and 49.6% female; the mean birth weight of children was 3419.9 g (SD = 452.5, median 3400) and the mean number of months with at least one portion of homogenized fish per week eaten by children was 2.7 (SD = 4.5) ([Table 1](#)).

Total and sex-stratified distributions of BSID-III Composite Scores and metal concentrations, by type of biological sample, are presented in [Table 2](#). Breast milk and cord blood samples were collected from 80.7% (N = 490) and 75.7% (N = 460) of our cohort, respectively. The average concentration of Zn was more than 5 times higher than that of Cu in breast milk (mean Zn-to-Cu in breast milk = 5.9; median 5.7) ratio and more than 3 times in cord blood (mean Zn-to-Cu in cord blood = 3.7; median 3.6) ([Table 2](#)).

[Tables 3–5](#) show results of simple and multiple linear regressions between cognitive, motor and language composite scores and Cu, Zn and Zn-to-Cu ratio concentrations, respectively, by biological sample. A total of 18 models are shown, without stratification by sex. We found no association between Cu nor Zn cord blood concentration and the cognitive composite score; similar results were found when we tested the association between Zn to Cu ratio and the cognitive score ([Table 3](#)). The cognitive composite score was not associated with Zn and Cu concentrations in breast milk either. ([Table 3](#)). Conversely, a higher motor composite score at 18 months was associated with higher Cu concentrations in cord blood in both the univariate and adjusted model: the β coefficient was 4.75 (95% CI 2.46; 7.05) for univariate model and 4.31 (95% CI 2.03; 6.59) for the full model adjusted for alcohol consumption by mother during pregnancy, indicating that the motor composite score increases by more than 4 points when the Cu concentration in cord-blood doubles ([Table 4](#)). A suggestive inverse association was found between Zn-to-Cu ratio concentration in cord blood and BSID-III motor composite score with an adjusted β of -2.00 (95% CI -3.84 ; -0.16)

Table 1
Sociodemographic characteristics of 607 children and their mothers of the Italian PHIME Cohort.

Maternal age at delivery, years, mean ± SD (median)	33.3 ± 4.3 (33.0)
Maternal BMI before pregnancy, kg/m ² , mean ± SD (median)	22.8 ± 3.8 (22.2)
Weight gain during pregnancy, kg, mean ± SD (median)	13.5 ± 4.4 (13.0)
Marital status of mother at delivery, n (%)	
Married/living with partner	542 (89.3)
Separated/divorced	20 (3.3)
Single	38 (6.3)
Not reported	7 (1.2)
Maternal educational level, n (%)	
Elementary School	5 (0.8)
Middle School	95 (15.7)
High School	293 (48.3)
University Degree	212 (34.9)
Not Reported	2 (0.3)
Maternal occupational status, n (%)	
Employed	510 (84.0)
Housewife	47 (7.7)
Seeking work	27 (4.5)
Student	6 (1.0)
Stopped working	8 (1.3)
Other/missing	9 (1.5)
Cigarettes smoked during pregnancy by mother, mean ± SD (median)	144.2 ± 559.6 (0.0)
Alcohol drinks per Week during pregnancy, mean ± SD (median)	1.6 ± 3.3 (0.35)
Maternal IQ*, mean ± SD (median)	119 ± 11 (125)
Months with at least one portion of fresh fish per week by child, mean ± SD (median)	9.1 ± 4.0 (10.0)
Months with at least one portion of homogenized fish per week by child, mean ± SD (median)	2.7 ± 4.5 (0.0)
Socioeconomic index of family, mean ± SD (median)	2.7 ± 0.9 (2.8)
House ownership by family, N (%)	
Yes	470 (77.4)
No	130 (21.4)
Not reported	7 (1.2)
Home size, N (%)	
<50 m ²	41 (6.8)
50–100 m ²	406 (66.9)
>100 m ²	153 (25.2)
Not reported	7 (1.2)
Sex of child, N (%)	
Male	306 (50.4)
Female	301 (49.6)
Birth weight of child, grams, mean ± SD (median)	3419.9 ± 452.5 (3400)
Gestational age, weeks, N (%)	
37	32 (5.3)
38	93 (15.3)
39	164 (27.0)
40	167 (27.5)
41	121 (19.9)
42	30 (4.9)

Abbreviation: SD, standard deviation. BMI, body mass index. *Raven's Progressive Matrices score.

(Table 4). Our results did not show convincing associations between Cu, Zn or Zn/Cu and language composite score, neither when metal concentrations were measured in cord blood nor in maternal milk (Table 5). Selected results stratified by sex are displayed in Table 6. A positive association between Cu cord blood concentration and motor composite score was found in the stratified analyses by sex, with a β equal to 5.49 in males (95% CI 2.15; 8.36) and 3.11 in females (95% CI 0.00; 6.22). On the other hand, in our cohort, sex proved to be an effect modifier when the association between Zn in breast milk and cognitive composite score was investigated. In fact, our models showed a suggestive direct association in girls ($\beta = 1.30$ 95% CI -0.08; 2.68) (Table 6), but not in boys. Similarly, for the language composite score, we found a direct association, in girls only, with Cu concentration in cord blood ($\beta = 5.60$ 95% CI 0.63; 10.57) and breast milk ($\beta = 3.04$ 95% CI 1.06; 5.03) (Table 6).

Table 2
Distribution of BSID-III composite scores assessed at 18 months and Cu, Zn and Zn-to-Cu ratio concentrations in different biological samples (n = 607).

	N	25th p	Median	Mean	75th p	Mean	SD
BSID-III Composite Score:							
Cognitive Composite Score	604	100	105	106	110	106	8
Sex of Child = Male	306	100	105	105	110	105	8
Sex of Child = Female	298	100	105	107	115	107	8
Motor Composite Score	604	97	100	101	107	101	5
Sex of Child = Male	306	97	100	101	103	101	6
Sex of Child = Female	298	97	100	101	107	101	5
Language Composite Score	604	91	97	97	103	97	8
Sex of Child = Male	306	91	94	95	100	95	8
Sex of Child = Female	298	94	100	100	106	100	8
Metal concentration (ng/g) in breast milk:							
Zn	490	2111	3136	3226	4184	3226	1428
Sex of Child = Male	253	2112	3101	3201	4096	3202	1435
Sex of Child = Female	237	2109	3167	3252	4217	3252	1423
Cu	490	436	545	607	639	607	498
Sex of Child = Male	253	426	543	601	642	601	443
Sex of Child = Female	237	447	545	613	622	613	551
Zn-to-Cu ratio	490	4.2	5.7	5.9	7.5	5.9	2.4
Sex of Child = Male	253	4.3	5.6	5.9	7.7	5.9	2.5
Sex of Child = Female	237	4.2	5.7	5.9	7.4	5.9	2.3
Metal concentration (ng/g) in cord blood:							
Zn	460	2142	2465	2538	2800	2538	589
Sex of Child = Male	240	2134	2458	2514	2778	2514	566
Sex of Child = Female	220	2159	2470	2565	2822	2565	613
Cu	460	619	683	699	758	699	129
Sex of Child = Male	240	619	681	696	750	696	114
Sex of Child = Female	220	617	687	703	762	703	144
Zn-to-Cu ratio	460	3.2	3.6	3.7	4.0	3.7	0.8
Sex of Child = Male	240	3.2	3.5	3.6	4.0	3.6	0.7
Sex of Child = Female	220	3.2	3.6	3.7	4.0	3.7	0.9

4. Discussion

We analyzed the relationship between Zn, Cu, and their ratio, and child neurodevelopment at 18 months of life in an Italian birth cohort that was evaluated previously in relation with other chemical exposures (Barbone et al., 2019; Castriotta et al., 2020). The main results of this analysis, showed a strong direct association between Cu concentration in cord-blood and motor composite score, suggesting the essential role of Cu in early life as trace element necessary for child neurodevelopment. In our cohort we did not find any detrimental effect of Cu concentrations in umbilical cord blood nor in maternal milk and child neurodevelopment at 18 months of life. A recent Spanish birth cohort study observed a negative association between maternal Cu concentrations and some domains of child neuropsychological development assessed at

Table 3

Results of simple and multiple linear regression analysis between BSID-III Cognitive Composite Score and Cu, Zn and Zn/Cu concentrations in Cord Blood and Breast Milk.

Log ₂ Metal concentrations (ng/g):	BSID-III Cognitive Composite Score						
	N	β (95% CI)	p	N	Adjusted β (95% CI)	p	Interaction p ^c
In Cord Blood:							
Cu ^a	458	0.50 (−2.80; 3.81)	0.766	430	2.81 (−0.57; 6.19)	0.103	0.367
Zn ^a	458	0.37 (−2.25; 2.90)	0.771	430	0.82 (−1.77; 3.42)	0.532	0.119
Zn/Cu ^a	458	0.09 (−2.55; 2.73)	0.947	430	−0.86 (−3.52; 1.80)	0.526	0.396
In Breast Milk:							
Cu ^b	488	1.04 (−0.18; 2.27)	0.096	456	1.14 (−0.16; 2.43)	0.085	0.684
Zn ^b	488	0.54 (−0.44; 1.52)	0.279	456	0.54 (−0.46; 1.55)	0.284	0.150
Zn/Cu ^b	488	−0.14 (−1.17; 0.90)	0.796	456	−0.15 (−1.22; 0.92)	0.781	0.172

^a Full model adjusted for: maternal IQ, sex of child, the number of months with at least one portion of fresh fish per week by child, socioeconomic index of family.

^b Full model adjusted for: maternal IQ, sex of child, the number of months with at least one portion of fresh fish per week by child, maternal age at delivery, socioeconomic index of family.

^c p-value of the interaction effect between the metal concentration in each biological sample and sex of the child.

Table 4

Results of simple and multiple linear regression analysis between BSID-III Motor Composite Score and Cu, Zn and Zn/Cu concentrations in Cord Blood and Breast Milk.

Log ₂ Metal concentrations (ng/g):	BSID-III Motor Composite Score						
	N	β (95% CI)	p	N	Adjusted β (95% CI)	p	Interaction p ^d
In Cord Blood:							
Cu ^a	458	4.75 (2.46; 7.05)	0.000	457	4.30 (2.02; 6.58)	0.000	0.295
Zn ^a	458	1.06 (−0.72; 2.84)	0.242	457	1.02 (−0.75; 2.78)	0.258	0.894
Zn/Cu ^b	458	−1.68 (−3.53; 0.17)	0.074	457	−2.10 (−3.94; −0.26)	0.025	0.671
In Breast Milk:							
Cu ^c	488	−0.48 (−1.35; 0.40)	0.282	485	−0.60 (−1.46; 0.26)	0.171	0.230
Zn ^c	488	−0.14 (−0.84; 0.56)	0.696	485	−0.24 (−0.93; 0.45)	0.493	0.619
Zn/Cu ^c	488	0.19 (−0.55; 0.92)	0.622	485	0.16 (−0.57; 0.89)	0.667	0.120

^a Full model adjusted for: sex of child, alcohol drinks per week during pregnancy by mother.

^b Full model adjusted for: sex of child, birth weight of child, alcohol drinks per week during pregnancy by mother.

^c Full model adjusted for: sex of child, maternal IQ, birth weight of child, alcohol drinks per week during pregnancy by mother.

^d p-value of the interaction effect between the metal concentration in each biological sample and sex of the child.

Table 5

Results of simple and multiple linear regression analysis between BSID-III Language Composite Score and Cu, Zn and Zn/Cu concentrations in Cord Blood and Breast Milk.

Log ₂ Metal concentrations (ng/g):	BSID-III Language Composite Score						
	N	β (95% CI)	p	N	Adjusted β (95% CI)	p	Interaction p ^c
In Cord Blood							
Cu ^a	458	1.36 (−2.18; 4.90)	0.452	430	2.71 (−0.80; 6.22)	0.130	0.040
Zn ^a	458	1.54 (−1.17; 4.24)	0.272	430	1.40 (−1.29; 4.09)	0.307	0.099
Zn/Cu ^a	458	0.81 (−2.02; 3.63)	0.575	430	−0.20 (−2.96; 2.57)	0.888	0.996
In Breast Milk							
Cu ^a	488	1.19 (−0.10; 2.49)	0.070	463	1.17 (−0.14; 2.48)	0.080	0.016
Zn ^a	488	0.98 (−0.05; 2.00)	0.063	463	0.86 (−0.14; 1.88)	0.091	0.467
Zn/Cu ^b	488	0.24 (−0.85; 1.33)	0.664	463	0.12 (−0.96; 1.20)	0.824	0.314

^a Full model adjusted for: sex of child, the number of months with at least one portion of fresh fish per week by child, the number of months with at least one portion of homogenized fish per week by child, socioeconomic index of family.

^b Full model adjusted for: sex of child, the number of months with at least one portion of fresh fish per week by child, socioeconomic index of family.

^c p-value of the interaction effect between the metal concentration in each biological sample and sex of the child.

12 months and 5 years of age (Amorós et al., 2019). However, our cohort showed lower Cu cord blood concentrations (699 ± 129 ng/g) compared with the Spanish cohort (mean = 1606 ± 272 µg/L). This may suggest that, according to an inverted U-shaped curve, detrimental effects in early life neurodevelopment might occur above certain concentrations of Cu in umbilical cord blood and that below these concentrations the relationship between Cu concentration in the cord blood and neurodevelopment is linear and directly associated with a more favorable motor developmental score. As with other essential trace elements, excess Cu is neurotoxic. Although the mean Cu cord blood concentration in our population was lower than concentrations of Cu in plasma or reported by several studies (Izquierdo Alvarez et al., 2007; Polanska et al., 2017; S.M. Awadallah et al., 2004; Zhang et al., 2013), Cu levels in

the umbilical cord blood from another recent study were very close to ours, with a mean concentration of 749.85 µg/L (SD = 203.86) (Grzesik-Gąsior et al., 2023). To date no consensus on Cu ranges for pregnancy has been defined. The role of prenatal or early postnatal Cu concentration on child neuropsychological development is scarce and the results are often controversial (Forns et al., 2014).

On the other hand, further analyses on outcomes other than the motor composite scores, were null findings. In fact, using a multiple linear regression model, we found that prenatal availability of Zn was not associated with cognitive composite score, and only a tendency of association, with language composite score at 18 months ($p = 0.091$). In our cohort the mean Zn cord blood concentration was 2538 ± 589 ng/g, slightly higher than that shown by the recent Slovenian study population

Table 6

Selected results of simple and multiple linear regression analysis between BSID-III Composite Scores and Cu, Zn and Zn/Cu concentrations in Cord Blood and Breast Milk, by sex.

Log ₂ Metal concentrations (ng/g):	N	β (95% CI)	p	N	Adjusted β (95% CI)	p
BSID-III Composite Cognitive Score						
In Breast Milk						
Zn, Sex of Child = Male ^a	253	−0.04 (−1.46; 1.38)	0.952	234	−0.24 (−1.69; 1.22)	0.750
Zn, Sex of Child = Female ^a	235	1.12 (−0.20; 2.45)	0.097	222	1.30 (−0.08; 2.68)	0.065
BSID-III Composite Motor Score						
In Cord Blood:						
Cu, Sex of Child = Male ^b	240	5.77 (2.40; 9.14)	0.001	240	5.49 (2.15; 8.36)	0.001
Cu, Sex of Child = Female ^b	218	3.15 (0.05; 6.25)	0.047	217	3.11 (0.00; 6.22)	0.050
Zn/Cu, Sex of Child = Male ^c	240	−2.17 (−4.91; 0.58)	0.121	240	−2.59 (−5.27; 0.08)	0.058
Zn/Cu, Sex of Child = Female ^c	218	−1.30 (−3.79; 1.18)	0.303	216	−1.62 (−4.16; 0.92)	0.209
BSID-III Composite Language Score						
In Cord Blood:						
Cu, Sex of Child = Male ^d	240	−2.83 (−7.62; 1.96)	0.245	225	−0.38 (−5.35; 4.59)	0.881
Cu, Sex of Child = Female ^d	218	5.36 (0.64; 10.08)	0.026	205	5.60 (0.63; 10.57)	0.027
In Breast Milk						
Cu, Sex of Child = Male ^d	253	0.27 (−1.48; 2.01)	0.764	239	−0.30 (−2.04; 1.45)	0.737
Cu, Sex of Child = Female ^d	235	2.08 (0.32; 3.83)	0.020	224	3.04 (1.06; 5.03)	0.003

^a Full model adjusted for: maternal IQ, the number of months with at least one portion of fresh fish per week by child, maternal age at delivery, socioeconomic index of family.

^b Full model adjusted for: alcohol drinks per week during pregnancy by mother.

^c Full model adjusted for: birth weight of child, alcohol drinks per Week during pregnancy by mother.

^d Full model adjusted for: the number of months with at least one portion of fresh fish per week by child, the number of Months with at least one portion of homogenized fish per week by child, socioeconomic index of family.

of mothers and their neonates recruited within the PHIME prospective cohort study (mean = 1910 ± 62.0 µg/L) (Osredkar et al., 2022). However, those concentrations were consistent with the ones showed by Grzesik-Gąsior et al. (2023) in a population of mothers and newborns in Poland (Zn cord blood mean 2025.24 ± 717.83). Assessing the contribution of Zn and Cu maternal milk concentration on child neurodevelopment may also be of global interest considering that the range of Zn and Cu maternal milk concentration may also vary widely by country. In brief, Osredkar et al. (2022) from Slovenia showed distributions that were very consistent with our results. In addition, several studies suggested that micronutrients as Zn and Cu also vary in breast milk during different stages of breastfeeding, with concentrations that decreased significantly at successive stages of lactation (Feeley et al., 1983; Maeda et al., 1990; Wasowicz et al., 2001). The same trend of decreasing of both Zn and Cu serum concentration has been seen in postpartum mothers, in different stages after delivery (Maeda et al., 1990). Our results showed that Zn and Cu concentrations in breastmilk

were consistent with the results obtained by Maeda et al. (1990) at the closest stages after delivery. Regarding the effect modification shown by sex stratified analyses, this could be explained with sex-based differences in biology, neurodevelopment, and vulnerability to environmental toxins that can lead to different exposure effects between boys and girls. In fact, absorption and metabolism of trace metals can differ between boys and girls due to hormonal and physiological variations, as well as toxicodynamic. Furthermore, it is known that boys and girls exhibit different patterns of brain development, with certain regions of the brain maturing at different rates depending on sex. Finally, several studies have shown that sex-specific susceptibility to environmental pollutants, including metals, is common in children and sex hormones, such as estrogen and testosterone, influence both neurodevelopment and the body's response to toxic substances (Goodman et al., 2023; Rechtman et al., 2020; Silveyra et al., 2021).

Previous studies conducted in our cohort showed that total mercury (THg) and selenium (Se) concentrations in biological samples from mothers and children were consistently low, and in this scenario of relatively low exposures, no association between THg and adverse effects on children cognitive development emerged (Castriotta et al., 2020). Overall, at low levels of THg exposure (THg in cord blood quintiles: 1st = 2.1 ng/g, 2nd = 3.4 ng/g, 3rd = 5.2 ng/g, 4th = 7.9 ng/g), the Italian component of the PHIME cohort, which has been included in this study, showed some limited evidence of detrimental effects at 18 months on developmental motor scores, however none on cognitive and language outcomes (Barbone et al., 2019). In a recent Slovenian study population of mothers and their neonates recruited within the PHIME prospective cohort study, Cu and Zn levels in cord blood and maternal milk and their association with gestational age, birth weight and maternal smoking has been investigated; however no investigations were conducted about the role of those metals and child neurodevelopment (Osredkar et al., 2022).

Our study has several limitations. This article focuses on a neurodevelopment assessment conducted at 18 months of age. Therefore, the full effects of prenatal micronutrients exposure may not be fully measurable yet. Furthermore, according to the overview paper by Puchkova et al. (2018), measuring Cu concentrations in 24-h breast milk samples without monitoring the corresponding daily volume of breast milk consumed by each baby may only provide approximate associations between child characteristics and Cu levels in breast milk. The volume of breast milk consumption compensated for differences in breast milk Cu (Puchkova et al., 2018). In addition, the relationship between Cu and neurodevelopment was only observed at a single time point (18 months). This limitation might affect the perceived impact of Cu on neurodevelopment or its apparent lack of relationship with Zn. On the other hand, although several covariates were considered to adjust beta coefficients in our final analysis, neurodevelopment is a multifactorial process and might be influenced by variables that are still not known or not measured, such as genetic susceptibility (Stiles and Jer-nigan, 2010). Additionally, it cannot be excluded that covariates used in final analyses could be misreported by mothers on the questionnaires (information bias), leading to residual confounding on the effect estimations. Also, the study was not originally powered to detect the possible effects of Zn and Cu on neurodevelopment; thus, we cannot exclude that our failure to find significant associations on outcomes other than the motor composite scores is due to chance. In our study, we only partially considered post-natal dietary intake (fish intake during the first 18 months of age of child), but we did not consider information on mother or child supplement intake: this may lead to a partially biased result. Lastly, with specific reference to metal concentrations in breast milk, single measurements were performed in our cohort, and were used in this study to evaluate the impact on child neurodevelopment. Measuring metals exposure at multiple time points can reduce exposure misclassification and increase the accuracy of early-life metals exposure estimates.

This study also includes several strengths. We considered three

different neurodevelopmental assessments at 18 age months: this allowed us to observe how prenatal exposure to the investigated micronutrients varied in the different components of neurodevelopment. Another strength is that the measurement of micronutrients in this study was assessed in different biological samples: this permitted us to better evaluate the effect of the micronutrients considered on neurodevelopment. In this study we also accounted for several covariates and assessed neurocognitive outcomes using validated instruments, administered by 2 trained psychologists that showed high levels of agreement. In addition, cord blood and breast milk metal concentrations are ideal sample matrices to assess prenatal and early-life exposure to these micronutrients, respectively, as they better represent actual exposure after having crossed the placenta and mammary glands.

5. Conclusions

Our cohort showed a strong direct association between prenatal availability of Cu and motor neurodevelopment at 18 months, suggesting the essential role of Cu in early life as a micronutrient necessary for the child's neurological development. However, as well as for other trace elements, only further studies fully adjusted for confounders and modifiers, that include observations at wide range of concentration, may provide conclusive evidence on the effects of Zn and Cu in neurodevelopment.

CRedit authorship contribution statement

Fabiano Barbiero: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Valentina Rosolen:** Writing – review & editing, Writing – original draft, Visualization, Data curation. **Dario Consonni:** Writing – review & editing, Writing – original draft. **Marika Mariuz:** Writing – review & editing, Investigation. **Maria Parpinel:** Writing – review & editing. **Luca Ronfani:** Writing – review & editing, Resources, Methodology. **Liza Vecchi Brumatti:** Writing – review & editing, Resources, Investigation. **Maura Bin:** Writing – review & editing, Investigation. **Luigi Castriotta:** Writing – review & editing. **Francesca Valent:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **D'Anna Little:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Janja Snoj Tratnik:** Writing – review & editing. **Darja Mazej:** Writing – review & editing. **Ingrid Falnoga:** Writing – review & editing. **Milena Horvat:** Writing – review & editing, Resources, Methodology, Investigation, Funding acquisition, Formal analysis. **Fabio Barbone:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Funding

This work was supported by the European Union through its Sixth Framework Programme for RTD (contract “PHIME” No. FOOD-CT-2006-016253). The present article reflects only the views of the authors. The European Union is not liable for any use of the information contained herein.

Declaration of competing interest

The authors declare no conflict of interest

Acknowledgements

The authors are grateful to the Slovenian funding agency ARIS (programme P1-0143)

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijheh.2024.114485>.

References

- Adamo, A.M., Oteiza, P.I., 2010. Zinc deficiency and neurodevelopment: the case of neurons. *Biofactors* 36, 117–124. <https://doi.org/10.1002/biof.91>.
- Amorós, R., Murcia, M., González, L., Soler-Blasco, R., Rebagliato, M., Iniguez, C., Carrasco, P., Vioque, J., Broberg, K., Levi, M., Lopez-Espinosa, M.-J., Ballester, F., Llop, S., 2019. Maternal copper status and neuropsychological development in infants and preschool children. *Int. J. Hyg Environ. Health* 222, 503–512. <https://doi.org/10.1016/j.ijheh.2019.01.007>.
- Awadallah, S.M., Abu-Elteen, K.H., Elkarmi, A.Z., Qaraein, S.H., Salem, N.M., Mubarak, M.S., 2004. Maternal and cord blood serum levels of zinc, copper, and iron in healthy pregnant Jordanian women. <https://doi.org/10.1002/jtra.10032>.
- Barbone, F., Rosolen, V., Mariuz, M., Parpinel, M., Casetta, A., Sammartano, F., Ronfani, L., Vecchi Brumatti, L., Bin, M., Castriotta, L., Valent, F., Little, D.L., Mazej, D., Snoj Tratnik, J., Miklavčič Višnjevec, A., Sofianou, K., Špirić, Z., Kršnik, M., Osredkar, J., Neubauer, D., Kodrić, J., Stropnik, S., Prpić, I., Petrović, O., Vlaisić-Cicvarić, I., Horvat, M., 2019. Prenatal mercury exposure and child neurodevelopment outcomes at 18 months: results from the Mediterranean PHIME cohort. *Int. J. Hyg Environ. Health* 222, 9–21. <https://doi.org/10.1016/j.ijheh.2018.07.011>.
- Bayley, N., 2006. *Bayley Scales of Infant and Toddler Development*, third ed. Harcourt Assessment, Inc, San Antonio, TX.
- Bennett, G.G., Wolin, K.Y., James, S.A., 2007. Lifecourse socioeconomic position and weight change among blacks: the Pitt County study. *Obesity* 15, 172–181. <https://doi.org/10.1038/oby.2007.522>.
- Bhatnagar, S., Taneja, S., 2001. Zinc and cognitive development. *Br. J. Nutr.* 85 (Suppl. 2), S139–S145. <https://doi.org/10.1079/bjn2000306>.
- Bhutta, A.T., Cleves, M.A., Casey, P.H., Cradock, M.M., Anand, K.J.S., 2002. Cognitive and behavioral outcomes of school-aged children who were born preterm: a meta-analysis. *JAMA* 288, 728–737. <https://doi.org/10.1001/jama.288.6.728>.
- Bilandžić, N., Sedak, M., Đokić, M., Varenina, I., Kolanović, B.S., Božić, D., Brstilo, M., Šimić, B., 2014. Determination of zinc concentrations in foods of animal origin, fish and shellfish from Croatia and assessment of their contribution to dietary intake. *J. Food Compos. Anal.* 35, 61–66. <https://doi.org/10.1016/j.jfca.2014.04.006>.
- Bjorklund, G., 2013. The role of zinc and copper in autism spectrum disorders. *Acta Neurobiol. Exp.* 73, 225–236. <https://doi.org/10.55782/ane-2013-1932>.
- Castriotta, L., Rosolen, V., Biggeri, A., Ronfani, L., Catelan, D., Mariuz, M., Bin, M., Brumatti, L.V., Horvat, M., Barbone, F., 2020. 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. *Int. J. Hyg Environ. Health* 230, 113604. <https://doi.org/10.1016/j.ijheh.2020.113604>.
- Classen, H.-G., Gröber, U., Löw, D., Schmidt, J., Stracke, H., 2011. [Zinc deficiency. Symptoms, causes, diagnosis and therapy]. *Med. Monatsschr. Pharm.* 34, 87–95.
- Cornelius, M.D., Day, N.L., 2009. Developmental consequences of prenatal tobacco exposure. *Curr. Opin. Neurol.* 22, 121–125. <https://doi.org/10.1097/WCO.0b013e328326f6dc>.
- Deroma, L., Bin, M., Tognin, V., Rosolen, V., Valent, F., Barbone, F., Carrozzi, M., 2013. [Interrater reliability of the Bayley III test in the Italian northern-adriatic cohort II]. *Epidemiol. Prev.* 37, 297–302.
- Ellingsen, D.G., Horn, N., Aaseth, J., 2007. Chapter 26 - copper. In: Nordberg, G.F., Fowler, B.A., Nordberg, M., Friberg, L.T. (Eds.), *Handbook on the Toxicology of Metals*, third ed. Academic Press, Burlington, pp. 529–546. <https://doi.org/10.1016/B978-012369413-3/50081-1>.
- Feeley, R.M., Eitenmiller, R.R., Jones, J.B.J., Barnhart, H., 1983. Copper, iron, and zinc contents of human milk at early stages of lactation. *Am. J. Clin. Nutr.* 37, 443–448. <https://doi.org/10.1093/ajcn/37.3.443>.
- Ferri, R., Orsini, A., Rea, M., Stoppa, E., Mascellani, F., 2015. *Bayley: Scales of Infant and Toddler Development-Terza Edizione. Contributo alla taratura italiana*.
- Forns, J., Fort, M., Casas, M., Cáceres, A., Guxens, M., Gascon, M., Garcia-Esteban, R., Julvez, J., Grimalt, J.O., Sunyer, J., 2014. Exposure to metals during pregnancy and neuropsychological development at the age of 4 years. *Neurotoxicology* 40, 16–22. <https://doi.org/10.1016/j.neuro.2013.10.006>.
- Gambling, L., Danzeisen, R., Fosset, C., Andersen, H.S., Dunford, S., Srai, S.K.S., Mcardle, H.J., 2003. Iron and copper interactions in development and the effect on pregnancy outcome. *J. Nutr.* 133, 1554S. <https://doi.org/10.1093/jn/133.5.1554S>.
- Georgieff, M.K., Ramel, S.E., Cusick, S.E., 2018. Nutritional influences on brain development. *Acta Paediatr.* 107, 1310–1321. <https://doi.org/10.1111/apa.14287>.
- Goodman, C.V., Green, R., DaCosta, A., Flora, D., Lanphear, B., Till, C., 2023. Sex difference of pre- and post-natal exposure to six developmental neurotoxins on intellectual abilities: a systematic review and meta-analysis of human studies. *Environ. Health* 22, 80. <https://doi.org/10.1186/s12940-023-01029-z>.
- Grantham-McGregor, S.M., Ani, C.C., 1999. The role of micronutrients in psychomotor and cognitive development. *Br. Med. Bull.* 55, 511–527. <https://doi.org/10.1258/0007142991902583>.
- Grzesik-Gąsior, J., Sawicki, J., Pieczykowski, A., Bień, A., 2023. Content of selected heavy metals in the umbilical cord blood and anthropometric data of mothers and newborns in Poland: preliminary data. *Sci. Rep.* 13, 14077. <https://doi.org/10.1038/s41598-023-41249-4>.

- Harris, E.D., 2001. Copper homeostasis: the role of cellular transporters. *Nutr. Rev.* 59, 281–285. <https://doi.org/10.1111/j.1753-4887.2001.tb07017.x>.
- Hibbeln, J.R., Davis, J.M., Steer, C., Emmett, P., Rogers, I., Williams, C., Golding, J., 2007. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. *Lancet* 369, 578–585. [https://doi.org/10.1016/S0140-6736\(07\)60277-3](https://doi.org/10.1016/S0140-6736(07)60277-3).
- Izquierdo Alvarez, S., Castañón, S.G., Ruata, M.L.C., Aragüés, E.F., Terraz, P.B., Irazabal, Y.G., González, E.G., Rodríguez, B.G., 2007. Updating of normal levels of copper, zinc and selenium in serum of pregnant women. *J. Trace Elem. Med. Biol.* 21 (Suppl. 1), 49–52. <https://doi.org/10.1016/j.jtemb.2007.09.023>.
- Krebs, N.F., Lozoff, B., Georgieff, M.K., 2017. Neurodevelopment: the impact of nutrition and inflammation during infancy in low-resource settings. *Pediatrics* 139, S50–S58. <https://doi.org/10.1542/peds.2016-2828G>.
- Liu, C., Huang, L., Huang, S., Wei, L., Cao, D., Zan, G., Tan, Y., Wang, S., Yang, M., Tian, L., Tang, W., He, C., Shen, C., Luo, B., Zhu, M., Liang, T., Pang, B., Li, M., Mo, Z., Yang, X., 2022. Association of both prenatal and early childhood multiple metals exposure with neurodevelopment in infant: a prospective cohort study. *Environ. Res.* 205, 112450. <https://doi.org/10.1016/j.envres.2021.112450>.
- Lockyer, F., McCann, S., Moore, S.E., 2021. Breast milk micronutrients and infant neurodevelopmental outcomes: a systematic review. *Nutrients* 13. <https://doi.org/10.3390/nu13113848>.
- Maeda, T., Tanaka, T., Ohshiro, H., Funakawa, K., Nose, T., Imai, S., Hayashi, Y., 1990. [Zinc and copper concentrations in breast milk and maternal serum in the postpartum period]. *Nihon Eiseigaku Zasshi* 45, 781–787. <https://doi.org/10.1265/jjh.45.781>.
- Mai, W., Wang, F., He, S., Wen, Y., Yu, G., Zhang, L., Dong, H., 2024. Zinc contents in foods and estimates of dietary intakes in Guangzhou, Guangdong Province, China. *Front. Nutr.* 11, 1364033. <https://doi.org/10.3389/fnut.2024.1364033>.
- Mousa, A., Naqash, A., Lim, S., 2019. Macronutrient and micronutrient intake during pregnancy: an overview of recent evidence. *Nutrients* 11. <https://doi.org/10.3390/nu11020443>.
- Oken, E., Radesky, J.S., Wright, R.O., Bellinger, D.C., Amarasiwardena, C.J., Kleinman, K.P., Hu, H., Gillman, M.W., 2008. Maternal fish intake during pregnancy, blood mercury levels, and child cognition at age 3 years in a US cohort. *Am. J. Epidemiol.* 167, 1171–1181. <https://doi.org/10.1093/aje/kwn034>.
- Osredkar, J., Gersak, Ž.M., Karas Kuzelić, N., Snoj Tratnik, J., Mazej, D., Falnoga, I., Horvat, M., Gersak, K., 2022. Association of Zn and Cu levels in cord blood and maternal milk with pregnancy outcomes among the slovenian population. *Nutrients* 14. <https://doi.org/10.3390/nu14214667>.
- Polanska, K., Hanke, W., Krol, A., Gromadzinska, J., Kuras, R., Janasik, B., Wasowicz, W., Mirabella, F., Chiarotti, F., Calamandrei, G., 2017. Micronutrients during pregnancy and child psychomotor development: opposite effects of Zinc and Selenium. *Environ. Res.* 158, 583–589. <https://doi.org/10.1016/j.envres.2017.06.037>.
- Puchkova, L.V., Babich, P.S., Zatulovskaia, Y.A., Ilyechova, E.Y., Di Sole, F., 2018. Copper metabolism of newborns is adapted to milk ceruloplasmin as a nutritive source of copper: overview of the current data. *Nutrients* 10. <https://doi.org/10.3390/nu10111591>.
- Raven, J., Raven, J.C., Court, J.H., 1998. *Raven Manual: Section 4, Advanced Progressive Matrices, 1998 Edition*. Oxford Psychologists Press Ltd, Oxford, UK.
- Rechtman, E., Curtin, P., Papazaharias, D.M., Renzetti, S., Cagna, G., Peli, M., Levin-Schwartz, Y., Placidi, D., Smith, D.R., Lucchini, R.G., Wright, R.O., Horton, M.K., 2020. Sex-specific associations between co-exposure to multiple metals and visuospatial learning in early adolescence. *Transl. Psychiatry* 10, 358. <https://doi.org/10.1038/s41398-020-01041-8>.
- Riley, E.P., Infante, M.A., Warren, K.R., 2011. Fetal alcohol spectrum disorders: an overview. *Neuropsychol. Rev.* 21, 73–80. <https://doi.org/10.1007/s11065-011-9166-x>.
- Rivera, H.M., Christiansen, K.J., Sullivan, E.L., 2015. The role of maternal obesity in the risk of neuropsychiatric disorders. *Front. Neurosci.* 9, 194. <https://doi.org/10.3389/fnins.2015.00194>.
- Ronfani, L., Vecchi Brumatti, L., Mariuz, M., Tognin, V., Bin, M., Ferluga, V., Knowles, A., Montico, M., Barbone, F., 2015. The complex interaction between home environment, socioeconomic status, maternal IQ and early child neurocognitive development: a multivariate analysis of data collected in a newborn cohort study. *PLoS One* 10, e0127052. <https://doi.org/10.1371/journal.pone.0127052>.
- Rossipal, E., Krachler, M., Li, F., Micetic-Turk, D., 2000. Investigation of the transport of trace elements across barriers in humans: studies of placental and mammary transfer. *Acta Paediatr.* 89, 1190–1195. <https://doi.org/10.1080/080352500750027556>.
- Sharma, R.K., Agrawal, M., 2005. Biological effects of heavy metals: an overview. *J. Environ. Biol.* 26, 301–313.
- Silveyra, P., Al Housseiny, H., Rebuli, M.E., 2021. Sex and gender differences in the susceptibility to environmental exposures. In: Silveyra, P., Tigno, X.T. (Eds.), *Sex-Based Differences in Lung Physiology*. Springer International Publishing, Cham, pp. 251–290. https://doi.org/10.1007/978-3-030-63549-7_9.
- Skalny, A.V., Mazaletskaia, A.L., Ajsuvakova, O.P., Bjørklund, G., Skalnaya, M.G., Chao, J.C.-J., Chernova, L.N., Shakieva, R.A., Kopylov, P.Y., Skalny, A.A., Tinkov, A. A., 2020. Serum zinc, copper, zinc-to-copper ratio, and other essential elements and minerals in children with attention deficit/hyperactivity disorder (ADHD). *J. Trace Elem. Med. Biol.* 58, 126445. <https://doi.org/10.1016/j.jtemb.2019.126445>.
- Stiles, J., Jernigan, T.L., 2010. The basics of brain development. *Neuropsychol. Rev.* 20, 327–348. <https://doi.org/10.1007/s11065-010-9148-4>.
- Valent, F., Horvat, M., Sofianou-Katsoulis, A., Spiric, Z., Mazej, D., Little, D., Prasouli, A., Mariuz, M., Tamburlini, G., Nakou, S., Barbone, F., 2013a. Neurodevelopmental effects of low-level prenatal mercury exposure from maternal fish consumption in a Mediterranean cohort: study rationale and design. *J. Epidemiol.* 23, 146–152. <https://doi.org/10.2188/jea.je20120030>.
- Valent, F., Mariuz, M., Bin, M., Little, D., Mazej, D., Tognin, V., Tratnik, J., McAfee, A.J., Mulhern, M.S., Parpinel, M., Carozzi, M., Horvat, M., Tamburlini, G., Barbone, F., 2013b. Associations of prenatal mercury exposure from maternal fish consumption and polyunsaturated fatty acids with child neurodevelopment: a prospective cohort study in Italy. *J. Epidemiol.* 23, 360–370. <https://doi.org/10.2188/jea.je20120168>.
- van Bakel, H.J.A., Riksen-Walraven, J.M., 2002. Parenting and development of one-year-olds: links with parental, contextual, and child characteristics. *Child Dev.* 73, 256–273. <https://doi.org/10.1111/1467-8624.00404>.
- Wasowicz, W., Gromadzinska, J., Szram, K., Rydzynski, K., Cieslak, J., Pietrzak, Z., 2001. Selenium, zinc, and copper concentrations in the blood and milk of lactating women. *Biol. Trace Elem. Res.* 79, 221–233. <https://doi.org/10.1385/BTER:79:3:221>.
- Wright, R.O., Baccarelli, A., 2007. Metals and neurotoxicology. *J. Nutr.* 137, 2809–2813. <https://doi.org/10.1093/jn/137.12.2809>.
- Zhang, Z., Yuan, E., Liu, J., Lou, X., Jia, L., Li, X., Zhang, L., 2013. Gestational age-specific reference intervals for blood copper, zinc, calcium, magnesium, iron, lead, and cadmium during normal pregnancy. *Clin. Biochem.* 46, 777–780. <https://doi.org/10.1016/j.clinbiochem.2013.03.004>.