THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Prenatal Se concentrations and anthropometry at birth in the INMA study

Citation for published version:

Lozano, M, Murcia, M, Soler-Blasco, R, Iñiguez, C, Irizar, A, Lertxundi, A, Basterrechea, M, Marina, LS, Amoros Salvador, R, Broberg, K, Ballester, F & Llop, S 2020, 'Prenatal Se concentrations and anthropometry at birth in the INMA study', *Environmental Research*, vol. 181.
<https://doi.org/10.1016/j.envres.2019.108943>

Digital Object Identifier (DOI):

[10.1016/j.envres.2019.108943](https://doi.org/10.1016/j.envres.2019.108943)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Environmental Research

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

1Prenatal Se concentrations and anthropometry at birth in the INMA study

2(Spain)

3Manuel Lozano^{1,8}, Mario Murcia^{2,3}, Raquel Soler-Blasco², Carmen Iñiguez^{3,9}, Amaia Irizar⁵, Aitana
4Lertxundi^{3,4,5}, Mikel Basterrechea^{3,4,5}, Loreto Santa Marina^{3,4,5}, Rubén Amorós⁶, Karin Broberg⁷, Ferran
5Ballester^{2,3,10}, Sabrina Llop^{*2,3}

6¹ Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health

7² Epidemiology and Environmental Health Joint Research Unit, FISABIO–Universitat Jaume I–Universitat de València,
8Valencia, Spain

9³ Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Madrid, Spain

10⁴ Preventive Medicine and Public Health Department. University of Basque Country, UPV/EHU, Leioa, Bizkaia, Spain

11⁵ Health Research Institute, BIODONOSTIA, San Sebastian, Gipuzkoa, Spain.

12⁶ School of Mathematics, Peter Guthrie Tait Road, University of Edinburgh, EH9 3FD Edinburgh, United Kingdom

13⁷ Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

14⁸ Preventive Medicine and Public Health, Food Sciences, Toxicology and Forensic Medicine Department. Universitat de
15València, València, Spain

16⁹ Department of Statistics and Computational Research. Universitat de València, València, Spain

17¹⁰ Nursing Department, Universitat de València, València, Spain

18

19Corresponding author

20*Sabrina Llop. Foundation for the Promotion of Health and Biomedical Research in the Valencian
21Region (FISABIO), Av. Catalunya 21, 46020 Valencia, Spain. E-mail address: llop_sab@gva.es

22Abstract

23We assessed whether prenatal selenium (Se) exposure is associated with anthropometry at
24birth, placental weight and gestational age. Study subjects were 1249 mother-child pairs from
25the Valencia and Gipuzkoa cohorts of the Spanish Childhood and Environment Project (INMA,
262003–2008). Se was determined in serum samples taken at the first trimester of pregnancy.
27Socio-demographic and dietary characteristics were also collected by questionnaires. Mean (SD)
28serum Se concentration was 79.57 (9.64) $\mu\text{g/L}$. Se showed weak associations with both head
29circumference and gestational age. The association between serum Se concentration and birth
30weight and length was negative, and direct for placental weight and probability of preterm birth,
31although the coefficients did not reach statistical significance. Individuals with total mercury
32(THg) levels $>15 \mu\text{g/L}$ reversed the serum Se concentration effect on head circumference.
33Significant interactions were found between sex and both gestational age and prematurity.
34Spontaneous birth gestational ages were estimated to be lower for males and their probability
35of prematurity was higher. In conclusion, prenatal Se exposure may be associated with lower
36head circumference and lower gestational ages at spontaneous birth. Interactions with THg
37exposure and gender should be considered when assessing these relationships.

38

39Keywords

40Selenium, prenatal exposure, birth anthropometry, gestational age, preterm birth

41Funding

42The authors declare no competing financial interest.

43This study was supported by grants from Instituto de Salud Carlos III [FIS-FEDER: 13/1944,
4416/1288, 17/00663 and 19/1338; Miguel Servet-FEDER: CP15/0025; Miguel Servet-FSE:
45MS15/0025], Department of Health of the Basque Government (2005111093), the Karolinska
46Institutet, Provincial Government of Gipuzkoa (DFG06/002), and annual agreements with the
47municipalities of the study area (Zumarraga, Urretxu, Legazpi, Azkoitia, Azpeitia and Beasain).

481 Introduction

49Selenium (Se) is an essential trace mineral of fundamental importance to human health, since it
50is involved in antioxidant processes and has therapeutic aspects due to its chemo-preventive,
51anti-inflammatory and antiviral properties (Rayman, 2012).

52Se is present in the soil and enters the food chain via plants in its inorganic form (Peters et al.,
532016). On a global scale, Se availability in the soil varies between areas. Low Se content is
54observed in volcanic regions and in regions with an acidic soil. Consequently, Se deficiency-
55linked disorders in humans (such as gastrointestinal and prostate cancer, cardiovascular disease,
56diabetes, or compromised fertility) have been documented in such areas, and more so if the
57food is produced mainly locally (Papp et al., 2007). In plants, Se is converted into organic forms
58such as selenomethionine (SeMet) and selenocysteine (Sec). SeMet is the major
59selenocompound in cereal grains and legumes, and it serves as a major precursor for Sec
60synthesis in animals (Peters et al., 2016). Sec is the most active biological form of Se and
61participates in the synthesis of selenoproteins, such as glutathione peroxidase (GPx),
62selenoprotein P (SeIP) and thioredoxin reductase (Trx), which play an important role in
63preventing damage from oxidative stress (Whanger, 2002).

64During a normal pregnancy, maternal oxidative stress rises and there is a systemic inflammatory
65response, largely triggered by the invasion of the placenta into maternal uterine tissue (Hung et
66al., 2010). The ability of selenoproteins to reduce oxidative stress, endoplasmic-reticulum stress
67and inflammation, and to protect the endothelium, control eicosanoid production, regulate
68vascular tone and reduce infection, is likely to be important for the health and development of
69the fetus (Rayman, 2016). However, maternal whole blood Se concentration falls substantially
70with increasing gestational age (e.g. by 12% between the first and third trimester) because of
71both plasma volume expansion and Se transfer to the fetus (Rayman et al., 2014).

72Previous research assessing the effect of Se concentrations on anthropometric birth outcomes is
73scarce and inconclusive. Thus, two studies have evaluated the association between decreasing
74maternal Se concentrations and the higher probability of preterm birth in 113 woman-and-child
75pairs from Nigeria (Okunade et al., 2018) and 1197 from Holland (Rayman et al., 2011), with
76statistically significant results. However, regarding the anthropometric birth outcomes such as
77low birth weight, birth length and head circumference the results were more heterogeneous.
78This inconsistency in results could be related to reduced sample size. Thus, maternal Se was
79positively associated with anthropometric birth outcomes in women from UK (n=233), China
80(n=408), Saudi Arabia (n=249) and USA (n=271) (Al-Saleh et al., 2014; Bogden et al., 2006; Sun
81et al., 2018; Wells et al., 2016a). Other studies with lower sample sizes have not shown any
82statistical relationship, such as a study carried out in 81 woman-and-child pairs from China (Hu
83et al., 2015) and another one conducted in 40 Chilean woman-and-child pairs (Llanos and
84Ronco, 2009).

85Birth anthropometric measures predict adverse pregnancy outcomes that might be associated
86with a high risk of mortality, morbidity and disability during infancy and childhood as well other
87chronic diseases later in life (Zheng et al., 2016). For example, fetal growth retardation has been
88associated with a relatively greater probability of coronary heart disease, hypertension and type
892 diabetes mellitus (Barker et al., 2005; Bhargava et al., 2004). Moreover, low birth weight has
90been related to cardiovascular disease (Osmond et al., 1993). Finally, prematurity has been
91associated with insulin resistance and glucose intolerance in pre-pubertal children (Hofman et
92al., 2004) that may track during young adulthood and which can be accompanied by elevated
93blood pressure (Hovi et al., 2007). Several studies have also found positive associations between
94birth weight and a higher probability of breast cancer (Dos Santos Silva et al., 2004). On the
95other hand, head circumference at birth and early childhood has been positively associated with
96a range of neuropsychological outcomes, including protective associations with ADHD symptoms
97(Ferrer et al., 2019).

98Recommended Se intake through diet is set at 70 µg/day and 55 µg/day for men and women,
99respectively, plus a further 10–20 µg/day during pregnancy and lactation (James, 2000; National
100Research Council (US) Subcommittee on the Tenth Edition of the Recommended Dietary
101Allowances, 1989). The most conservative reference of Se in plasma is 70 µg/L, which is
102considered the minimum value to reach maximum GPx activity (López-Bellido Garrido and López
103Bellido, 2013). In Spain, the mean Se intake range is 21–75 µg/day and serum mean $\pm$ standard
104deviation (SD) is 80.7 ± 10 µg/L (Rivas et al., 2012), which is close to the recommendations.
105Most dietary Se comes from bread, cereal, meat, fish and poultry and, increasingly in recent
106times, dietary supplements (Alexander, 2015). In Spain, the Mediterranean dietary pattern
107highlights fish consumption (Sofi et al., 2013; Willett et al., 1995), which is a substantial source
108of Se and Hg. Se content in fish varies from about 12 µg/100 g in lean fish to over 70 µg/100 g in
109canned tuna (Hatfield et al., 2016). However, the bioavailability of Se (14% of Se content in
110ingested food) may be modified by the presence of heavy metals, such as mercury (Hg), which
111may decrease Se absorption via chelation and precipitation (Peters et al., 2016; Wells et al.,
1122016a). THg is also able to affect Se transport across membranes and tissue distribution by
113binding to inorganic Se and forming an insoluble, stable and inert Hg:Se complex, as well as
114binding to the Se site in selenoproteins and permanently inhibiting their function, thereby
115disrupting the intracellular redox environment (Spiller, 2018).

116The *INMA – Infancia y Medio Ambiente* (Environment and Childhood) Project
117(<http://www.proyectoinma.org/>) is a network of seven birth cohorts in Spain (Valencia, Granada,
118Menorca, Sabadell, Ribera d’Ebre, Gipuzkoa and Asturias) that aims to study the role of
119environmental pollutants in air, water and diet during pregnancy and early childhood in relation
120to child growth and development (Guxens et al., 2012). The objective of the present study was
121to evaluate the association between maternal Se serum levels and anthropometric birth
122outcomes in the INMA-Valencia and INMA-Gipuzkoa cohorts.

123 **2 Materials and Methods**

124 **2.1 Study population**

125 Study subjects were participants in those two cohorts of the INMA Project with available serum
126 Se data. Between 2003 and 2008, 1493 pregnant women were recruited at the beginning of
127 their pregnancy in the geographical areas of Valencia and Gipuzkoa, Spain. The inclusion criteria
128 were: at least 16 years of age, 10–13 weeks of gestation, singleton pregnancy, intention of
129 undergoing follow-up and delivery in the corresponding center of reference, non-assisted
130 conception and no impediment for communication. A final sample of 1249 mother-child pairs
131 (83.7%, Valencia n=655, Gipuzkoa n=594), for whom serum Se concentrations and new-born
132 anthropometric data were available, completed the study until delivery. The Hospital Ethics
133 Committees of each area approved these research protocols and informed consent was
134 obtained from all participants.

135 **2.2 Se and THg concentrations**

136 Se concentrations were determined in serum samples taken at the end of the first trimester of
137 pregnancy (mean (SD) = 13.1 (1.2) weeks of gestation). After separation of serum by
138 centrifugation, samples were stored at –80°C and transported frozen to the Karolinska Institutet,
139 Sweden, for analysis. The serum Se concentrations were determined by inductively coupled
140 plasma mass spectrometry (ICPMS); Agilent 7700x, Agilent Technologies, Tokyo, Japan) with
141 the collision/reaction cell system in hydrogen mode. Approximately 120 µg of serum was diluted
142 1:25 in an alkaline solution containing 1% ammonium hydroxide (NH₄OH) (Merck, Darmstadt,
143 Germany), 2% w/w 1-butanol, 0.05% ethylenediaminetetraacetic acid (EDTA), 0.05% Triton X-
144 100 and 20 ng/g of internal standards (Sc-45, Ge-72, Rh-103; CPI International, Amsterdam,
145 Netherlands). Samples were then sonicated and centrifuged for 5 min each. Analytical quality
146 control was performed by inclusion of reference materials (Seronorm™: Trace Elements serum
147 lot MI0181, Trace Elements whole blood L-1 lot 1406263 and L-2 lot 1406264, and Medisafe

148serum L-2 lot 28342). The values obtained were within the analytical range or within 20% of the
149analytical value for all reference materials (Amorós et al., 2018a). The limit of detection was 0.03
150µg/L and no samples had concentrations below this value serum Se concentrations were
151corrected according to the variations in three daily measures of the Seronorm™ (lot MI0181)
152reference material. The correction was performed by adding to each measure the difference
153between the daily mean of the reference measures and the overall mean of the reference
154measures (Amorós et al., 2018a, 2018b). Cord blood total mercury (THg) concentrations were
155determined as explained elsewhere (Murcia et al., 2016).

1562.3 Birth anthropometric measures

157Gestational age was defined on the basis of the self-reported last menstrual period. An early
158crown-rump length measurement was also available and was used for gestational dating when
159the difference with the last menstrual period was ≥ 7 days (12% of the cases). Women for whom
160this difference exceeded 3 weeks (0.7%) were removed from the study to avoid possible bias.
161Preterm births were those with a gestational age below 37 weeks. Type of delivery was
162categorized as spontaneous, induced or caesarean, while births were divided into preterm
163versus full-term.

164Birth weight was measured by the midwife attending the birth, whereas birth length and head
165circumference (HC) were measured by a nurse when the new-born arrived at the hospital ward
166within the first 12 hours of life. Placental weight was recorded by the midwife after birth.
167Growth curves for anthropometric measures and placental weight were fitted to further
168standardize them to week 40 of gestation using the Box-Cox power exponential method (Rigby
169and Stasinopoulos, 2004) adjusting by cohort and sex.

1702.4 Covariates and potential confounders

171The recruited women completed two questionnaires during their pregnancy, one at the first
172trimester (mean (SD) = 13.2 (1.6) weeks of gestation) and the other at the third trimester
173(mean (SD) = 32.6 (2.3) weeks of gestation). The questionnaires were administered by trained
174interviewers and focused on dietary, socio-demographic, environmental and lifestyle information
175during pregnancy. The maternal covariates and potential confounders collected were selected
176based on previous INMA studies on Se (Amorós et al., 2018a, 2018b): country of birth (Spain,
177other), age (quantitative and categorized as age <25, 25–29, 30–34, ≥ 35 years), weight, height,
178body mass index before pregnancy, level of education (up to primary studies, secondary studies,
179university), parity (0, 1, ≥ 2), type of delivery (spontaneous, induced and caesarean), area of
180residence (urban, semi-urban, rural), employment during pregnancy (non-worker, worker),
181smoker at the first trimester (no, yes), overall exposure to smoking (number of exposure
182environments such as home, workplace, restaurant services and leisure areas), season of blood
183sampling, and the newborn's sex. We also obtained data on paternal age, height, body mass
184index, employment and level of education. Parental social class was defined from the maternal
185or paternal occupation during pregnancy with the highest social class, according to a widely
186used Spanish adaptation of the International Standard Classification of Occupations, approved in
1871988 (ISCO88) (Class I + II: managerial jobs, senior technical staff and commercial managers;
188class III: skilled non-manual workers; and class IV + V: manual and unskilled workers).

189Information on diet during pregnancy was collected in order to assess potential dietetic sources
190of serum Se concentrations by using a semi-quantitative food frequency questionnaire (FFQ) in
191the first trimester. This FFQ was validated in this population of pregnant women, with good
192reproducibility being observed for nutrient and food intake (Vioque et al., 2013).

193We obtained data (expressed in grams and servings per week) on the intake of seafood, meat,
194cereals and pasta, legumes, nuts, fruits, vegetables, eggs, dairy products, potatoes and bread.

195Due to the large range of both Se and THg concentrations depending on the type of fish,
196seafood was included as four different categories based on the regular consumption of the
197Spanish population: lean fish, oily fish, canned tuna and processed lean fish. Self-reported
198maternal consumption of multivitamin supplements was obtained from an additional
199questionnaire at the first trimester of pregnancy. Se supplementation was also categorized into
200two groups (Se supplementation vs. no Se supplementation).

201We categorized this variable (< 15 vs. ≥ 15 $\mu\text{g/L}$) according to the equivalent for the WHO
202Provisional Tolerable Weekly Intake (1.6 $\mu\text{g/kg}$ of body weight per week) ("Scientific Opinion on
203the risk for public health related to the presence of mercury and methylmercury in food," 2012).

2042.5 Statistical Analysis

205We analyzed the association between serum Se concentrations and anthropometric measures
206using multivariable generalized linear regressions models (GLM): linear regression models for
207continuous Gaussian outcomes (birth weight, birth length, head circumference, and placental
208weight) and logistic regression models for the binary outcome (prematurity). Cox regression
209(proportional hazards model) for the length of gestation was also used due to the lack of
210normality of the outcome and the presence of censoring. Spontaneous birth was therefore
211defined as the event in Cox regression, while elective delivery was considered to be censoring
212(Murcia et al., 2016). Survival curves were built for descriptive purposes, with maternal serum
213Se concentrations categorized as < 70 or ≥ 70 $\mu\text{g/L}$ (James, 2000).

214We adjusted confounders and predictors of the outcomes in accordance with the following
215procedure: (1) Basal models were built following a backward elimination procedure considering
216all the 1249 mother-child pairs: starting from models including all covariates related to each
217response at $p < 0.20$ in univariate analysis, we sequentially excluded those variables with an
218adjusted $p > 0.10$. Cohort was included in all models regardless of their statistical significance. (2)

219Serum Se concentration was then incorporated and possible confounders were subsequently
220included if they changed the magnitude of the main effect in a significant way with a 5%
221significance level (Lee, 2014). In cases in which they were not included in the basal models,
222maternal age, country of birth, maternal education, season of blood sampling, and seafood
223consumption were assessed as potential confounders on the basis of previous knowledge
224regarding their potential relation with maternal Se status (Al-Saleh et al., 2014; Amorós et al.,
2252018a). Energy-adjusted intakes were computed using the residual method, where the
226residuals were calculated from a linear regression, with the natural logarithm of the food group
227modeled as the dependent variable and the natural logarithm of total energy intake as the
228independent variable.

229Generalised additive models (GAM) using natural cubic smoothing splines (one or two internal
230knots) were employed to assess the linearity of the relationship between each outcome and
231serum Se concentrations by graphical observation and the Akaike information criterion (AIC).
232Segmented models were used to determine possible break-points in the linear association
233between each outcome and serum Se concentrations, and the Davis test was also used to find
234the potential location of these break-points (Muggeo, 2003).

235Effect modifications (interaction) by child sex, categorised cord blood THg concentrations and
236season of sampling were assessed. To do so, the interaction effect of these variables with serum
237Se concentration was tested using AIC scores between the GLM and GAM models with and
238without interaction.

239Finally, sensitivity analyses were performed to assess changes in serum Se concentrations by
240building a multivariate model only using participants without Se supplementation and excluding
241preterm births. Analyses were conducted with the statistical software R, version 3.4.3 (R core
242Team, 2017).

2433 Results

244Descriptive statistics of the study variables are displayed in Table 1. Mean age of mothers
245included in the study at the time of conception was 30.7 years, 91.8% were born in Spain, 85.6%
246were working at the first trimester of pregnancy, 54.4% were primiparous, 18.7% smoked at
247sampling and 65.9% were exposed to sources of smoke, and 7.5% lived in rural areas. The mean
248birth weight was 3,343 g and the mean gestational length was 39.7 weeks, with 4.2% preterm
249births.

250The mean (SD) maternal serum Se concentration was 79.57 (9.64) µg/L. Lower serum Se levels
251were found in Gipuzkoa (77.08 (10.62) µg/L) than in Valencia (81.93 (7.92) µg/L). Statistically
252significant differences were found between the two cohorts (t-test p-value <0.001). The
253geometric mean (SD) THg in cord blood was 8.45 (2.17) µg/L.

254Table 2 shows correlations between serum Se concentrations, THg and the four seafood intake
255variables, which are likely to contain Hg, calculated considering the whole sample and adjusted
256for cohort. The table also shows that the correlation between Se and Hg also remains significant
257after mutual adjustment for Se, Hg, and seafood intake. The correlation between serum Se
258concentrations and seafood intake was significant in the Gipuzkoa cohort.

259The variables included in the multivariate models for each anthropometric outcome can be
260observed in Figure 1. Multivariate GLMs showed a fit improvement (lower AIC score) compared
261to the GAM for the association between maternal serum Se concentrations and each outcome.
262Direct observation of the estimated splines of the GAMs did not show any non-linear
263associations, and the Davis test did not find any break-point with statistical significance.

264The serum Se concentrations effect was marginally statistically significant in both head
265circumference ($\beta = -0.007$; p-value = 0.08) and gestational age (HR 1.007; p-value = 0.06) (Table
2663). The positive relation between serum Se concentrations and gestational age should be

267considered as an increased risk of spontaneous delivery (event of survival analysis). The
268association between maternal serum Se concentrations and other anthropometric outcomes
269was negative for birth weight and length and direct for probability of preterm birth and placental
270weight, although the coefficients did not reach statistical significance.

271Table 3 also shows serum Se concentrations effect modifications (interactions) by categorised
272cord blood THg concentrations, sex of the child and season of sampling. Individuals with THg
273levels > 15 µg/L reversed the serum Se concentrations effect on head circumference with
274marginal significance (Figure 2). A slight improvement in terms of AIC was found on adding the
275interaction term to the GLM in the model (AIC 3783.90 and 3784.07 with and without
276interaction term, respectively). Child's sex also showed a significant serum Se concentrations
277effect modification on both gestational age (risk of spontaneous birth) and prematurity
278(Supplemental Figure S3 and S4, respectively). Females had a lower risk of spontaneous birth (β
279interaction term -0.017 , p -value = 0.02) than males, the AIC improved after adding the
280interaction term in the model (AIC 10783.57 and 10786.95 with and without interaction term,
281respectively). Direct observation of the survival curves for both sexes showed significant
282differences only for females whose mother's serum Se concentration was < 70 µg/L (p -value of
283log-rank test = 0.0074). Female sex had a lower probability of prematurity (β interaction term $-$
2840.068, p -value = 0.02), the AIC improved after adding the interaction term in the model (AIC
285421.76 and 424.18 with and without interaction term, respectively). No seasonal interactions
286were found.

287Sensitivity analyses were also carried out. Figure 2 shows adjusted associations between
288maternal serum Se concentrations and birth anthropometric measures: (1) raw models
289considering only exposure; (2) models adjusted by covariates as described in Figure 1; (3)
290models adjusted for covariates and confounders, as shown in Table 3; (4) models adjusted for
291covariates and confounders including interaction terms for sex, sampling season and THg, the

numeric results of which are also shown in Table 3; (5) models adjusted for covariates and confounders excluding 142 mothers who consumed dietary supplements that contained Se, which did not affect the estimates meaningfully, although the slight loss of serum Se concentrations significance in the head circumference model might be related to the chemical form of Se in dietary supplements; (6) models adjusted for covariates and confounders excluding 69 preterm births, which did not affect the estimates either; and (7) models adjusted for covariates and confounders with THg as an additional confounder in order to assess changes in Se coefficients, which also showed similar estimates. As can be seen in Supplemental Table S1, THg did not confound the relationship between serum Se concentrations and the outcomes. When models were additionally adjusted for THg, an apparent slight and non-significant change in the serum Se coefficients was observed. However, these changes are due exclusively to the reduction in the sample size and the consequent loss of statistical power. All models were adjusted for cohort.

4 Discussion

In this birth cohort study, we observed a weak inverse association between maternal serum Se concentrations in serum and child's head circumference at birth. Additionally, serum Se concentrations showed a marginally significant direct relation to a higher risk of spontaneous birth at lower gestational ages. The relation between maternal serum Se concentrations and other anthropometric outcomes was negative for birth weight and length, and direct for probability of preterm birth and placental weight, although the coefficients did not reach statistical significance. Maternal serum Se concentrations were 79.57 (9.64) µg/L at the first trimester of gestation, which are close to the recommendations set at 70 µg/L during pregnancy. Previous research concerning the effect of Se on anthropometric birth outcomes is scarce and inconclusive. These studies have mainly evaluated the association between Se concentrations

and child birth weight, showing a protective effect of Se but showing some inconsistencies in results which may be due to different sources of dietary intake, sample sizes, matrices used to collect Se concentrations or residual confounding. For example, positive associations were found between maternal Se concentrations and birth weight in United Kingdom (mean (SD): 106.24 (2.99) $\mu\text{g/L}$ in serum at the first trimester, $n=233$) (Bogden et al., 2006), as well as in China (geometric mean (95% confidence intervals (CI)): 156.58 (145.82–167.34) $\mu\text{g/L}$ in maternal blood at the third trimester, $n=209$) (Sun et al., 2014). Considering cord blood, positive associations were also found with birth weight in Saudi Arabia (67.618 (12.897) $\mu\text{g/L}$, $n=249$) (Al-Saleh et al., 2014) and in USA (70.0 (68.5–71.4) $\mu\text{g/L}$, $n=271$) (Wells et al., 2016b). However, other studies with lower sample sizes have not shown any statistical relationship, such as a study carried out in 81 woman-and-child pairs from China in both maternal and cord blood (Hu et al., 2015) and another one conducted in 40 Chilean woman-and-child pairs by using placenta samples (Llanos and Ronco, 2009). The relationship between Se and other anthropometric outcomes such as length, head circumference, placental weight, as well as gestational age and prematurity has been barely studied to date. A weak but significant positive association between Se and gestational age ($\beta = 0.31$ days, 95% CI = 0.18–0.45) was also highlighted in the study carried out in USA mentioned above (Wells et al., 2016b). Furthermore, a positive association between cord blood Se and child's head circumference and placental weight was also observed in the Saudi study (Al-Saleh et al., 2014). However, an inverse relation between Se concentrations in placenta and placental weight was found in a small sample ($n = 20$) of Polish women (Zadrozna et al., 2009).

Contrary results to ours regarding prematurity were observed in two studies conducted in Holland ($n=1167$) and Nigeria ($n=113$). Women with Se concentrations below 72.6 $\mu\text{g/L}$ in Holland (Rayman, 2016) and 70 $\mu\text{g/L}$ in Nigeria (Okunade et al., 2018) had a higher probability of giving birth prematurely. The discrepancies between those results and present data could be explained by a non-linear relationship, a possibility that was not explored in any of the above

342mentioned studies. However, the AIC analyses performed in our study indicated that the
343relationships between Se and anthropometric outcome were linear.

344In the present study we have also observed a differentiated relationship between maternal
345serum Se levels and the newborn's head circumference at birth according to the level of THg in
346cord blood. The association between serum Se concentrations and the newborn's head
347circumference was negative for those with cord blood THg below 15 µg/L and positive for those
348with THg levels above 15 µg/L. The interaction p-value was marginally statistically significant.
349The Saudi study mentioned above also indicated marginally significant Hg–Se antagonistic
350interactions in placental samples ($p = 0.091$) that may have moderated the toxic effect of Hg on
351head circumference. However, other studies have also assessed interactions between the two
352metals without finding any significant effects. For example, in a study carried out in 15,444
353Japanese women, which specifically assessed the relationship between maternal Se and THg
354concentrations and birth outcomes, a reduction in birth head circumference by 0.073 cm (95%
355CI: -0.134, -0.011) was observed in mothers with the highest THg levels, but without Se
356interaction (Kobayashi et al., 2019). Similarly, the study conducted in 271 children from USA
357mentioned earlier showed that interaction between Se and methylmercury had no significant
358effect on birth outcomes (Wells et al., 2016b). A recent review proposed that Hg interacts with
359Se by binding with both the inorganic Se form and Se sites in selenoproteins, which might
360explain why Hg causes oxidative stress (Spiller, 2018). According to this, results shown in this
361study regarding the inverse relationship between serum Se concentrations and children's head
362circumference would be supported.

363We have also observed a differentiated association between maternal serum Se levels and
364outcomes according to the child's sex, since a higher probability of spontaneous birth and
365prematurity was seen in males. Possible explanations of this sex effect are greater body weight,
366increased susceptibility to complications of pregnancy, sex-linked biochemical processes, and

earlier conception in the fertile cycle (Zeitlin et al., 2002). The lower probability of prematurity observed for females in the present study has also been found in several studies. Among the most recent, a Turkish study carried out in 29,528 pregnant women, 7,382 of whom had preterm deliveries, showed a significantly lower prematurity in 24.3% of female versus 25.7% of male newborns (p -value = 0.004) (Kanmaz et al., 2019). However, no significant association between sex and preterm births was found in a sample of 2,505 pregnant women from UK, 230 of whom had spontaneous deliveries < 37 weeks (males n = 108 (8.7%), females n = 122 (9.6%); Relative Risk (95% CI) = 1.10 (0.86–1.41), p -value = 0.45) (Teoh et al., 2018).

Furthermore, our sensitivity analyses excluding participants who consumed dietary supplements and preterm births obtained similar estimates. However, these analyses resulted in the loss of serum Se significance regarding both the newborn's head circumference and a higher probability of spontaneous birth (versus caesarean or induced among non-preterm births) because of the decrease in the sample. Results with respect to head circumference were also observed in the Saudi study mentioned previously (Al-Saleh et al., 2014), since after the exclusion of preterm births Se significance disappeared. Authors suggest that Se may play a noteworthy role in the etiology of preterm births. Nevertheless, the US study (Wells et al., 2016b) which observed an association between Se and gestational age did not assess sensitivity analyses excluding preterm births and no other research about this association has been found.

There are some limitations in the present study: (1) there was a lack of Se speciation data. Associations observed in this study could be related to one or several of the Se forms present in the body and this fact could in turn be related to the specific form used in supplements, which have shown a specific compromise of Se significance in sensitivity analyses; (2) although we used a validated FFQ administered at the same time as blood sampling, some degree of misclassification in the maternal food intake is likely to be expected; and (3) the number of children with cord blood THg data available was lower than with maternal serum Se

392 concentrations data and this could affect the proper interpretation of statistical models with
393 THg. This reduction affects the power of these models.

394 The main strengths of this study are: (1) the large size of the whole sample built with two
395 distanced cohorts with differences in social class and education (higher in the Gipuzkoa cohort)
396 and dietary pattern. Regional differences in the consumption of big oily fish species (higher in
397 Valencia) could explain, in part, the different mean concentrations between cohorts. In our FFQ
398 this category included fresh tuna and swordfish, the fish species with higher Se content (Olmedo
399 et al., 2013) (2) the novelty of the statistical approach, which assesses both linear and non-linear
400 associations between serum Se concentrations and anthropometric measures, and also the use
401 of time-to-event analysis to control for the presence of censoring (induced births) in the analysis
402 of the length of gestation, while also avoiding an inaccurate assumption of normality. The
403 statistical analyses also include the performance of sensitivity analysis, confounding assessment
404 and interaction analysis; and (3) the study's prospective design, which made it possible to obtain
405 detailed information concerning maternal and child socio-demographic, dietary and life-style
406 characteristics that may affect anthropometric outcomes.

407 **5 Conclusion**

408 We observed a weak linear association between maternal serum Se concentrations and lower
409 circumference of the child's head at birth and higher risk of spontaneous birth (lower gestational
410 age at birth), despite the fact that serum Se concentrations were within the range considered to
411 be appropriate to ensure antioxidant capability. The non-significant tendencies observed with
412 birth weight and length, as well as prematurity, show consistency regarding a detrimental effect
413 of Se. Further studies are necessary in order to confirm these results.

4146 Acknowledgments

415We are grateful to all the participants and their families for taking part in this study. We also
416appreciate the generous contribution in the study of all INMA members. A full roster of the
417INMA Project Investigators can be found at [http://www.proyectoinma.org/presentacion-](http://www.proyectoinma.org/presentacion-inma/listado-investigadores/en_listado-investigadores.htm)
418inma/listado-investigadores/en_listado-investigadores.htm

4197 **References**

- 420 Alexander, J., 2015. Selenium, in: Handbook on the Toxicology of Metals. Elsevier, pp. 1175–
421 1208. <https://doi.org/10.1016/B978-0-444-59453-2.00052-4>
- 422 Al-Saleh, I., Al-Rouqi, R., Obsum, C.A., Shinwari, N., Mashhour, A., Billedo, G., Al-Sarraj, Y.,
423 Rabbah, A., 2014. Mercury (Hg) and oxidative stress status in healthy mothers and its
424 effect on birth anthropometric measures. International Journal of Hygiene and
425 Environmental Health 217, 567–585. <https://doi.org/10.1016/j.ijheh.2013.11.001>
- 426 Amorós, R., Murcia, M., Ballester, F., Broberg, K., Iñiguez, C., Rebagliato, M., Skröder, H.,
427 González, L., Lopez-Espinosa, M.-J., Llop, S., 2018a. Selenium status during pregnancy:
428 Influential factors and effects on neuropsychological development among Spanish infants.
429 Science of The Total Environment 610–611, 741–749.
430 <https://doi.org/10.1016/j.scitotenv.2017.08.042>
- 431 Amorós, R., Murcia, M., González, L., Rebagliato, M., Iñiguez, C., Lopez-Espinosa, M.-J., Vioque,
432 J., Broberg, K., Ballester, F., Llop, S., 2018b. Maternal selenium status and
433 neuropsychological development in Spanish preschool children. Environmental Research
434 166, 215–222. <https://doi.org/10.1016/j.envres.2018.06.002>
- 435 Barker, D.J.P., Osmond, C., Forsén, T.J., Kajantie, E., Eriksson, J.G., 2005. Trajectories of Growth
436 among Children Who Have Coronary Events as Adults. New England Journal of Medicine
437 353, 1802–1809. <https://doi.org/10.1056/NEJMoa044160>
- 438 Bhargava, S.K., Sachdev, H.S., Fall, C.H.D., Osmond, C., Lakshmy, R., Barker, D.J.P., Biswas, S.K.D.,
439 Ramji, S., Prabhakaran, D., Reddy, K.S., 2004. Relation of Serial Changes in Childhood
440 Body-Mass Index to Impaired Glucose Tolerance in Young Adulthood. New England
441 Journal of Medicine 350, 865–875. <https://doi.org/10.1056/NEJMoa035698>
- 442 Bogden, J.D., Kemp, F.W., Chen, X., Stagnaro-Green, A., Stein, T.P., Scholl, T.O., 2006. Low-normal
443 serum selenium early in human pregnancy predicts lower birth weight. Nutrition Research
444 26, 497–502. <https://doi.org/10.1016/j.nutres.2006.08.008>
- 445 Dos Santos Silva, I., De Stavola, B.L., Hardy, R.J., Kuh, D.J., McCormack, V.A., Wadsworth, M.E.J.,
446 2004. Is the association of birth weight with premenopausal breast cancer risk mediated

447 through childhood growth? *British Journal of Cancer* 91, 519–524.
448 <https://doi.org/10.1038/sj.bjc.6601972>

449 Ferrer, M., García-Esteban, R., Iñiguez, C., Costa, O., Fernández-Somoano, A., Rodríguez-Delhi, C.,
450 Ibarluzea, J., Lertxundi, A., Tonne, C., Sunyer, J., Julvez, J., 2019. Head circumference and
451 child ADHD symptoms and cognitive functioning: results from a large population-based
452 cohort study. *European Child and Adolescent Psychiatry* 28, 377–388.
453 <https://doi.org/10.1007/s00787-018-1202-4>

454 Guxens, M., Ballester, F., Espada, M., Fernández, M.F., Grimalt, J.O., Ibarluzea, J., Olea, N.,
455 Rebagliato, M., Tardón, A., Torrent, M., Vioque, J., Vrijheid, M., Sunyer, J., 2012. Cohort
456 Profile: The INMA—Infancia y Medio Ambiente—(Environment and Childhood) Project.
457 *International Journal of Epidemiology* 41, 930–940. <https://doi.org/10.1093/ije/dyr054>

458 Hatfield, D.L., Schweizer, U., Tsuji, P.A., Gladyshev, V.N., 2016. Selenium: Its molecular biology
459 and role in human health, fourth edition, *Selenium: Its Molecular Biology and Role in*
460 *Human Health, Fourth Edition*. <https://doi.org/10.1007/978-3-319-41283-2>

461 Hofman, P.L., Regan, F., Jackson, W.E., Jefferies, C., Knight, D.B., Robinson, E.M., Cutfield, W.S.,
462 2004. Premature birth and later insulin resistance. *New England Journal of Medicine* 351,
463 2179–2186. <https://doi.org/10.1056/NEJMoa042275>

464 Hovi, P., Andersson, S., Eriksson, J.G., Järvenpää, A.-L., Strang-Karlsson, S., Mäkitie, O., Kajantie,
465 E., 2007. Glucose regulation in young adults with very low birth weight. *New England*
466 *Journal of Medicine* 356, 2053–2063. <https://doi.org/10.1056/NEJMoa067187>

467 Hu, X., Zheng, T., Cheng, Y., Holford, T., Lin, S., Leaderer, B., Qiu, J., Bassig, B.A., Shi, K., Zhang, Y.,
468 Niu, J., Zhu, Y., Li, Y., Guo, H., Chen, Q., Zhang, J., Xu, S., Jin, Y., 2015. Distributions of heavy
469 metals in maternal and cord blood and the association with infant birth weight in China.
470 *Journal of Reproductive Medicine* 60, 21–29.

471 Hung, T.-H., Lo, L.-M., Chiu, T.-H., Li, M.-J., Yeh, Y.-L., Chen, S.-F., Hsieh, T.-T., 2010. A longitudinal
472 study of oxidative stress and antioxidant status in women with uncomplicated
473 pregnancies throughout gestation. *Reprod Sci* 17, 401–409.
474 <https://doi.org/10.1177/1933719109359704>

475 James, J.S., 2000. Selenium: important new review of health findings. *AIDS treatment news* 3–4.

476 Kanmaz, A.G., İnan, A.H., Beyan, E., Karataşlı, V., Çakır, İ., Budak, A., Emirdar, V., 2019. Effects of
 477 fetal gender and low first trimester aneuploidy screening markers on preterm birth.
 478 Journal of Gynecology Obstetrics and Human Reproduction.
 479 <https://doi.org/10.1016/j.jogoh.2019.01.011>

480 Kobayashi, S., Kishi, R., Saijo, Y., Ito, Y., Oba, K., Araki, A., Miyashita, C., Itoh, S., Minatoya, M.,
 481 Yamazaki, K., Ait Bamai, Y., Sato, T., Yamazaki, S., Nakayama, S.F., Isobe, T., Nitta, H., The,
 482 J.E. and C.S.G., 2019. Association of blood mercury levels during pregnancy with infant
 483 birth size by blood selenium levels in the Japan Environment and Children's Study: A
 484 prospective birth cohort. Environment International 418–429.
 485 <https://doi.org/10.1016/j.envint.2019.01.051>

486 Lee, P.H., 2014. Is a Cutoff of 10% Appropriate for the Change-in-Estimate Criterion of
 487 Confounder Identification? Journal of Epidemiology 24, 161–167.
 488 <https://doi.org/10.2188/jea.JE20130062>

489 Llanos, M.N., Ronco, A.M., 2009. Fetal growth restriction is related to placental levels of
 490 cadmium, lead and arsenic but not with antioxidant activities. Reproductive Toxicology 27,
 491 88–92. <https://doi.org/10.1016/j.reprotox.2008.11.057>

492 López-Bellido Garrido, F.J., López Bellido, L., 2013. Selenium and health; reference values and
 493 current status of Spanish population. Nutricion Hospitalaria 28, 1396–1406.
 494 <https://doi.org/10.3305/nh.2013.28.5.6634>

495 Muggeo, V.M.R., 2003. Estimating regression models with unknown break-points. Statistics in
 496 Medicine 22, 3055–3071. <https://doi.org/10.1002/sim.1545>

497 Murcia, M., Ballester, F., Enning, A.M., Iñiguez, C., Valvi, D., Basterrechea, M., Rebagliato, M.,
 498 Vioque, J., Maruri, M., Tardon, A., Riaño-Galán, I., Vrijheid, M., Llop, S., 2016. Prenatal
 499 mercury exposure and birth outcomes. Environmental Research 151, 11–20.
 500 <https://doi.org/10.1016/j.envres.2016.07.003>

501 National Research Council (US) Subcommittee on the Tenth Edition of the Recommended
 502 Dietary Allowances., 1989. Recommended Dietary Allowances - 10th Edition. National
 503 Academies Press (US), Washington (DC).

504 Okunade, K.S., Olowoselu, O.F., Osanyin, G.E., John-Olabode, S., Akanmu, S.A., Anorlu, R.I., 2018.
 505 Selenium deficiency and pregnancy outcome in pregnant women with HIV in Lagos,

506 Nigeria. *International Journal of Gynecology & Obstetrics* 142, 207–213.
 507 <https://doi.org/10.1002/ijgo.12508>

508 Olmedo, P., Hernández, A.F., Pla, A., Femia, P., Navas-Acien, A., Gil, F., 2013. Determination of
 509 essential elements (copper, manganese, selenium and zinc) in fish and shellfish samples.
 510 Risk and nutritional assessment and mercury–selenium balance. *Food and Chemical*
 511 *Toxicology* 62, 299–307. <https://doi.org/10.1016/j.fct.2013.08.076>

512 Osmond, C., Barker, D.J.P., Winter, P.D., Fall, C.H.D., Simmonds, S.J., 1993. Early growth and death
 513 from cardiovascular disease in women. *British Medical Journal* 307, 1519–1524.

514 Papp, L.V., Lu, J., Holmgren, A., Khanna, K.K., 2007. From selenium to selenoproteins: Synthesis,
 515 identity, and their role in human health. *Antioxidants and Redox Signaling* 9, 775–806.
 516 <https://doi.org/10.1089/ars.2007.1528>

517 Peters, K.M., Galinn, S.E., Tsuji, P.A., 2016. Selenium: Dietary Sources, Human Nutritional
 518 Requirements and Intake Across Populations, in: Hatfield, D.L., Schweizer, U., Tsuji, P.A.,
 519 Gladyshev, V.N. (Eds.), *Selenium: Its Molecular Biology and Role in Human Health*.
 520 Springer International Publishing, Cham, pp. 295–305. [https://doi.org/10.1007/978-3-](https://doi.org/10.1007/978-3-319-41283-2_25)
 521 [319-41283-2_25](https://doi.org/10.1007/978-3-319-41283-2_25)

522 Rayman, M.P., 2016. Is Adequate Selenium Important for Healthy Human Pregnancy?, in:
 523 Hatfield, D.L., Schweizer, U., Tsuji, P.A., Gladyshev, V.N. (Eds.), *Selenium: Its Molecular*
 524 *Biology and Role in Human Health*. Springer International Publishing, Cham, pp. 353–364.
 525 https://doi.org/10.1007/978-3-319-41283-2_30

526 Rayman, M.P., 2012. Selenium and human health. *The Lancet* 379, 1256–1268.
 527 [https://doi.org/10.1016/S0140-6736\(11\)61452-9](https://doi.org/10.1016/S0140-6736(11)61452-9)

528 Rayman, M.P., Searle, E., Kelly, L., Johnsen, S., Bodman-Smith, K., Bath, S.C., Mao, J., Redman,
 529 C.W.G., 2014. Effect of selenium on markers of risk of pre-eclampsia in UK pregnant
 530 women: a randomised, controlled pilot trial. *Br. J. Nutr.* 112, 99–111.
 531 <https://doi.org/10.1017/S0007114514000531>

532 Rayman, M.P., Wijnen, H., Vader, H., Kooistra, L., Pop, V., 2011. Maternal selenium status during
 533 early gestation and risk for preterm birth. *CMAJ* 183, 549–555.
 534 <https://doi.org/10.1503/cmaj.101095>

535 Rigby, R.A., Stasinopoulos, D.M., 2004. Smooth centile curves for skew and kurtotic data
 536 modelled using the Box–Cox power exponential distribution. *Statistics in Medicine* 23,
 537 3053–3076. <https://doi.org/10.1002/sim.1861>

538 Rivas, A., Romero, A., Mariscal-Arcas, M., Monteagudo, C., López, G., Lorenzo, M.L., Ocaña-
 539 Peinado, F.M., Olea-Serrano, F., 2012. Association between dietary antioxidant quality
 540 score (DAQs) and bone mineral density in Spanish women. *Nutricion Hospitalaria* 27,
 541 1886–1893. <https://doi.org/10.3305/nh.2012.27.6.6039>

542 Scientific Opinion on the risk for public health related to the presence of mercury and
 543 methylmercury in food, 2012. . *EFSA Journal* 10. <https://doi.org/10.2903/j.efsa.2012.2985>

544 Sofi, F., Macchi, C., Abbate, R., Gensini, G.F., Casini, A., 2013. Mediterranean diet and health.
 545 *BioFactors* 39, 335–342. <https://doi.org/10.1002/biof.1096>

546 Spiller, H.A., 2018. Rethinking mercury: the role of selenium in the pathophysiology of mercury
 547 toxicity. *Clinical Toxicology* 56, 313–326.
 548 <https://doi.org/10.1080/15563650.2017.1400555>

549 Sun, H., Chen, W., Wang, D., Jin, Y., Chen, X., Xu, Y., 2014. The effects of prenatal exposure to
 550 low-level cadmium, lead and selenium on birth outcomes. *Chemosphere* 108, 33–39.
 551 <https://doi.org/10.1016/j.chemosphere.2014.02.080>

552 Sun, X., Jiang, Y., Xia, W., Jin, S., Liu, W., Lin, X., Liu, H., Chen, X., Peng, Y., Li, H., Lu, B., Xu, S., Li, Y.,
 553 Shen, X., 2018. Association between prenatal nickel exposure and preterm low birth
 554 weight: possible effect of selenium. *Environmental Science and Pollution Research* 25,
 555 25888–25895. <https://doi.org/10.1007/s11356-018-2622-x>

556 Teoh, P.J., Ridout, A., Seed, P., Tribe, R.M., Shennan, A.H., 2018. Gender and preterm birth: Is
 557 male fetal gender a clinically important risk factor for preterm birth in high-risk women?
 558 *European Journal of Obstetrics & Gynecology and Reproductive Biology* 225, 155–159.
 559 <https://doi.org/10.1016/j.ejogrb.2018.04.025>

560 Vioque, J., Navarrete-Muñoz, E.-M., Gimenez-Monzó, D., García-de-la-Hera, M., Granado, F.,
 561 Young, I.S., Ramón, R., Ballester, F., Murcia, M., Rebagliato, M., Iñiguez, C., INMA-Valencia
 562 Cohort Study, 2013. Reproducibility and validity of a food frequency questionnaire among
 563 pregnant women in a Mediterranean area. *Nutrition Journal* 12, 26.
 564 <https://doi.org/10.1186/1475-2891-12-26>

565 Wells, E.M., Herbstman, J.B., Lin, Y.H., Jarrett, J., Verdon, C.P., Ward, C., Caldwell, K.L., Hibbeln,
566 J.R., Witter, F.R., Halden, R.U., Goldman, L.R., 2016a. Cord Blood Methylmercury and Fetal
567 Growth Outcomes in Baltimore Newborns: Potential Confounding and Effect Modification
568 by Omega-3 Fatty Acids, Selenium, and Sex. *Environmental Health Perspectives* 124, 373–
569 379. <https://doi.org/10.1289/ehp.1408596>

570 Wells, E.M., Herbstman, J.B., Lin, Y.H., Jarrett, J., Verdon, C.P., Ward, C., Caldwell, K.L., Hibbeln,
571 J.R., Witter, F.R., Halden, R.U., Goldman, L.R., 2016b. Cord Blood Methylmercury and Fetal
572 Growth Outcomes in Baltimore Newborns: Potential Confounding and Effect Modification
573 by Omega-3 Fatty Acids, Selenium, and Sex. *Environmental Health Perspectives* 124, 373–
574 379. <https://doi.org/10.1289/ehp.1408596>

575 Whanger, P.D., 2002. Selenocompounds in Plants and Animals and their Biological Significance.
576 *Journal of the American College of Nutrition* 21, 223–232.
577 <https://doi.org/10.1080/07315724.2002.10719214>

578 Willett, W.C., Sacks, F., Trichopoulou, A., Drescher, G., Ferro-Luzzi, A., Helsing, E., Trichopoulos,
579 D., 1995. Mediterranean diet pyramid: A cultural model for healthy eating. *American*
580 *Journal of Clinical Nutrition* 61, 1402S–1406S. <https://doi.org/10.1093/ajcn/61.6.1402S>

581 Zadrozna, M., Gawlik, M., Nowak, B., Marcinek, A., Mrowiec, H., Walas, S., Wietecha-Postuszny,
582 R., Zagrodzki, P., 2009. Antioxidants activities and concentration of selenium, zinc and
583 copper in preterm and IUGR human placentas. *Journal of Trace Elements in Medicine and*
584 *Biology* 23, 144–148. <https://doi.org/10.1016/j.jtemb.2009.02.005>

585 Zeitlin, J., Saurel-Cubizolles, M.-J., De Mouzon, J., Rivera, L., Ancel, P.-Y., Blondel, B., Kaminski, M.,
586 2002. Fetal sex and preterm birth: Are males at greater risk? *Human Reproduction* 17,
587 2762–2768.

588 Zheng, T., Zhang, J., Sommer, K., Bassig, B.A., Zhang, X., Braun, J., Xu, S., Boyle, P., Zhang, B., Shi,
589 K., Buka, S., Liu, S., Li, Y., Qian, Z., Dai, M., Romano, M., Zou, A., Kelsey, K., 2016. Effects of
590 Environmental Exposures on Fetal and Childhood Growth Trajectories. *Annals of Global*
591 *Health, Children's Health in a Changing Global Environment* 82, 41–99.
592 <https://doi.org/10.1016/j.aogh.2016.01.008>

Figure 1. Variables included in each of the multivariate models for the anthropometric outcomes and associated p-value range.

595

596

597

598 **Figure 2.** GLM for the association between maternal Se concentrations (measured at the first trimester
599 of gestation) and child's head circumference at birth. INMA Project (2003–2008).

600

601

602

603**Figure 3.** Sensitivity analysis: raw model adjusted only for cohort, main model adjusted by covariates as Figure 1, main model adjusted by covariates and confounders,
604interaction analysis, and exclusion or inclusion of Hg, supplements consumption and preterm births. Adjusted association (95% CI) between maternal Se
605concentrations and outcome variables. INMA Project, 2004–2008 (Spain).

606

607All models adjusted for cohort.

608Birth weight linear model additionally adjusted for covariates: parity, maternal smoking and exposure to smoking sources, maternal BMI, height, and country of birth at evaluation, child sex, paternal BMI and height; and additionally adjusted for confounders:
609maternal age and lean fish consumption.

57

58

29

610Birth length linear model additionally adjusted for covariates: parity, maternal smoking and exposure to smoking sources, height, child sex and paternal height; and additionally adjusted for confounders: maternal age, country of birth, educational level and
611gestational age at evaluation.

612Head circumference linear model additionally adjusted for covariates: parity, maternal smoking, height and educational level at evaluation, and child sex; and additionally adjusted for confounders: maternal age, country of birth, lean and oily fish consumption.

613Placental weight linear model additionally adjusted for covariates: maternal BMI before pregnancy, parity, maternal working status at evaluation, parental social class, type of zone, maternal and paternal height; and additionally adjusted for confounders:
614maternal country of birth and educational level, lean and oily fish consumption.

615Prematurity linear model additionally adjusted for covariates: maternal age, parity, maternal educational level. Without any significant confounders.

616Gestational age COX model additionally adjusted for covariates: parity, type of zone, maternal height; and additionally adjusted for confounders: blood sampling season and lean fish consumption.

Table 1. Partial correlation matrix including outcome variables, Se and seafood intake variables.^a

-0.032

618

618
619 Abbreviations: BW, birth weight; BL, birth length; ; HC, head circumference; PW, placental weight; GL, gestational length.

620^aSeafood intake variables (servings per day).

621^bTHg was log₂-transformed.

622. p-value <0.10; * p-value <0.05; ** p-value <0.01; *** p-value <0.001.

Table 2. Characteristics of mother-child pairs participating in the study. INMA Project, 2004–2006, Valencia and Gipuzkoa cohorts (Spain).

		N	%	Se (µg/L) Mean (SD)	ANOVA p-value
Cohort					
	Gipuzkoa	1249	47.6	77.22 (10.61)	<0.001
	Valencia		52.4	81.94 (7.92)	
Maternal variables					
Age (years)		1246	30.70 (4.08) ^a	0.055 ^b	0.053 ^c
Age (categorised)					
	< 25 years	1246	6.3	77.71 (8.13)	0.170
	25–29 years		32.2	79.40 (8.97)	
	30–34 years		44.8	80.00 (9.46)	
	≥ 35 years		16.7	80.25 (11.38)	
Country of birth					
	Spain	1249	91.8	79.87 (9.70)	0.028
	Other		8.2	77.70 (8.06)	
Education					
	Up to primary	1247	22.9	79.58 (8.54)	0.106
	Secondary		39.7	79.58 (8.95)	
	University		37.4	79.84 (10.79)	
Social class					
	I. II (highest)	1249	33.4	79.63 (10.32)	0.879
	III		25	79.93 (9.92)	
	IV. V (lowest)		41.6	79.60 (8.76)	
Employed during pregnancy					
	No	1249	14.4	79.27 (8.82)	0.415
	Yes		85.6	79.77 (9.71)	
Maternal height (cm)		1246	163.05 (6.14) ^a	-0.024 ^b	0.401 ^c
Pre-pregnancy BMI (kg/m²)		1249	23.39 (4.19) ^a	0.014 ^b	0.614 ^c
Parity					
	0	1249	54.4	79.69 (8.73)	0.973
	1		38.4	79.72 (10.58)	
	≥2		7.2	79.60 (10.32)	
Type of delivery					
	Spontaneous	1182	79.0	79.46 (9.86)	0.015
	Induced		17.6	81.59 (8.59)	
	Caesarean		3.4	79.32 (8.85)	
Smoking at first trimester					
	No	1230	81.3	79.77 (9.71)	0.888
	Yes		18.7	79.87 (9.02)	
Exposure to sources of smoking					
	No exposure	1223	34.1	79.68 (10.42)	0.688
	1 exposure		40.7	79.53 (9.30)	
	2 exposures		22.4	80.45 (8.86)	
	≥3 exposures		2.8	78.85 (6.60)	
Area of residence					
	Rural	1249	7.5	77.99 (11.65)	<0.001
	Semi-urban		24.2	78.47 (8.79)	
	Urban		68.3	80.31 (9.56)	

Season of blood sampling				
	Spring		26.3	80.43 (9.13)
	Summer	1249	27.1	78.36 (8.72)
	Autumn		23.5	77.80 (9.67)
	Winter		23.1	82.35 (10.31)
Gestational age at sampling		1249	13.08 (1.21) ^a	-0.199
Seafood consumption (sv/d)				
	Lean fish		0.19 (0.20)	0.098 ^b
	Oily fish	1232	0.09 (0.10)	0.095 ^b
	Canned tuna		0.21 (0.21)	-0.014 ^b
	Processed lean fish		0.05 (0.13)	0.070 ^b
Se supplementation (µg/d)		1249	1.88 (6.20) ^a	0.077 ^b
Se supplementation (categorised)				
	Yes	1249	11.4	82.76 (7.86)
	No		88.6	79.30 (9.72)
Paternal variables				
Education				
	Up to primary		35.5	80.32 (8.72)
	Secondary	1241	43.7	79.25 (9.93)
	University		20.8	79.53 (10.22)
Paternal height (cm)		1242	176.30 (6.96) ^a	-0.021 ^b
Paternal BMI (kg/m ²)		1235	25.72 (3.35) ^a	0.059 ^b
Birth variables				
Sex				
	Female	1249	48.1	79.50 (9.91)
	Male		51.9	79.87 (9.29)
Season of delivery				
	Spring		23.3	78.26 (9.83)
	Summer	1249	22.4	81.77 (9.89)
	Autumn		27	80.90 (9.35)
	Winter		27.3	78.02 (9.35)
Hg (µg/L) in cord blood		983	11.20 (8.91) ^a	0.231 ^b
Categorised Hg in cord blood				
	< 15 µg/L	983	75.5	78.95 (9.55)
	≥15 µg/L		24.5	82.81 (9.14)
Outcome variables				
Gestational length (weeks)		1246	39.66 (1.56) ^a	-0.025 ^b
Preterm (<37 weeks of gestation)				
	No	1249	95.8	79.61 (9.55)
	Yes		4.2	81.52 (10.32)
Anthropometry				
	Birth weight (g)	1239	3342.67 (404.02) ^a	-0.020 ^b
	Birth length (cm)	1200	49.91 (1.88) ^a	0.087 ^b
	Birth head circumference (cm)	1201	34.51 (1.31) ^a	-0.072 ^b
Placental weight (g)		1144	609.82 (120.56) ^a	0.043 ^b

625^a Mean (standard deviation)

626^b Pearson correlation coefficient

627^c p-value of Pearson's test

Table 3. Multivariate linear regression analysis between maternal Se concentrations and anthropometric outcomes (n = 1249) with and without interaction with THg, sex, and season of blood collection.

	Main Se effect beta (95% CI), p	Se*THg p	Se*Sex p	Se*Season p
Birth weight (g)	-1.316 (-3.625 , 0.994), 0.264	0.164	0.590	0.766
Birth length (cm)	-0.001 (-0.012 , 0.009), 0.805	0.319	0.509	0.327
Head circumference (cm)	-0.007 (-0.014 , 0.001), 0.083•	0.075•	0.233	0.238
Placental weight (g)	0.026 (-0.712 , 0.764), 0.944	0.607	0.740	0.677
	Odds Ratio, p	Se*THg p	Se*Sex p	Se*Season p
Prematurity (yes/no)	1.015 (0.986 , 1.045), 0.312	0.767	0.025**	0.214
	Hazard Ratio, p	Se*THg p	Se*Sex p	Se*Season p
Gestational age (weeks)	1.007 (0.999 , 1.014), 0.061•	0.587	0.017**	0.827

p: p-value.

Values in parentheses are at 95% Confidence Interval

THg: total mercury. Categorised as < 15 vs. ≥15 µg/L according to the equivalent for the WHO Provisional Tolerable Weekly Intake (1.6 µg/kg of body weight per week).

All models adjusted for cohort.

Birth weight linear model additionally adjusted for parity, maternal smoking and exposure to smoking sources, age, BMI, height, and country of birth at evaluation, child sex, paternal BMI and height, and lean fish consumption.

Birth length linear model additionally adjusted for parity, maternal smoking and exposure to smoking sources, height, age, country of birth and educational level at evaluation, child sex and gestational age at evaluation, paternal height, blood sampling season, and lean, oily, canned tuna and processed lean fish consumption.

Head circumference linear model additionally adjusted for parity, maternal smoking, height, age, country of birth and educational level at evaluation, child sex, lean and oily fish consumption.

Placental weight linear model additionally adjusted for maternal BMI before pregnancy, parity, maternal working status at evaluation, parental social class, type of zone, maternal and paternal height, maternal country of birth and educational level, lean and oily fish consumption.

Prematurity logistic linear model additionally adjusted for maternal age, parity, maternal educational level.

Gestational age COX model additionally adjusted for parity, type of zone, maternal height, blood sampling season and lean fish consumption.

• p-value <0.10; * p-value <0.05; ** p-value <0.01; *** p-value <0.001.

648