Trace elements and APOE polymorphisms in pregnant women and their new-borns

Trdin, Ajda; Snoj Tratnik, Janja; Stajnko, Anja; Marc, Janja; Mazej, Darja; Sešek Briški, Alenka; Kastelec, Damijana; Prpić, Igor; Petrović, Oleg; Špirić, Zdravko; ...

Source / Izvornik: **Environment International, 2020, 143**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

https://doi.org/10.1016/j.envint.2020.105626

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:184:052289

Environment International 143 (2020) 105626

Contents lists available at ScienceDirect

Environment International

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

Trace elements and *APOE* polymorphisms in pregnant women and their new-borns

Ajda Trdin[a,b], Janja Snoj Tratnik[a], Anja Stajnko[a,b], Janja Marc[c], Darja Mazej[a], Alenka Sešek Briški[d], Damijana Kastelec[e], Igor Prpić[f,g], Oleg Petrović[f], Zdravko Špirić[h], Milena Horvat[a,b], Ingrid Falnoga[a,*]

[a] Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia
[b] Jožef Stefan International Postgraduate School, Ljubljana, Slovenia
[c] Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia
[d] Institute of Clinical Chemistry and Biochemistry, University Medical Centre Ljubljana, Ljubljana, Slovenia
[e] Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia
[f] Department of Paediatrics, University Hospital Centre Rijeka, Rijeka, Croatia
[g] Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[h] Green Infrastructure Ltd., Zagreb, Croatia

ARTICLE INFO

Handling Editor: Adrian Covaci

Keywords:
Apolipoprotein E polymorphism
ε4 allele
Pregnancy
Selenium
Mercury
Trace elements

ABSTRACT

We investigated the relationship between lipid binding glycoprotein apolipoprotein E (apoE; gene *APOE*) polymorphisms (*ε4* allele carriers versus no carriers = *ε4+/ε4−*) and trace elements (TEs) (e.g., (methyl) mercury, arsenic, lead, cadmium, selenium, manganese, copper, and zinc) in mothers (N = 223) and their new-borns (N = 213) exposed to potentially toxic metal(loid)s from seafood consumption. The apoE isoform encoded by the *ε4* allele is believed to have beneficial effects in early life but represents a risk factor for age-associated diseases. Under certain conditions *ε4* carriers are more susceptible to oxidative stress and metal(loid) toxicity. DNA from Croatian pregnant women (N = 223, third trimester) and their new-borns (N = 176), was genotyped for *APOE* by TaqMan® SNP assay – rs429358 and rs7412. Seafood intake data and TE levels in maternal urine, milk, hair, peripheral venous blood, mixed cord blood, and new-borns' urine were available from previous studies. We compared TEs between *ε4+* and *ε4−* carriers using Mann-Whitney U tests and applied multiple linear regression models to analyse the TE's dependence on the presence of allele *ε4* (genotypes *ε3/ε4*, *ε4/ε4*) in combination with other explanatory variables. We identified 17% (n = 37) and 20% (n = 35) *ε4* allele carriers in mothers and new-borns, respectively. The Mann-Whitney *U* test showed that mothers with the *ε4* allele had significantly higher mean levels of (methyl)mercury in peripheral venous blood, cord blood, and hair; arsenic in urine and cord blood; and selenium in peripheral venous blood and plasma. However, taking confounders into account, only the maternal plasma selenium remained statistically significant in the linear regression models (*ε4* carriers *vs* non-carriers: 62.6 *vs* 54.9 ng/mL, $p < 0.001$). Literature suggestions of possible *ε4* allele impact on Hg levels were not observed, while superior selenium status observed in healthy pregnant women carrying allele *ε4* could be linked to the proposed *APOE ε4* beneficial effects early in life.

1. Introduction

Apolipoprotein E (apoE, gene *APOE*) is a pleiotropic lipid binding plasma (and cellular) glycoprotein that plays a central role in general and neuronal lipid metabolism by directing lipid transfer, uptake, and excretion (Giau et al., 2015; Huang and Mahley, 2014). Some studies also point to its antioxidative, metal-binding, and immunomodulatory/anti-inflammatory roles (Egert et al., 2012; Jofre-Monseny et al., 2008; Vitek et al., 2009). It has three major isoforms — apoE2, apoE3, and apoE4 — encoded by the alleles *ε2*, *ε3*, and *ε4*, respectively. In general, the alleles frequencies are from 0 to 14% for *ε2*, 49–90% for *ε3* and 5–37% for *ε4* (Giau et al., 2015). Protein isoforms differ in one amino acid at two positions (apoE2: 112cys, 158cys; apoE3: 112cys, 158arg; and apoE4: 112arg, 158arg) and differently affect peripheral lipid and brain neuronal homeostasis (Huang and Mahley, 2014). The apoE4 isoform is believed to have several age-related disadvantages over

* Corresponding author.
E-mail address: ingrid.falnoga@ijs.si (I. Falnoga).

https://doi.org/10.1016/j.envint.2020.105626
Received 10 October 2019; Received in revised form 28 February 2020; Accepted 29 February 2020

apoE3 and apoE2, mostly attributed to its comparatively lower antioxidative effect (Miyata and Smith, 1996; Xu et al., 2014), and lower degradation and clearance capacity for β-amyloid in the brain, with the consequent higher risk of developing Alzheimer's disease (Giau et al., 2015). Alternatively, many consider apoE4 to be a global activator of the innate immune function, providing a high survival value in a population exposed to high levels of infectious diseases (Vitek et al., 2009). Further, experimental and epidemiological evidence suggest that apoE allele ε4 is associated with higher levels of serum cholesterol, vitamin D (Huebbe et al., 2011), and higher bone Ca assimilation (Egert et al., 2012) in the general population, and higher progesterone levels in women (Jasienska et al., 2015). These factors can be beneficial in early life and promote higher fertility in women. Specifically, optimal cholesterol levels are important for prenatal and postnatal neurodevelopment (myelination, synaptogenesis) and steroid hormone and vitamin production; while higher vitamin D and calcium levels are essential for bone growth. Smith et al. (2019) and Tuminello and Han (2011) recently published literature reviews of studies that support the hypothesis of the *ε4* allele's beneficial effects during different life stages. However, a few studies have suggested that *APOE* allele *ε4* carriers may be more susceptible to (methyl)mercury toxicity during early neuronal development (Ng et al., 2013; Snoj Tratnik et al., 2017), whereas the opposite was found for lead (Pb) (Wright et al., 2003). Similar inconsistencies relating to other apoE effects suggest that either apoE4 or *APOE ε4* may interact with various other factors (e.g., related gene polymorphisms, epigenetics, essential nutrient deficiency, pollutant exposure, or viral infections); consequently, its effects on a particular condition, such as Alzheimer's disease (AD) or neurodevelopment, will depend on a person's risk profile (Haas and Lathe, 2018; Nehls, 2016; Rea et al., 2016; Tuminello and Han, 2011).

The potential higher susceptibility to metal toxicity and oxidative stress of *ε4* carriers is based on experimental studies. *In vitro* studies have shown that different isoforms have different antioxidant properties for protecting against hydrogen-peroxide-induced oxidative stress, with *ε4* having the least and *ε2* having the most protective effect. Further, experimental studies also showed that apoE can bind metal ions such as copper, zinc, and iron (Miyata and Smith, 1996) and modify the expression of metal-regulating proteins, metallothioneins (Augsten et al., 2011; Florianczyk, 2007; Graeser et al., 2012). Previous research also suggests that apoE isoforms can affect the metabolic fate of metals and/or metal-related oxidative stress involved in AD and other disease aetiology (Egert et al., 2012; Jofre-Monseny et al., 2008; Xu et al., 2014). Mercury is among the metals of interest (Godfrey et al., 2003; Mutter et al., 2004). The general population is exposed to inorganic mercury (iHg) through dental amalgam fillings and to methylmercury (MeHg) through seafood consumption. Marine fish have some of the highest levels of nonessential metal(loid)s such as arsenic (although mostly in the nontoxic arsenobetaine form), mercury (mostly as methylmercury), and moderate amounts of lead and cadmium (Bosch et al., 2016). Marine fish also contain high levels of beneficial nutrients such as long-chain polyunsaturated fatty acids, zinc, and vitamin D, which are important for optimal prenatal neurodevelopment (Julvez et al., 2016) and the health of ageing individuals (Morris et al., 2016). Fish are also a source of the essential element selenium (Morris et al., 2016), which has several important physiological functions mediated by selenoproteins in the form of selenocysteine(s) (Pieczyńska and Grajeta, 2015; Rayman, 2012), such as redox regulation (i.e., thyoredoxine reductases), antioxidative functions (i.e., glutathione peroxidases, selenoprotein P), selenium transfer (selenoprotein P) and thyroid hormone regulation (i.e., iodothyronine deiodinases). Furthermore, the well-known mutual antagonism between selenium and arsenic or between selenium and mercury can diminish the toxic effects of mercury and arsenic (Falnoga and Tušek-Žnidarič, 2007; La Port, 2011; Liu et al., 2018; Zeng et al., 2005; Zhang et al., 2014).

Given the suggested mutual metabolic interferences between apoE and metals, and the impact of either on maternal health during pregnancy and prenatal neurodevelopment, we focused our attention on apoE isoforms and various trace elements (TEs). This study aimed to assess whether there is a relationship between *APOE* gene polymorphisms (*ε4* carriers versus no carriers) and trace elements in Croatian pregnant mothers and their new-borns from the coastal region of the Adriatic Sea, who have been chronically exposed to low to moderate amounts of environmental mercury and arsenic from seafood consumption (Miklavčič et al., 2013; Bernhard, 1988; Kosta et al., 1978). The trace elements included in this study were mercury (Hg; as total mercury (THg) and methyl mercury (MeHg)), arsenic (As), lead (Pb), and cadmium (Cd), along with the essential TEs selenium (Se), copper (Cu), zinc (Zn), manganese (Mn), calcium (Ca), magnesium (Mg), and iron (Fe),

2. Materials and methods

2.1. Study population

Mothers (n = 223; 19–44 years) and their new-borns (n = 213; 102 girls, 101 boys, 10 with missing sex classification) from the Adriatic coastal region of Croatia (Rijeka and its surroundings) were recruited in 2007–2009 as part of the wider birth cohort study PHIME (EU 6th Framework Programme).

The PHIME project (Public Health Impact of Long-term Low-level Mixed Element Exposure in Susceptible Population Strata) was designed to study metal exposure and its possible negative effects in people living in the Mediterranean area. Participants were recruited from four countries (Slovenia, Croatia, Italy, and Greece) (Valent et al., 2013b). In our previous investigations, we used cord blood Hg levels to estimate the impact of prenatal mercury exposure on motor, cognitive, and language performance in 18-month old children of the Croatian participants (Prpić et al., 2017), combined Slovenian-Croatian (Snoj Tratnik et al., 2017) and all PHIME participants (Barbone et al., 2019). Studies confirmed slight statistically significant alterations in fine motor skills associated with Hg levels in the Croatian and Slovenian-Croatian cohorts (Barbone et al., 2019; Prpić et al., 2017; Snoj Tratnik et al., 2017) and Hg-associated cognitive score alterations together with a co-effect of children's *APOE ε4* allele in the Slovenian-Croatian cohort (Snoj Tratnik et al., 2017). In the full set of PHIME participants, a similar alteration was observed in gross motor skills, but the significance was borderline (Barbone et al., 2019). Regarding other exposure biomarkers, a positive relationship was found between language composite scores and maternal hair Hg (in the full PHIME dataset) and with maternal venous blood Hg (Croatian sample) (Barbone et al., 2019). However, the maternal *APOE* allelic effect on Hg or other TE levels was not tested in any of the previous studies.

In the present study we used only the Croatian dataset, which has the most complete data. The study design (settings, recruitment, exclusion criteria, questionnaires, and biological sampling) are described in detail in previous publications (Miklavčič et al., 2013; Valent et al., 2013a, 2013b). The study was conducted in accordance with the Declaration of Helsinki and its later amendments; the Ethics Committee of the University Hospital Centre Rijeka approved the protocol for the Croatian participants (No. 2170-29-02/1-07-1). Mothers gave their informed consent; recruitment and sampling took place at the University Hospital Rijeka. Participants were sampled during the third trimester of pregnancy for maternal peripheral venous blood (whole blood, plasma, and serum), morning urine, and hair (1–3 cm closest to scalp); at delivery for mixed cord blood (whole blood, plasma and serum), cord tissue, and new-borns' first urine; and one month after delivery for maternal breast milk.

2.2. Study database

Table 1 summarises the basic study protocols for the collected samples, including sampling time, analysed TEs with limits of

Table 1
Basic sampling and analytical data for Croatian cohort of mothers (N = 223) and their new-borns (N = 213) (PHIME project subgroup).

Sampling time and participants' samples	TE analysed$_{(LOD)}$	DNA extraction	N of analysed samples (%)
3rd TRIMESTER			
Maternal hair	$Hg_{(0.2\ ng/g)}$, $MeHg^{d}_{(0.02\ ng/g)}$		222 (99.6)
Maternal blood[a]	$Hg_{(0.02\ ng/g)}$, $MeHg_{(0.02\ ng/g)}$, $Pb_{(1.3\ ng/g)}$, $Cd_{(0.12\ ng/g)}$, $As_{(0.13\ ng/g)}$, $Se_{(5\ ng/g)}$, $Mn_{(2\ ng/g)}$, $Cu_{(11ng/g)}$, $Zn_{(20\ ng/g)}$		223 (100)
Maternal plasma	$Se_{(0.16\ ng/mL)}$, $Zn_{(1.6\ ng/mL)}$		212 (95.1)
Maternal serum	Fe, Mg, Ca		209 (93.7)
Maternal leukocytes	–	Blood DNA	223 (100)
Maternal urine[b]	$Hg_{(0.01\ ng/mL)}$, $MeHg^{d}_{(0.003\ ng/mL)}$, $Pb_{(0.1\ ng/mL)}$, $Cd_{(0.04ng/mL)}$, $As_{(0.4\ ng/mL)}$, $Se_{(2.8\ ng/mL)}$, $Mn_{(0.2\ ng/mL)}$, $Cu_{(1\ ng/mL)}$, $Zn_{(2\ ng/mL)}$, SG,		220 (98.7)
AT DELIVERY			
Cord blood[c]	$Hg_{(0.02\ ng/g)}$, $MeHg_{.(0.02\ ng/g)}$ $Pb_{(1.3\ ng/g)}$, $Cd_{(0.12\ ng/g)}$, $As_{(0.13\ ng/g)}$, $Se_{(5\ ng/g)}$, $Mn_{(2\ ng/g)}$, $Cu_{(11\ ng/g)}$, $Zn_{(20\ ng/g)}$		205 (91.9)
Cord plasma	$Se_{(0.16\ ng/mL)}$, $Zn_{(1.6\ ng/mL)}$		190 (85.2)
Cord serum	Fe, Mg, Ca		184 (82.5)
Cord tissue	–	Tissue DNA	176 (82.6)
New-born's first urine	$Hg_{(0.02\ ng/mL)}$, SG		126 (59.2)
1 MONTH POST-DELIVERY			
Maternal breast milk	$Hg_{(0.045\ ng/g)}$, $MeHg^{d}_{(0.003\ ng/g)}$, $Pb_{(0.4\ ng/g)}$, $Cd_{(0.04\ ng/g)}$, $As_{(0.04\ ng/g)}$, $Se_{(0.9\ ng/g)}$, $Mn_{(0.4\ ng/g)}$, $Cu_{(6\ ng/g)}$, $Zn_{20ng/g)}$		123 (55.2)

N – Sample size; LOD – Limit of detection; SG – Specific gravity; a – Peripheral venous blood;
b – Morning urine; c – Mixed venous-arterial blood; d – MeHg analysed in cases when total Hg in maternal hair exceeded 1 μg/g.

detection, and DNA extracts. The obtained TE database consisted of nonessential Hg (as THg and MeHg), As, Pb, and Cd, and essential Se, Cu, Zn, Mn, Ca, Mg, and Fe. The TE levels were determined at the Jožef Stefan Institute (JSI, Ljubljana, Slovenia) and the University Medical Centre Ljubljana (UMCL, Institute of Clinical Chemistry and Biochemistry, Ljubljana, Slovenia) using the methods described below.

Hg levels in maternal hair, blood, and cord blood and in the new-borns' urine were determined by thermal combustion at 650 °C, amalgamation and analysis using a direct mercury analyser (DMA; Milestone, USA) (EPA and US EPA (US Environmental Protection Agency), 2007; Miklavčič et al., 2013). Hg in maternal milk and urine was determined using a semi-automated mercury analyser based on cold vapour atomic absorption spectrometry (CVAAS, Model Hg-201; Sanso Seisakusho Co. Ltd., Japan) (Miklavčič et al., 2013).

MeHg in hair was measured by gas chromatography with electron capture detection (GC-ECD, Hewlett-Packard Model 5890; HP/Agilent Technologies; USA) (Horvat and Byrne, 1990). MeHg in maternal blood, cord blood, and breast milk was measured by cold vapour atomic fluorescence detection (CV AFS, Tekran 2700 instrument; Tekran Instruments Corporation, Canada and Brooks Rand Model III, Brooks Rand Instruments, USA) (Liang et al., 1994).

Hg^{2+} levels in blood, cord blood, hair, and milk were obtained by subtracting MeHg from THg.

As, Cd, Pb, Se, Cu, and Zn in blood, cord blood, breast milk, and urine were previously prepared (Barany et al., 1997; Miklavčič et al., 2013) and analysed by inductively coupled plasma mass spectrometry (ICP-MS; 7500ce, Agilent, Tokyo, Japan) equipped with an ASX-510 Auto sampler (Cetac). The mass spectral interferences were eliminated using an Octapole Reaction System (ORS) with helium, or hydrogen in case of Se determination.

Se in plasma was measured using a Zeeman electrothermal AAS (Varian SpektrAA-800 ETAA spectrometer; Varian Australia Pty. Ltd., Mulgrave, Victoria, Australia) (Kobal et al., 2004).

Zn in plasma was measured by flame AAS (FAAS, deuterium) using a Varian SpektrAA-250 Plus FAAS (Varian Australia Pty. Ltd., Mulgrave, Victoria, Australia) (Tsalev and Zaprianov, 1983).

Ca, Mg, and Fe(III) in serum were determined spectrophotometrically using their complexes with diazonium salt xilidyl blue (for Mg at 505–660 nm), o-cresolphtalein (for Ca at 546 and 660 nm) and ferrozine (for Fe at 570 nm). All measurements were performed on a Roche Hitachi 917 analyser calibrated by C.f.a.s. (Roche, Switzerland). To control the method accuracy and precision, we used Control Sera presenting normal ranges (PerciNorm U, Roche) and pathological ranges (PerciPath U, Roche) of the analysed essential elements.

Urine concentrations were adjusted for specific gravity (SG) using an Atago® PAL-10S Refractometer (Japan). Adjustments were performed according to Santonen et al. (2015) as follows: $c_s = c_o \times (SG_{ref} - 1.000)/(SG_o - 1.000)$, where c_s is the corrected concentration; c_o, the observed concentration; SG_{ref}, the SG reference value; and SG_o, the observed specific gravity. SG_{ref} is a population reference value representing normal or undiluted urine. SG_{ref} was 1.021, that is, the middle value of the SG reference range of 1.018–1.024 for the general population. For multivariate statistics, we used raw urine data SG_o as the independent variable.

All measurements were performed under strict quality control procedures and gave comparable results. The limits of detection (LODs), calculated as three times the standard deviation of the blank sample, are included in Table 1. The reference materials Seronorm™ Trace Elements in Whole Blood L-1, and Seronorm™ Trace Elements in Serum L-1 were used to check the accuracy of the methods. Becton Dickinson tubes for trace elements (7 mL) were used for blood sampling.

Personal characteristics (e.g., age, pre-pregnancy body mass index (BMI), parity, estimated gestation week at sampling (EGW) and at delivery (EGA)), and lifestyle data (e.g., seafood consumption, smoking habits, education, employment, supplement intake) were obtained through two subsequent questionnaires: the first during pregnancy and the second during breastfeeding. Parity was defined as the number of times a participant carried a pregnancy to a viable stage (> 20 weeks of gestation). Self-reported smokers during pregnancy (8.5%) and self-reported former smokers (32%; defined as those quitting smoking immediately before or at the beginning of the pregnancy) were combined in the group 'ever-smokers' (40.5%). Daily seafood intake was estimated from the second questionnaire with several questions on seafood that addressed the frequency (never, 1x/month, 1–3x/month, 1x/week, 2–4x/week, 5–6x/week, 1x/day, 2–3x/day, > 3x/day) that 150 g servings of fish, crustaceans, molluscs (boiled, grilled, fried, baked, or in oil) was consumed. Selected characteristics data stratified by maternal genotyping results (presence versus absence of *ε4* allele) are presented in the results (Section 3.2).

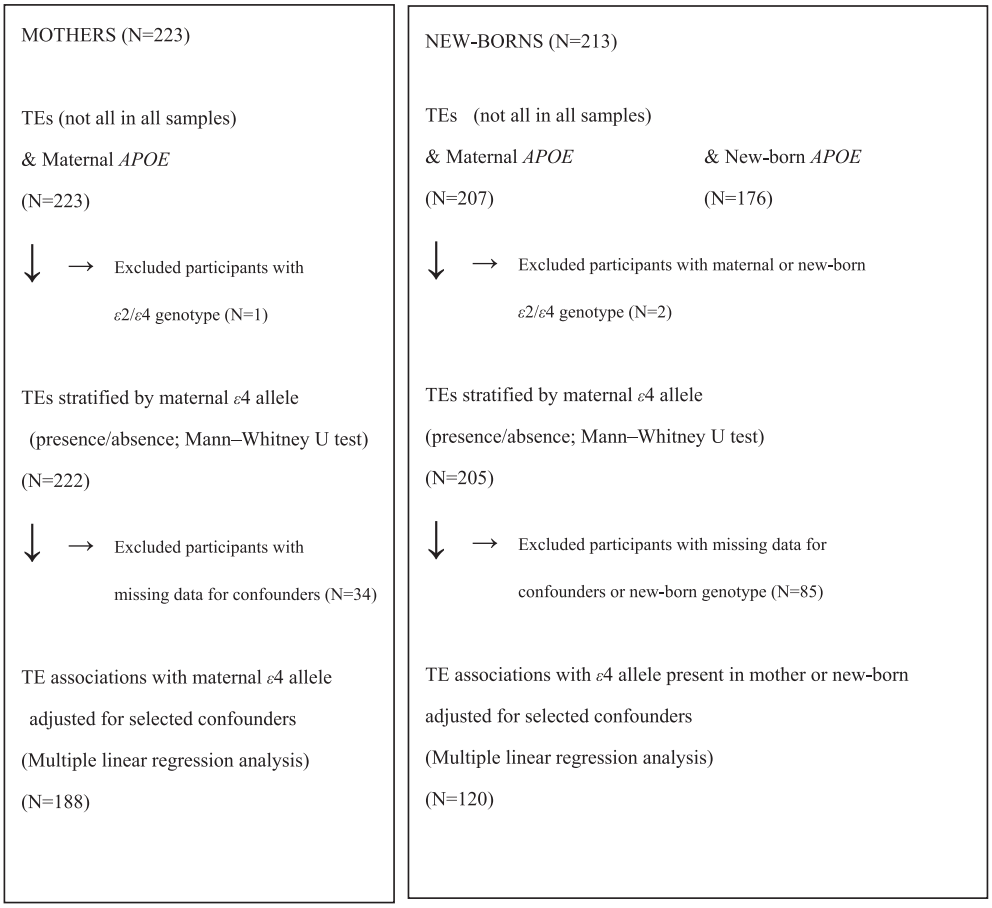

Fig. 1. Flow chart on participants included in statistical analyses.

2.3. APOE genotyping

Archived high molecular DNA extracts (stored at −80 °C) from maternal leukocytes and umbilical cord tissues were provided by the Faculty of Pharmacy, University of Ljubljana. DNA extraction was performed using a High Pure PCR Template Preparation Kit (Roche) and a QIA amp DNA Mini Kit (Qiagen, USA). The DNA was genotyped using a TaqMan® pre-designed SNP genotyping assay (small scale) with C_3084793_20 for rs429358 and C_904973_10 for rs7412 (Applied Biosystems, Foster City, Ca, USA) according to the given procedure. A LightCycler 480 II (Roche) was used to identify polymorphisms: rs429358 (c.334 T > C; Cys112Arg) and rs7412 (c.472C > T; Arg158Cys). Based on these results, we determined the genotype of each individual. The distribution of the *APOE* genotype frequencies were in Hardy-Weinberg equilibrium ($p > 0.05$; Chi-square test). Mothers and new-borns were then divided into *ε4* carriers (genotypes *APOE ε3/ε4* and *ε4/ε4*) versus *ε4* non-carriers (genotypes *APOE ε3/ε3*, *ε3/ε2*, and *ε2/ε2*). Carriers of the genotype *ε2/ε4* were excluded from both groups because specific properties of *ε4* are weakened when the *ε4* allele is combined with the *ε2* allele; apoE2/4 proteins are suspected to functionally resemble apoE3/3 proteins.

2.4. Statistical analyses

First, we divided all samples into two groups, *ε4+* and *ε4−*, according to maternal genotype. The distribution of TEs in the two groups was compared using the Mann-Whitney *U* test. The influence of *ε4* on TE concentration controlling for the other explanatory variables (obtained through questionnaires) was examined using multiple linear regression models. All dependent variables, such as TE levels in maternal blood, serum/plasma, hair, urine, and milk were normal log-transformed to avoid heteroscedasticity. The independent variables included the presence of maternal *APOE* allele *ε4*, presence of parity, maternal pre-pregnancy BMI, maternal age, seafood intake (servings/day), ever-smoking, and urine SG_o. We extended independent variables for mixed cord blood and cord plasma/serum with newborns' characteristics: EGA, birth weight, and sex. According to Gundacker et al. (2000), *"the maternal genotype determines the placental environment (…), while the foetal genotype determines the capacity of the placenta to cope with this environment"*. Given the supposed complementary effects of maternal/foetal genotype on (mixed) cord blood and placenta, we analysed the combined effect of maternal/foetal *APOE* allele ε4 using the presence of at least one allele *ε4* in either the mother or new-born as an explanatory variable. A flow chart of the number of participants included in the statistical analyses is shown in Fig. 1.

Multiple regression model diagnostics were performed based on an analysis of the residuals. All models were checked for collinearity. The *p*-values for the individual effect of each explanatory variable were corrected according to the simultaneous testing of numerous null hypotheses using the glht function from the multcomp package in the R statistical package (Team, 2017). All TEs were log-transformed for regression modelling, which means that an estimate of the model parameter multiplied by 100 represents the percentage change in the TE levels for a one-unit increase in each numeric explanatory variable (e.g., 1 seafood serving/day, 1 year for age, etc.) given that all other explanatory variables remain constant. For binary explanatory variables (presence/absence), an estimate of the model parameter multiplied by 100 represents the percentage difference between the two groups. The coding for binary variables (0/1; no/yes) was as follows: *APOE ε4* = 0 for non-carriers, and *APOE ε4* = 1 for carriers; parity = 0

Table 2
Selected characteristics for Croatian cohort of mothers and new-borns stratified by maternal *APOE* ε4 allele*.

Participants	All	N (%)	ε4+	N (%)	ε4−	N (%)	p
	x ± SD (range)		x ± SD (range)		x ± SD (range)		
MOTHERS (m)		222		37		185	
mAge	30.1 ± 4.8	209	30.8 ± 4.6	36	29.9 ± 4.8	173	0.143
(years)	(19 – 44)		(20 – 38)		(19 – 44)		
mBMI	23.0 ± 4.2	220	22.3 ± 3.2	36	23.2 ± 4.3	184	0.334
(kg/m^2)	(17 – 41)		(18 – 32)		(17 – 41)		
mSeafood intake	0.45 ± 0.3	195	0.54 ± 0.3	33	0.43 ± 0.3	162	**0.038**
(servings/day)	(0 – 2.2)		(0.1 – 1.2)		(0 – 2.2)		
mParity**	(0 – 3)	220	(0 – 2)	37	(0 – 3)	183	0.835
0		125 (56.8)		21 (56.8)		104 (56.8)	
1–3		95 (43.2)		16 (43.2)		79 (43.2)	
mEver-smoking		222		37		185	0.463
Yes		90 (40.5)		13 (35.1)		77 (41.6)	
No		132 (59.5)		24 (64.9)		108 (58.4)	
mEducation***		195		32		163	0.352
Primary		4 (2.1)		0 (0)		4 (2.5)	
Secondary or more		191 (97.9)		32 (100)		159(97.5)	
mEmployment		192		31		161	0.556
Yes		174 (91.6)		29 (93.5)		145 (90.1)	
No		18 (9.4)		2 (6.5)		16 (9.9)	
mUse of supplements (yes)		139		24		115	
mEGW	37 ± 2.5	148	36.9 ± 1.8	22	37.1 ± 2.6	126	0.770
(weeks)	(35 – 41)		(35 – 40)		(35 – 41)		
NEW-BORNS (nb)		207		34		173	
nbEGA	39.4 ± 1.2	165	39.1 ± 1.4	25	39.5 ± 1.2	140	0.205
(weeks)	(35 – 41)		(36 – 41)		(35 – 41)		
nbBW	3.52 ± 0.5	192	3.60 ± 0.6	33	3.50 ± 0.4	159	0.704
(kg)	(1.8 – 4.8)		(2.5 – 4.7)		(2.4–4.8)		
nbSex		203					
Boys		101 (49.8)		18 (50)		83 (49.7)	
Girls		102 (50.2)		18 (50)		84 (50.3)	

x – arithmetical mean; SD – standard deviation; *p* – statistically significant difference between groups according to maternal genotype (*ε4+* and *ε4−*), Mann-Whitney *U* test for all except for parity, education, employment, and ever-smokers, where χ^2 test was used; BMI – pre-pregnant body mass index; EGW – estimated gestation week at maternal sampling at 3rd trimester; EGA – estimated gestational age at delivery; BW – birth weight.

* Excluded were one mother with maternal *ε2/ε4* genotype and three new-borns with either maternal or new-born's *ε2/ε4* genotype.

** Parity – number of deliveries after 20th gestation week.

*** Education defined by two groups: primary with elementary diploma and secondary or higher (middle school diploma + high school diploma + university degree).

for absence of deliveries, and parity = 1 for 1–3 deliveries; ever-smoking = 0 for non-smoking, and ever-smoking = 1 for smoking during or before pregnancy; and sex = 0 for boys, and 1 for girls.

EGW at sampling in the third semester, supplement intake, education level, and employment data (yes/no) were not included in the explanatory variables to avoid over-fitting the regression models or diminishing the number of participants. EGW and supplement intake were available for a relatively low subset of samples (148 and 139, respectively). Further, we had a population with known low social differences: the majority of participants were employed (78%) and had finished at least middle school (98%). All participants had finished elementary school. In pre-testing models, the addition of EGW or education level (primary versus secondary or more) together with employment (yes/no) or supplement intake did not modify the associations between *APOE* and TEs.

3. Results

3.1. Frequencies of APOE genotypes and alleles

Frequencies of six possible genotypes (% *ε2/ε2*, % *ε2/ε3*, % *ε2/ε4*, % *ε3/ε3*, % *ε3/ε4*, % *ε4/ε4*) and individual alleles (% *ε2*, % *ε3*, % *ε4*) are listed in the supplemental material (Table S1). The frequencies were similar for mothers and their new-borns and geographically matched those reported in the literature (Giau et al., 2015; Huebbe and Rimbach, 2017). Genotype *ε3/ε3* was the most prevalent (74% of mothers, 68.2% of new-borns) followed by *ε3/ε4* (16.1% of mothers, 17.6% of new-borns) and *ε2/ε3* (8.5% of mothers, 10.8% of new-borns). The other three genotypes, *ε2/ε2*, *ε2/ε4* and *ε4/ε4* were present in minor or even zero percentages (0.5%, 0.5%, and 0.5% of mothers; 0%, 1.1% and 2.4% of new-borns). Excluding the *ε2/ε4* genotype (n = 3), we identified 16.7% (n = 37) and 20.1% (n = 35) of mothers and new-borns, respectively, to be *ε4* allele carriers.

3.2. Participants' personal characteristics stratified by maternal ε4 allele

Table 2 lists the basic personal characteristics of the mothers and new-borns. Only one mother and two new-borns were omitted for having the genotype *ε2/ε4*. For new-borns, data for all cases with the identified genotype of their mother (but their own genotype could be missing) were included. The average maternal age was 30.1 ± 4.7 years and EGA at delivery was 39.4 ± 1.2 weeks. The majority of women were *nulliparous* or *primiparous* (56% and 37%, respectively). Except for seafood intake (servings/day), the distribution of the personal characteristics did not differ significantly by the presence or absence of maternal *ε4* alleles. The estimated seafood frequency intake was higher in mothers carrying the *ε4* allele (p = 0.038, Table 2). Employment and education status were relatively high overall, as mentioned in Section 2.4.

3.3. TE levels stratified by maternal ε4 allele

Table 3 lists mothers' and new-borns' TE levels stratified by the presence or absence of maternal *ε4* allele. The mean blood levels for

Table 3
TE levels in samples for Croatian cohort of mothers and their new-borns stratified by maternal *APOE* ε4 allele[a].

TE	*ε4+*	*ε4−*	All	p
	GM, range, Me, (N)	GM, range, Me, (N)	GM, (N)	
MATERNAL BLOOD				
mB $Hg_{ng/g}$	**2.67, 0.59–9.83, 2.85 (37)**	**2.03, 0.55–20.5, 1.93 (184)**	**2.12 (221)**	**0.0111**
mB $MeHg_{ng/g}$	**2.13, 0.12–8.90, 2.84 (37)**	**1.38, 0.03–19.6, 1.64 (184)**	**1.47 (221)**	**0.0105**
mB $IHg_{ng/g}$	0.17, 0.01–1.19, 0.33 (37)	0.18, 0.01–12.6, 0.37 (184)	0.12 (221)	0.8554
mB $Pb_{ng/g}$	12.8, 5.25–53.6, 12.78 (35)	12.0, 3.58–87.6, 11.55 (180)	12.1 (215)	0.4909
mB $Cd_{ng/g}$	0.50, 0.06–3.70, 0.47 (35)	0.47, 0.06–3.69, 0.51 (180)	0.48 (215)	0.7294
mB $As_{ng/g}$	**3.31, 0.42–29.2, 3.96 (35)**	**2.02, 0.25–37.3, 1.76 (180)**	**2.19 (215)**	**0.0144**
mB $Se_{ng/g}$	**99.4, 67.9–142, 99.4 (35)**	**90.1, 42.4–182, 89.2 (180)**	**91.6 (215)**	**0.0080**
mB $Mn_{ng/g}$	13.7, 7.45–26.5, 13.8 (35)	15.3, 3.80–49.4, 15.0 (180)	15.1 (215)	0.0850
mB $Cu_{μg/g}$	1.49, 1.06–2.02, 1.53 (35)	1.56, 0.91–2.51, 1.56 (180)	1.55 (215)	0.2719
mB $Zn_{μg/g}$	6.21, 4.58–10.27, 6.03 (35)	6.19, 2.90–11.04, 6.12 (180)	6.19 (215)	0.7283
MATERNAL PLASMA				
mP $Se_{ng/mL}$	**61.7, 41.0–96.0, 62.0 (36)**	**53.7, 33.0–90.0, 54.0 (176)**	**55.0 (212)**	**0.0006**
mP $Zn_{μg\ /mL}$	0.75, 0.52–1.08, 0.74 (36)	0.76, 0.46–1.14, 0.73 (176)	0.76 (212)	0.8137
MATERNAL SERUM				
mS $Ca_{μg\ /mL}$	89.4, 29.7–104.6, 92.2 (35)	89.8, 50.5–117.4, 91.0 (174)	89.7 (209)	0.2357
mS $Mg_{μg\ /mL}$	16.4, 5.10–20.7, 16.8 (35)	16.6, 9.48–20.9, 16.8 (174)	16.6 (209)	0.5745
mS $Fe_{μg\ /mL}$	0.79, 0.20–4.94, 0.83 (35)	0.85, 0.22–2.94, 0.86 (174)	0.84 (209)	0.4169
MATERNAL URINE (SG corrected)				
mU $Hg_{ng/mL}$	0.81, 0.16–5.94, 0.69 (33)	0.73, 0.03–30.8, 0.66 (141)	0.74 (180)	0.5431
mU $Pb_{ng/mL}$	0.83, 0.08–3.58, 0.93 (31)	0.73, 0.04–8.33, 0.95 (141)	0.75 (172)	0.9238
mU $Cd_{ng/mL}$	0.38, 0.03–1.48, 0.44 (31)	0.40, 0.04–1.98, 0.42 (141)	0.40 (172)	0.9033
mUAs $_{ng/mL}$	**42.6, 2.83–260, 66.4 (31)**	**23.3, 1.58–1679, 22.5 (141)**	**26.0 (172)**	**0.0233**
mU $Se_{ng/mL}$	25.2, 5.98–56.6, 28.5 (31)	26.5, 3.71–73.8, 28.6 (141)	26.3 (172)	0.9381
mU $Mn_{ng/mL}$	0.52, 0.08–19.1, 0.41 (31)	0.40, 0.06–22.7, 0.36 (144)	0.42 (176)	0.9142
mU $Cu_{μg/mL}$	0.017, 0.002–0.057, 0.018 (31)	0.020, 0.008–0.545, 0.019 (141)	0.019 (172)	0.4245
mU $Zn_{μg/mL}$	0.37, 0.59–1.36, 0.34 (31)	0.49, 0.066–2.96, 0.49 (141)	0.46 (172)	0.0828
mU SG	1.0163, 1.002–1.029, 1.016 (33)	1.0159, 1.005–1.035, 0.016 (149)	1.016 (182)	0.6529
MATERNAL MILK				
mM $Hg_{ng/g}$	0.19, 0.04–1.82, 0.20 (25)	0.13, 0.01–2.36, 0.13 (98)	0.14 (123)	0.1411
mM $MeHg_{ng/g}$	0.17, 0.09–0.33, 0.17 (6)	0.14, 0.04–0.55, 0.14 (19)	0.15 (25)	
mM $IHg_{ng/g}$	0.09, 0.01–0.46, 0.12 (6)	0.07, 0.01–0.9, 0.09 (19)	0.08 (25)	
mM $Pb_{ng/g}$	0.92, 0.20–5.70, 1.08 (24)	0.54, 0.20–11.1, 0.52 (97)	0.60 (121)	0.0572
mM $Cd_{ng/g}$	0.12, 0.02–1.12, 0.12 (24)	0.10, 0.02–6.54, 0.10 (97)	0.10 (121)	0.3690
mM $As_{ng/g}$	0.25, 0.02–2.18, 0.21 (23)	0.21, 0.02–19.0, 0.21 (97)	0.22 (120)	0.6412
mM $Se_{ng/g}$	18.6, 8.70–29.0, 18.5 (24)	18.3, 8.43–48.8, 17.5 (97)	18.4 (121)	0.4908
mM $Mn_{ng/g}$	3.38, 1.10–22.0, 2.94 (24)	2.66, 0.67–17.1, 2,82 (97)	2.79 (121)	0.2608
mM $Cu_{μg/mL}$	0.55, 0.24–0.86, 0.61 (24)	0.48, 0.14.1.05, 0.50 (97)	0.49 (121)	0.0898
mM $Zn_{μg/mL}$	2.92, 0.73–9.73, 3.15 (24)	2.48, 0.21–9.37, 2.63 (97)	2.56 (121)	0.1936
MATERNAL HAIR				
mH $Hg_{μg/g}$	**0.74, 0.08–3.45, 0.84 (37)**	**0.48, 0.02–8.71, 0.56 (185)**	**0.51 (222)**	**0.0107**
mH $MeHg_{μg/g}$	1.76, 1.15–3.33, 1.74 (10)	1.78, 0.93–8.71, 1.65 (35)	1.78 (45)	
mH $IHg_{ng/g}$	1.82; 0.1–135; 4.0 (10)	5.30; 0.1–1302; 30.5 (35)	4.18 (45)	
CORD BLOOD				
cB Hg $_{ng/g}$	**4.01, 0.82–21.8, 3.96 (34)**	**2.74, 0.33–32.3, 2.83 (171)**	**2.92 (205)**	**0.0137**
cB $MeHg_{ng/g}$	**3.59, 0.29–21.7, 3.96 (34)**	**2.26, 0.16–31.9, 2.70 (171)**	**2.44 (205)**	**0.0181**
cB $IHg_{ng/g}$	0.07, 0.01–2.60, 0.08 (34)	0.07, 0.01–4.79, 0.10 (171)	0.07 (205)	0.7897
cB $Pb_{ng/g}$	8.64, 3.23–17.7, 877 (34)	7.87, 1.82–34.1, 8.19 (168)	8.00 (202)	0.9407
cB $Cd_{ng/g}$	0.08, 0.06–0.30, 0.06 (34)	0.09, 0.06–1.21, 0.06 (168)	0.09 (202)	0.9407
cB $As_{ng/g}$	**2.60, 0.29–25.7, 2.59 (34)**	**1.84, 0.33–31.8, 1.42 (168)**	**1.95 (202)**	**0.0475**
cB $Se_{ng/g}$	94.4, 64.7–132, 90.1 (34)	94.4, 54.8–163, 96.0 (168)	94.4 (202)	0.9231
cB $Mn_{ng/g}$	29.7, 16.0–53.9, 29.2 (34)	30.7, 7.23–77.3, 31.6 (168)	30.5 (202)	0.5034
cB $Cu_{μg/g}$	0.69, 0.50–0.99, 0.68 (34)	0.69, 0.36–1.16, 0.69 (168)	0.69 (202)	0.9078
cB$Zn_{μg/g}$	**2.13, 1.58–3.18, 2.07 (33)**	**2.30, 1.16–4.72, 2.21 (167)**	**2.27 (200)**	**0.0456**
CORD PLASMA				
cP $Se_{ng/mL}$	41.1, 25.0–65.0, 41.5 (30)	40.8, 24.0–71.0, 42.0 (160)	40.8 (190)	0.9509
cP $Zn_{μg\ /mL}$	0.91, 0.55–1.38, 0.92 (30)	0.99, 0.39–1.77, 0.99 (159)	0.98 (198)	0.1023
CORD SERUM				
cS $Ca_{μg\ /mL}$	101.5, 63.3–144.7, 104.6 (31)	101.5, 60.1–127.1, 103.4 (153)	101.5 (184)	0.7631
cS $Mg_{μg\ /mL}$	20.3, 16.3–27.0, 19.7 (31)	19.9, 14.6–27.2, 19.7 (152)	19.9 (183)	0.6713
cS $Fe_{μg\ /mL}$	1.45, 0.75–2.87, 1.44 (31)	1.33, 0.28–2.45, 1.37 (153)	1.35 (184)	0.3207
NEW-BORN URINE (SG corrected)				
nb U $Hg_{ng/mL}$	0.45, 0.10–1.76, 0.60 (15)	0.31, 0.04–8.64, 0.37 (73)	0.33 (88)	0.0542
nb U SG	1.010, 1.002–1.019, 1.009 (18)	1.012, 1.002–1.029, 1.013 (83)	1.012 (101)	0.1285

GM – geometrical mean; Me – median; N – sample size; SG – specific gravity; p – statistical significance of difference between *ε4* carriers and non-carriers obtained using the Mann-Whitney *U* test; bolded p $<$ 0.05; m – maternal; c – cord; nb – new-born child; B – blood; P – plasma; S – serum; U – urine; M – milk; H – hair.

* Excluded were one mother with maternal *ε2/ε4* genotype and three new-borns with either maternal or new-born *ε2/ε4* genotype.

potentially toxic metals such as Hg, Cd, and Pb were low in general. MeHg accounted for an average of 80% of the total mercury in maternal and cord blood (mean value). Almost all urine arsenic was in the nontoxic arsenobetaine form (90% of total As) (Stajnko et al., 2019). Breast milk had low mean levels of Hg, Pb, Cd, and As, meaning that breastfed infants were not at risk in either group. Selenium levels in breast milk in both groups were almost equal. The mean values of all essential elements were within UMCL laboratory reference levels.

Statistically significant differences in the distribution of TEs between the maternal *ε4+* and *ε4−* groups were observed for As, (Me)Hg, Se, and Zn (Table 3). Mothers with the *ε4* allele (genotypes *APOE* ε3/ε4 and *ε4/ε4*) had significantly higher levels ($p < 0.05$) of (Me)Hg, As, and Se in blood; Se in plasma; Hg in hair; As in urine; and (Me)Hg and As in cord blood. Although Zn levels in cord blood were significantly lower in the *ε4+* group, this has low importance because plasma/serum levels are the only recommended biochemical indicator for Zn status assessment (Gibson et al., 2008). Fig. 2 shows the distribution of each mentioned TE in boxplots. There was little difference in the Hg and As levels in maternal blood (2.67 *vs* 2.03 ng/g for Hg and 3.31 *vs* 2.02 ng/g for As (geometrical means)), while the differences in Se were more pronounced (99.4 *vs* 90.1 ng/mL for maternal blood and 61.7 *vs* 53.7 ng/mL for maternal plasma). Larger differences were observed in the distribution of Hg in maternal hair (0.74 *vs* 0.48 μg/g) and As in maternal urine (42.6 *vs* 23.3 ng/mL).

3.4. Linear regression models

Tables 4 and 5 summarise the statistically significant associations between TEs and *APOE ε4* and other independent variables obtained by the linear regression models. For maternal samples, we used the presence of the maternal *ε4* allele; for new-borns, the combined involvement of maternal and foetal *ε4* allele was considered (at least one *ε4* allele in mother or child versus none). Estimates for TEs without any statistically significant or marginally significant association are not presented (e.g., Pb, Mn, Cu, Zn, Ca, Mg, and Fe).

The differences previously observed by simple comparison shown in Fig. 2 and Table 3 (Mann-Whitney *U* test) were nearly all lost in the regression models, except for maternal plasma selenium. The association between the ε4 allele and maternal plasma selenium persisted even after considering the influence of parity, maternal BMI, age, seafood intake, and ever-smoking (Table 4). The presence of the *ε4* allele was associated with a 12% increase in the mean selenium level in maternal plasma (95% CI 1.2–22.7%). Further, the regression models did not reveal any new influence of the maternal and/or new-born *ε4* allele on the TE levels in any sample type (Tables 4 and 5). However, they did confirm modifying effects of five explanatory variables on TE levels: positive effects of seafood intake $>$ ever-smoking $\gg$ and age, and negative effect of parity $\gg$ and BMI on (Me)Hg, As, Se, or Cd levels.

In total, the models explained 10%–39% of the variation (R^2) in the TE levels: ~30% in the case of (Me)Hg in maternal and cord blood and maternal hair, ~20% for As in maternal and cord blood and maternal urine, and 12% for As in breast milk. They also explained 14% and 10% of the variation of Se in maternal blood and plasma, respectively, and ~22% and ~33% of the variation of Cd in maternal blood and urine, respectively.

The associations between *APOE* polymorphisms and metalloids were additionally tested by considering the presence of either foetal (new-born) or maternal *ε4* allele separately (data not shown). No significant associations were observed.

3.4.1. Influence of confounders

Seafood intake had a significant positive influence on (Me)Hg levels in maternal and cord blood and on Hg in maternal hair. It also had a significant positive influence on As levels in maternal and cord blood, maternal urine, and breast milk. The influence of seafood on As excretion through urine and milk was most likely from biologically inactive and readily excreted arsenobetaine, known to be present in seafood (Taylor et al., 2017) and previously identified in high percentages of total As in the urine samples of the present study (Stajnko et al., 2019). In general, we estimated that an extra serving of seafood per day (150 g) resulted in a 92.4% (95% CI 53.5–131.3%) increase in maternal blood Hg levels. For other mentioned samples and both metals, As and (Me)Hg, the increase was similar or even higher.

The impact of ever-smoking resulted in a 60.4% (95% CI 31.5–89.3%) increase in the blood Cd level. At the same time, an increase in age (1 year) resulted in a 5.3% increase in urine Cd levels (95% CI 1.4–9.2%).

Inverse associations were observed for parity (with maternal blood Hg, Se, and Cd and cord blood Hg) and to a lesser degree for BMI (with hair Hg, blood Hg, and blood As). The presence of previous deliveries (1–3) was associated with a 31.5% decrease in the average Hg level in maternal blood and a 47.8% decrease in cord blood. The decreased level of maternal blood Cd was almost the same, 30.2%, although only marginally significant, and that of maternal blood Se was lower (10.4%). Notably, parity did not affect maternal plasma Se levels; therefore, erythrocytes are the main candidates for Se decrease in maternal blood. They contain Gpx1; when the Se supply for selenoprotein synthesis is limited, Gpx1 is among those selenoproteins that can disappear fast (Brigelius-Flohe and Maiorino, 2013; Burk and Hill, 2015).

4. Discussion

4.1. TEs and APOE

APOE gene isoforms are supposed to behave differently in relation to metal kinetics, oxidative stress, age-related neurodegeneration, and (neonatal) neurodevelopment (Cardoso et al., 2017; Jofre-Monseny et al., 2008; Miyata and Smith, 1996; Ng et al., 2013; Smith et al., 2019; Wright et al., 2003; Xu et al., 2014). Studies suggest that young individuals with at least one copy of the *ε4* allele may have several advantages that could facilitate survival in harsh environments (Smith et al., 2019), while older individuals may be at higher risk for age-related diseases, such as AD and cardiovascular disease (Smith et al., 2019). Modifying affects or interactions of the *ε4* allele related to various metal(loid)s are less clear. In the present study, we observed significant differences between Hg, As, Se, and Zn distributions in the peripheral venous blood, peripheral venous plasma, hair, urine, and cord blood of Croatian pregnant mothers carrying the *APOE ε4* allele compared to those without the *APOE ε4* allele (but not for all elements in all samples) (Table 3, Fig. 2). The mean blood levels for potentially toxic metals such as Hg, Cd, and Pb in maternal or cord blood in either group did not exceed the existing guideline values for pregnant women (5.8 ng/mL for Hg, 1 ng/mL for Cd, 50 ng/mL for Pb) (Taylor et al., 2014) and As blood levels were also similar to those of the general population. Only four mothers (from the *ε4* group) exceeded the threshold or intervention blood value for Hg (15 ng/g) (Ewers et al., 1999). Furthermore, after controlling for seafood intake and other confounders, multiple linear regression analysis revealed no associations between investigated TEs and the presence of the *ε4* allele, except for Se in peripheral venous plasma (Table 4 and 5). This positive association was even stronger when we included only nulliparous women (data not shown) and excluded multiparous; but the number of nulliparous was too small for reliable assessment. The observed association is interesting, although it requires further study involving larger populations and attention on *nulliparous* cohorts to omit the masking effects of the inverse association found between the parity and blood elements Se, Hg, and Cd.

Parity affects the placenta by improving placental function via increased foetal-maternal contact, higher vascularisation, increased perfusion, and increased villi surface density (Prior et al., 2014). It can also have an independent positive effect on foetal weight and pregnancy; pregnancy complications associated with placental dysfunction occur

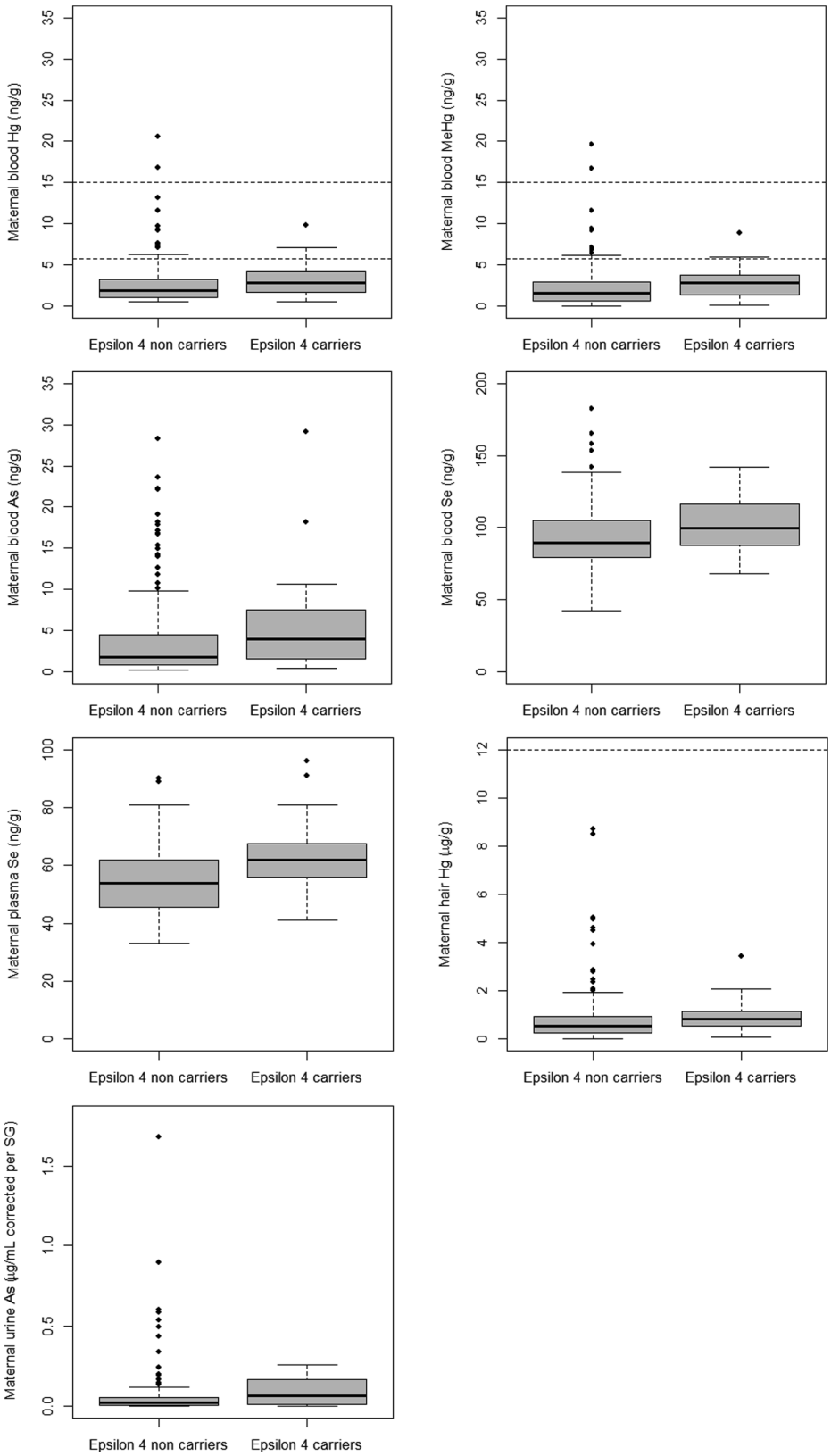

Fig. 2. TEs in maternal blood, plasma, hair, urine, and cord blood stratified by the presence of maternal *APOE ε4 allele.* Boxplot data distribution showing the median, 25th, and 75th percentiles and outliers; horizontal dotted lines represent alert and intervention levels for maternal blood (5.8 ng/mL; 15 ng/mL) and hair Hg (> 12 µg/g) regarding the new-born's health (Taylor et al., 2014; Ewers et al., 1999; JECFA, n.d.).

more frequently in *nulliparous* than in *parous* women. Thus, the parity-related decrease in maternal and cord blood (Me)Hg, Cd, and Se levels found in the present study may have resulted from an increase in the placenta's ability to detoxify, perhaps by accumulating and/or excreting various stress and foetal waste products including nonessential TEs, possibly also at the cost of essential ones (e.g., Se) needed for

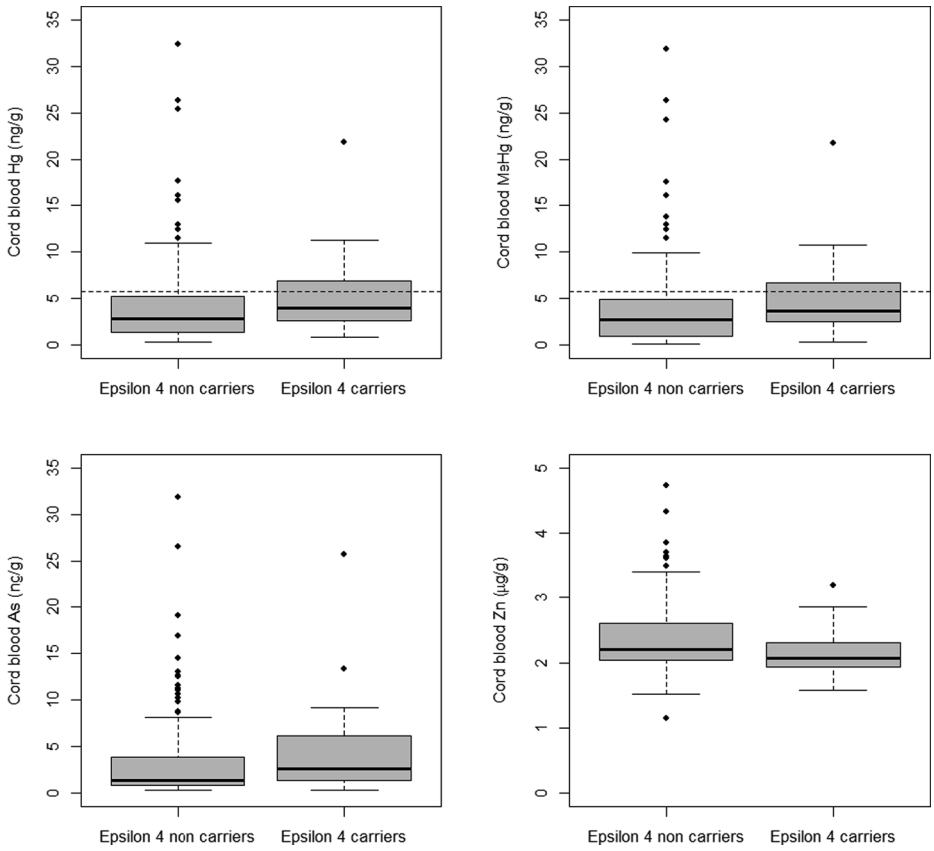

Fig. 2. (*continued*)

detoxification. For instance, Kuhnert et al. (1982) observed a parity-related increase in Cd levels in the placenta of pregnant women together with a reduced amount of Zn; and Horvat et al. (1988) reported a tendency for Hg accumulation and relatively high Se levels in the placental tissues of pregnant women. By contrast, higher placental efficiency is also known to reduce systemic oxidative stress, which is believed to be present during normal and pathological pregnancy (Leal et al., 2011). Such reduction might decrease demands for Se and might explain the observed inverse relationship between parity (0–3) and whole blood Se.

4.1.1. Se and APOE during pregnancy

In the present study, the maternal plasma selenium level means (61.7 ng/g for $\varepsilon 4+$, 53.7 ng/g for $\varepsilon 4-$) were within the European mean levels for pregnant women and close to the level believed to be sufficient for normal GPx activity (2/3 of maximal activity, 62 ng/mL) (Thomson, 2004).

Higher Se levels in pregnant women (in blood or plasma) were evidented to have several beneficial effects. Selenium was found to have a positive influence on reproduction, pregnancy-related diseases such as pre-eclampsia, miscarriage, preterm birth, thyroid autoimmunity, and neonatal diseases accompanied by oxidative stress (Pieczyńska and Grajeta, 2015; Rayman, 2016). Its antioxidative effect is known to be of great importance during embryogenesis (Ufer and Wang, 2011). It should also be pointed out that in a previous study of the present population group, authors observed a positive correlation between maternal blood selenium levels and a child's cognitive abilities (BSID-III scores) ($n = 154$; $r = 0.176$, $p = 0.029$; multivariate analyses were not performed) (Močenić et al., 2019).

Similarly, positive influences have been suggested for the ε4 allele, mostly attributed to higher cholesterol levels, vitamin D levels, and bone Ca assimilation, which also can have a positive influence on fertility (particularly in highly infectious environments), neurodevelopment, bone growth, and lower susceptibility to infectious diseases (Dieckmann et al., 2012; Huebbe et al., 2011; Jasienska et al., 2015; Smith et al., 2019). Accordingly, it is possible that sufficient nutritional intake of Se is a necessary condition for optimal functioning of redox and antioxidative processes and protection against lower antioxidative capacity of *ε4* allele. In other words, Se deficiency could pose a trigger for negative effects of *APOE ε4*.

4.1.2. Maternal versus foetal APOE

In two recent studies, the authors investigated the role of child *APOE* polymorphism and the relationship between prenatal Hg exposure (mixed cord blood levels) on child behaviour or neurodevelopment at around age two (Ng et al., 2013; Snoj Tratnik et al., 2017). Various confounders, including mixed cord blood Pb, mixed cord blood Se, and mixed cord plasma Se, were considered. However, because of the mixed cord blood/plasma samples the results remain debatable. It is possible that in such cases maternal levels of trace elements would be more powerful confounders. Unfortunately, the importance of maternal characteristics (Gundacker et al., 2000) for a child's prenatal neurodevelopment are often underestimated. In addition, in these studies we (Snoj Tratnik et al., 2017), and others (Ng et al., 2013), did not consider the maternal *APOE* genotype, whose important role in foetal lipid metabolism has been discussed in some older studies (Descamps et al., 2004; Witsch-Baumgartner et al., 2004; Witsch-Baumgartner and Lanthaler, 2015). For example, Witsch-Baumgartner et al. (2004) noted that the importance of the maternal genotype is, "*consistent with the finding that there is no difference in cholesterol concentrations of cord blood from new-borns with different APOE genotypes, suggesting that the embryo's APOE genotype does not significantly modulate lipoprotein concentrations prenatally*". Descamps et al. (2004) supported this suggestion, observing that maternal genetic variations of *APOE* and some other genes influence foetal lipoprotein concentrations independently of the genetic status of the foetus. However, in the case of pathological maternal

Table 4
Influence of maternal APOE allele (*ε4+*), parity, BMI, age, seafood intake, and ever-smoking on mothers' TE levels (estimated by multiple regression models).

Metal(loid)	m*ε4+*	mParity	mBMI	mAge	mSeafood intake	mEver-smoking	Constant	N	R^2	Chi^2
	(yes)	(yes)	(kg/m^2)	(years)	(serv./day)	(yes)			(%)	
mB Hg	0.123	**−0.315****	−0.026°	**0.039*****	**0.924*****	−0.010	−0.103	187	31	**69.2*****
	(0.119)	**(−0.569, −0.060)**	(−0.053, 0.002)	**(0.012, 0.066)**	**(0.535, 1.313)**	(0.091)	(0.391)			(df = 6)
mB MeHg	0.162	−0.357°,	**−0.050****	**0.064*****	**1.414*****	−0.016	−0.819	187	33	**75.5*****
	(0.177)	(−0.734, 0.020)	**(−0.091, −0.009)**	**(0.024, 0.104)**	**(0.838, 1.990)**	(0.136)	(0.580)			(df = 6)
mH Hg	0.183,	−0.252	**−0.053****	**0.052****	**1.239*****	0.036	5.450	188	28	**61.8*****,
	(0.178)	(0.143)	**(−0.100, −0.012)**	**(0.011, 0.092)**	**(0.656, 1.823)**	(0.030)	(0.586)			(df = 6)
mB As	0.329	−0.400	−0.045°	0.037	**1.248*****	−0.048	0.326	183	23	**48.5*****
	(0.211)	(0.168)	(−0.093, 0.003)	(0.018)	**(0.567, 1.930)**	−(0.158)	(0.677)			(df = 6)
mU As	0.435	−0.227	−0.064	0.028	**1.210***	−0.151	−71.07	148	19	**30.4*****
	(0.290)	(0.242)	(0.028)	(0.027)	**(0.133, 2.288)**	(0.233)	(18.7)			(df = 7)#
mM As	0.136	−0.412	0.008	−0.009	**2.325****	−0.002	−2.215	109	12	**14.4***
	(0.438)	(0.378)	(0.042)	(0.041)	**(0.561, 4.088)**	(0.377)	(1.550)			(df = 6)
mB Se	0.077	**−0.104***	0.004	**0.012****	0.114	0.010	4.138	183	14	**27.0*****
	(0.042)	**(−0.193, −0.016)**	(0.003)	**(0.003, 0.021)**	(0.051)	(0.032)	(0.135)			(df = 6)
mP Se	**0.120***	−0.032	0.001	0.007	0.079	0.024	3.717	179	10	**18.9****
	(0.012, 0.227)	(0.033)	(0.004)	(0.004)	(0.050)	(0.031)	(0.138)			(df = 6)
mB Cd	0.059	−0.302°	−0.019	0.003	0.137	**0.604*****	−0.468	183	22	**45.5*****
	(0.145)	(−0.608, 0.003)	(0.012)	(0.012)	(0.176)	**(0.315, 0.893)**	(0.464)			(df = 6)
mU Cd	−0.096	−0.110	−0.002	**0.053****	−0.069	0.292	87.3	148	39	**73.8*****
	(0.157)	(0.130)	(0.015)	**(0.014, 0.092)**	(0.217)	(0.126)				(df = 7)#

Summarised are data with statistically significant effects of independant variables on TE levels: 95% confidence limits for the parameters are added if the effect was statistically significant and the standard error of the estimated parameter otherwise; R^2 – percentage of variability of metal(loid) level explained by the model; Chi^2 – significance of the model; excluded was one mother with maternal *ε2/ε4* genotype; m – maternal; B – blood; P – plasma; M – milk; U – urine; H – hair; N – number of observations; # for urine samples, an additional independent variable SG was included in the multiple regression models; its effects are **73.1***** (95% CI: 23.5–122.7) for As, **83.1***** (95% CI: 56.3–110.0) for Cd; statistically significant results are indicated in bold.

$^{\circ}$ $p < 0.10$.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 5
Influence of maternal and/or new-born APOE allele (*ε4+*), parity, m BMI, m age, seafood intake, m ever-smoking, nb birth weight, nb sex, nb EGA on newborns' TE levels in cord blood (estimated by multiple regression models).

	cB Hg	cB MeHg	cB As
m or/and nb *ε4+*	−0.025	−0.030	−0.074
(yes)	(0.167)	(0.203)	(0.218)
mParity	**−0.478****	**−0.551***	−0.264
(yes)	**(−0.883, −0.073)**	**(−1.042, −0.059)**	(0.194)
mBMI	−0.026	−0.037	−0.043
(kg/m^2)	(0.016)	(0.020)	(0.021)
mAge	0.035	0.040	0.042
(years)	(0.016)	(0.019)	(0.021)
mSeafood intake	**1.143*****	**1.341*****	**0.895***
(serv./day)	**(0.536, 1.751)**	**(0.604, 3.078)**	**(0.095, 1.695)**
mEver-smoking	−0.097	−0.083	−0.076
(yes)	(0.146)	(0.176)	(0.192)
nbEGA	−0.074	−0.097	0.035
(weeks)	(0.067)	(0.080)	(0.087)
nbBirth weight	0.0002	0.0003	0.0004
(kg)	(0.0002)	(0.0002)	(0.0002)
nbSex	0.037	−0.021	0.017
(girl)	(0.142)	(0.172)	(0.188)
Constant	2.700	3.088	−2.508
	(2.526)	(3.061)	(3.290)
N	120	120	119
R^2	32	31	19
(%)			
Chi^2	**46.4*****	**45.3*****	**24.4*****
(df = 9)			

Summarised are data with statistically significant effects of independant variables on TE levels: 95% confidence limits for the parameters are added if the effect was statistically significant and standard error of the estimated parameter otherwise; R^2 – percentage of variability of level explained by the model; Chi^2 – significance of the model; * excluded were one mother with maternal *ε2/ε4* genotype and three new-borns with either maternal or new-born's *ε2/ε4* genotype; cB – cord blood; m – maternal; nb – new-born; N – number of observations; statistically significant results are indicated in bold.

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

levels, the foetal genotype may modulate maternal lipoprotein metabolism (Descamps et al., 2005). It should also be noted that, according to emerging data, the foetal apoE role differs from the new-born apoE role, and a lot remains to be discovered (Augsten et al., 2011).

4.2. Metabolic pathways of Se and apolipoprotein E

We also tried to elucidate a possible link between metabolic pathways of Se and apolipoprotein E. There are only a few studies covering selenium's relationship with apolipoproteins, including apoE and the lipoprotein receptors (apoER2, gene *LRP8*; megalin, gene *LRP2*) (Burk et al., 2014; Cardoso et al., 2017; Gabre-Medhin et al., 1988). It is also interesting that in some tissues, selenoprotein P (the main supplier of Se to extrahepatic tissues) and apoE are using the same two receptors, but different binding sites, for transfer of selenium and lipids, respectively. That is, there is apoER2 in the brain, placenta, bone marrow, and testis (Burk et al., 2014; Burk and Hill, 2015; Mao et al., 2016; Rayman, 2016), and megalin in the kidneys (renal proximal convoluted cells) and brain (Burk and Hill, 2015), and probably also in the placenta (Storm et al., 2016). Further, vitamins A and D regulate megalin expression (Liu et al., 1998). Regulation of megalin (mRNA and protein expression) was studied in a cell line derived from rat kidney proximal tubule cells, in human JEG-3 cells, and in the mouse embryonal carcinoma cell line F9. Animal studies have also shown that megalin (expressed by renal PCT cells) is important for retaining both vitamin D *via* the vitamin D binding protein (Kim and Kim, 2014) and selenium in the form of selenoprotein P (Burk and Hill, 2015). Accordingly, if kidney megalin is induced by vitamin D in humans (being higher in *ε4* allele carriers), this could be one reason for the observed association between Se and *APOE* allele *ε4*.

Overall, we concluded that the superior Se status observed in healthy pregnant women carrying the *ε4* allele could be linked to suggested beneficial effects of *APOE ε4* during pregnancy. We also suppose that the reported apoE4 associations with low nail Se levels in an elderly Chinese population (Gao et al., 2009) and low post-mortem brain Se levels of AD patients (Cardoso et al., 2017) could be related to their general poor nutritional status including low Se and vitamin D levels. As already discussed by other research groups, it seems that the *APOE* allele *ε4* could be a risk allele either for reproduction, neurodevelopment, or age-related neurodegenerative diseases, especially, if not alone, in combination with several other factors (Cardoso et al., 2017; Morris et al., 2016; Nehls, 2016; Rea et al., 2016) including epigenetics, polymorphisms in related genes, and the simultaneous deficiency of basic nutrients such as vitamin D and essential fatty acids, and/or selenium.

4.3. Study limitation

The main shortcomings of our study are the relatively low number of participants, low concentration levels of potentially toxic elements, and mixed cord blood/serum/plasma samples. Given the difficulties in obtaining separated venous/arterial cord samples in wider epidemiological studies with low metal(loid) exposure, mixed samples are usually used as an approximation of *in utero* exposure. There is no consensus regarding the type of studies for which mixed samples are acceptable and when they are likely to introduce unacceptable biases. Accordingly, for mixed cord blood and, in particular, mixed cord plasma/serum levels, elaborate comments on distribution differences in trace elements between *ε4+/ε4−* carriers are not possible. Although parity-related higher perfusion in the placenta (Prior et al., 2014) may lead to an increase in Se transfer to the foetus, we did not observe any effect of parity or apoE on Se levels in cord blood or cord plasma (Tables 4 and 5). Better insight would be possible with data on TE levels in the placenta.

For those nonessential elements that accumulate mostly in erythrocytes (e.g., Hg, Pb, and Cd), the mixed cord blood levels can be taken as a possible approximation, although erythrocyte levels would be more reliable. In contrast, mixed cord plasma/serum samples are more problematic. Individual cord venous/arterial differences are lost; therefore, the samples are not truly representative and thus more or less unsuitable for reliable comparison/association studies, although they are used in many studies as a rough substitute.

Additionally, Vitamin D status (25-hydroxysterol measurement), uncertainty of self-reported data for smoking and seafood intake, and selenoproteins gene polymorphisms may have introduced residual confounding. For instance, a recent study by Mao et al. (2016) in the UK, *"shows that women who carry the SEPP1 rs3877899 A allele were better able to maintain selenium status during pregnancy"*; however, they did not follow either the vitamin D status or the presence of the *ε4* allele.

5. Conclusion

Our study indicates that genetic variations in the *APOE* gene (*ε4+* versus *ε4−*) potentially influence selenium levels in pregnant women at the third trimester, while the effect of maternal/foetal *APOE* variations on cord blood should be re-evaluated on separated venous/arterial cord blood samples. Previous research suggestions of possible *ε4* allele impact on Hg levels were not observed, while superior selenium status observed in healthy pregnant women carrying allele *ε4* could be linked to the *APOE ε4* beneficial effects early in life. Follow-up research with the same and larger *nonparous* cohorts with additional potential

confounders are ongoing.

Acknowledgements

This work was supported by the Slovenian Research Agency, Slovenia (research programme P1 0143 and project J7-9400); University of Rijeka, Croatia (grant no. 13.06.1.2.25); and the Ministry of Science and Education of Republic of Croatia, Croatia (grant no. 062-0000000-3395). We would like to thank Dr. Mateja Blaš for assisting Prof. Dr. Damijana Kastelec and all other co-workers and participants involved in this study.

Declaration of Competing Interest

The authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105626.

References

Augsten, M., Hackl, H., Ebner, B., Chemelli, A., Glatter, O., Marsche, G., Lang, U., Desoye, G., Wadsack, C., 2011. Fetal HDL/apoE: a novel regulator of gene expression in human placental endothelial cells. Physiol. Genomics 43, 1255–1262. https://doi.org/10.1152/physiolgenomics.00109.2011.

Barany, E., Bergdahl, I.A., Schütz, A., Skerfving, S., Oskarsson, A., 1997. Inductively Coupled Plasma Mass Spectrometry for Direct Multi-Element Analysis of Diluted Human Blood and Serum. J. Anal. Spectrom. 12, 1005–1009. https://doi.org/10.1039/a700904f.

Barbone, F., Rosolen, V., Mariuz, M., Parpinel, M., Casetta, A., Sammartano, F., Ronfani, L., Vecchi, L., Bin, M., Castriotta, L., Valent, F., Latesha, D.A., Mazej, D., Snoj Tratnik, J., Miklavčič, A., So, K., Špirič, Z., Krsnik, M., Neubauer, D., Kodrič, J., Prpič, I., Petrovič, O., Vlašič, I., Horvat, M., 2019. Prenatal mercury exposure and child neurodevelopment outcomes at 18 months: Results from the Mediterranean PHIME cohort. Int. J. Hyg. Environ. Heal. 222, 9–21. https://doi.org/10.1016/j.ijheh.2018.07.011.

Bernhard, M., 1988. UNEP Report: Mercury in the Mediterranean.

Bosch, A.C., O'Neill, B., Sigge, G.O., Kerwath, S.E., Hoffman, L., 2016. Heavy metals in marine fish meat and consumer health: a review. J. Sci. Food Agric. 96, 32–48. https://doi.org/10.1002/jsfa.7360.

Brigelius-Flohe, R., Maiorino, M., 2013. Glutathione peroxidases. Biochim. Byophys. Acta 1839, 3289–3303. https://doi.org/10.1016/j.bbagen.2012.11.012.

Burk, R.F., Hill, K.E., 2015. Regulation of selenium metabolism and transport. Annu. Rev. Nutr. 35, 109–134. https://doi.org/10.1146/annurev-nutr-071714-034250.

Burk, R.F., Hill, K.E., Motley, A.K., Winfrey, V.P., Kurokawa, S., Mitchell, S.L., Zhang, W., 2014. Selenoprotein P and apolipoprotein E receptor-2 interact at blood brain barrier and also within the brain to maintain an essential selenium pool that protects against neurodegeneration. FASEB J. 28, 3579–3588. https://doi.org/10.1096/fj.14-252874.

Cardoso, B.R., Hare, J.D., Lind, M., McLean, C.A., Volitakis, I., Laws, S.M., Masters, C.L., Bush, A.I., Roberts, B.R., 2017. The APOE ε4 allele is associated with lower selenium levels in the brain: Implications for Alzheimer's disease. Neuroscience 8, 1459–1464. https://doi.org/10.1021/acschemneuro.7b00014.

Descamps, O.S., Bruniaux, M., Guilmot, P.F., Tonglet, R., Heller, F., 2004. Lipoprotein concentrations in newborns are associated with allelic variations in their mothers. Atherosclerosis 172, 287–298. https://doi.org/10.1016/j.atherosclerosis.2003.11.002.

Descamps, O.S., Bruniaux, M., Guilmot, P.R., Tonglet, R., Heller, F.R., 2005. Lipoprotein metabolism of pregnant women is associated with both their genetic polymorphisms and those of their newborn children. JLR 46, 2405–2414. https://doi.org/10.1194/jlr.M500232-JLR200.

Dieckmann, M., Beil, T.F., Mueller, B., Bartelt, A., Marshall, R.P., Koehne, T., Amling, M., Ruether, W., Cooper, J.A., Humphries, S.E., Herz, J., Niemeier, A., 2012. Human apolipoprotein E isoforms differentially affect bone mass and turnover in vivo. J. Bone Min. Res. https://doi.org/10.1002/jbmr.1840.

Egert, S., Rimbach, G., Huebbe, P., 2012. ApoE genotype : from geographic distribution to function and responsiveness to dietary factors. In: Proceedings of the Nutrition Society Proceedings of the Nutrition Society. Proc. Nutr. Soc. pp. 410–424. https://doi.org/10.1017/S0029665112000249.

EPA, US EPA (US Environmental Protection Agency), 2007. Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophometry – method 7473 – total Mercury. SW-846, Test Methods Eval. Solid Waste, Phys. Methods 1–17.

Ewers, U., Krause, C., Schulz, C., Wilhellm, M., 1999. Reference values and human biological monitoring values for environmental toxins – Report on the work and recommendations of the Commission on Human Biological Monitoring of the German Federal Environmental Agency. Int. Arch. Occup. Environ. Heal. 72, 255–260. https://doi.org/10.1007/s004200050369.

Falnoga, I., Tušek-Žnidarič, M., 2007. Selenium-Mercury Interactions in Man and Animals. Biol. Trace Elem. Res. 119, 212–220. https://doi.org/10.1007/s12011-007-8009-3.

Florianczyk, B., 2007. Metallothioneins and its role in metal regulation, binding of reactive oxygene species, apoptosis and cell differentiation. JPCCR 016–018.

Gabre-Medhin, M., Ewald, U., Tuvemo, T., 1988. Serum selenium is related to low density lipoproteins in healthy children but not in children with diabetes. Ups. J. Med. Sci. 93, 57–62. https://doi.org/10.1517/03009734000000038.

Gao, S., Jin, Y., Hall, K.S., Liang, C., Unverzagt, F.W., Ma, F., Cheng, Y., Shen, J., Cao, J., Matesan, J., Li, P., Bian, J., Hendrie, H.C., Murrell, J., 2009. Selenium level is associated with apoE epsilon4 in rural elderly Chinese. Public Heal. Nutr 12, 2371–2376. https://doi.org/10.1017/S1368980009005102.

Giau, V.V., Bagyinszky, E., An, S.S.A., Kim, S., 2015. Role of apolipoprotein E in neurodegenerative diseases. Neuropsychiatr. Dis. Treat. 11, 1723–1737. https://doi.org/10.2147/NDT.S84266.

Gibson, R.S., Hess, S.Y., Holtz, C., Brown, K.H., 2008. Indicators of zinc status at the population level: a review of the evidence. Br. J. Nutr. 99. https://doi.org/10.1017/S0007114508006818.

Godfrey, M.E., Wojcik, D.P., Krone, C.A., 2003. Apolipoprotein E genotyping as potential biomarker for mercury neurotoxicity. J. Alzheimer's Dis. 5, 189–195. https://doi.org/10.3233/JAD-2003-5303.

Graeser, A., Huebbe, P., Storm, N., Hoppner, W., Wagner, A.E., Rimbach, G., Doring, F., 2012. Apolipoprotein E genotype affects tissue metallothionein levels : studies in targeted gene replacement mice. Genes. Nutr. 7, 247–255. https://doi.org/10.1007/s12263-012-0282-x.

Gundacker, C., Neesen, J., Straka, E., Ellinger, I., Dolzing, H., Hengestschläger, M., 2000. Genetics of the human placenta: implications for toxicokinetics. Arch. Toxicol. 90, 2563–2581. https://doi.org/10.1006/s00204-016-1816-6.

Haas, J.G., Lathe, R., 2018. Microbes and Alzheimer's Disease: New findings call for a Paradigm Change. Trends Neurosci. 41570–41573. https://doi.org/10.1016/j.tins.2018.07.001.

Horvat, M., Byrne, A.R., 1990. A modified method for the determination of methylmercury by gas chromatography. Talanta 37, 207–212. https://doi.org/10.1016/0039-9140(90)80024-A.

Horvat, M., Stegnar, P., Byrne, A.R., Dermelj, M., 1988. A study of trace elements in human placenta, blood and hair from the Yugoslav Central Adriatic. In: Brätter, P., Schramel, P. (Eds.), Trace Element Analytical Chemistry in Medicine and Biology, 5. Proceedings of the Fifth International Workshop. Walter de Gruyter, Berlin New York, pp. 243–550.

Huang, Y., Mahley, R.W., 2014. Apolipoprotein E: Structure and function in lipid metabolism, neurobiology, and Alzheimer's diseases. Neurobiol. Dis. 72, 3–12. https://doi.org/10.1016/j.nbd.2014.08.025.

Huebbe, P., Nebel, A., Siegert, S., Moehring, J., Boesch-Saadatmandi, C., Most, E., Pallauf, J., Egert, S., Müller, M.J., Schreiber, S., Nöthlings, U., Rimbach, G., 2011. APOE ε4 is associated with higher vitamin D levels in targeted replacement mice and humans. FASEB J. 25, 3262–3270. https://doi.org/10.1096/fj.11-180935.

Huebbe, P., Rimbach, G., 2017. Evolution of human apolipoprotein E (APOE) isoforms: Gene structure, protein function and interaction with dietary factors. Ageing Re Rev. 37, 146–161. https://doi.org/10.1016/j.arr.2017.06.002.

Jasienska, G., Ellison, P.T., Galbarczyk, A., Jasienski, M., Kalemba-Drozdz, M., Nenko, I., Thune, I., Ziomkiewicz, 2015. Apolipoprotein R (ApoE) polymorphism is related to differences in potential fertility in women: a case of antagonistic pleotropy? A Proc. R Soc. 282 https://doi.org/20142395.

JECFA, n.d. Summary and conclusions of the sixty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives. [WWW Document].

Jofre-Monseny, L., Minihane, A.M., Rimbach, G., 2008. Impact of apoE genotype on oxidative stress, inflammation and disease risk. Mol. Nutr. Food Res. 52, 131–145. https://doi.org/10.1002/mnfr.200700322.

Julvez, J., Mèndez, M., Fernandez-Barres, S., Romaguera, D., Vioque, J., Llop, S., Ibarluzea, J., Guxen, M., Avella-Garcia, C., Tardón, A., Riano, I., Andiarena, A., Robinson, O., Arija, V., Esnaola, M., Ballester, F., Sunyer, J., 2016. Maternal Consumption of seafood in pregnancy and child neuropsychological development: A longitudinal study based on population with high consumption levels. Am. J. Epidemiol. 3, 169–182. https://doi.org/10.1093/aje/kwv195.

Kim, C.S., Kim, S.W., 2014. Vitamin D and chronic kidney disease. Korean J. Intern. Med. 29, 414–427. https://doi.org/10.3904/kjim.2014.29.4.416.

Kobal, A.B., Horvat, M., Prezelj, M., Sešek Briški, A., Kersnik, M., Dizdarevič, T., Mazej, D., Falnoga, I., Sibilj, V., Arnerič, N., Kobal, N., Osredkar, J., 2004. The impact of long-term past exposure to elemental mercury on antioxidative capacity and lipid peroxidation in mercury miners. J. Trace Elem. Med Biol. 17, 261–274.

Kosta, L., Ravnik, V., Byrne, A.R., Štirn, J., Dermelj, M., Stegnar, P., 1978. Some trace elements in the waters, marine organisms and sediments of the Adriatic by neutron activation analysis. J. Radioanal. Chem. 44, 317–332.

Kuhnert, B.R., Bottoms, S.F., Erhard, P., 1982. Cadmium levels in maternal blood, fetal cord blood, and placental tissues of pregnant women who smoke. Am. J. Obs. Gynecol. 142 2021–1025.

La Port, P., 2011. Selenium in the detoxification of arsenic: Mechanisms and clinical efficiency. Dissertation. The University of Chicago.

Leal, C.A.M., Schetinger, M.R.C., Leal, D.B.R., Morsch, V.M., Schafer da Silva, A., Rezer, J.F.P., 2011. Oxidative stress and antioxidant defenses in pregnant women. Free Radic Res. Commun. 16, 230–236. https://doi.org/10.1179/1351000211Y.0000000013.

Liang, L., Bloom, N.S., Horvat, M., 1994. Simultaneous determination of mercury speciation in biological materials by GC/CVAFS after ethylation and room-temperature precollection. Clin. Chem. 40, 602–607.

Liu, W., Carling, T., Rastad, J., Akerström, G., Yu, W.R., Juhlin, C., Ridefelt, P., Hellman,

P., 1998. Regulation of gp330/megalin expression by vitamins A and D. Eur. J. Clin. Investig. 28, 100–107. https://doi.org/10.1046/j.1365-2362.1998.00253.x.
Liu, Y., Zhang, W., Zhao, J., Lin, X., Liu, J., Cui, L., Gao, Y., Zhang, T.L., Li, B., Li, Y.F., 2018. Selenoprotein P as the major transporter for mercury and serum from methylmercury-poisoned rats. J. Trace Elem. Med Biol. 50, 589–595. https://doi.org/10.1016/j.jtemb.2018.04.013.
Mao, J., Vanderlelie, J.J., Perkins, A.V., Redman, C.G.W., Ahmadi, K.R., Rayman, M., 2016a. Genetic polymorphisms that affect selenium status and response to selenium supplementation in United Kingdom pregnant women. Am. J. Clin. Nutr. 104, 100–106. https://doi.org/10.3945/ajcn.115.114231.
Mao, J., Vanderlelie, J.J., Perkins, A.V., Redman, C.W.G., Ahmadi, K.R., Rayman, M.P., 2016b. Genetic polymorphisms that affect selenium status and response to selenium supplementation in United Kingdom pregnant women. Am. J. Clin. Nutr. https://doi.org/10.3945/ajcn.115.114231.
Miklavčič, A., Casetta, A., Snoj Tratnik, J., Mazej, D., Krsnik, M., Mariuz, M., Sofianou, K., Špirić, Z., Barbone, F., Horvat, M., 2013. Mercury, arsenic and selenium exposure levels in relation to fish consumption in the Mediterranean area. Environ. Res. 120, 7–17. https://doi.org/10.1016/j.envres.2012.08.010.
Miyata, M., Smith, J.D., 1996. Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and beta-amyloid peptides. Nat. Genet. 14, 55–61. https://doi.org/10.1038/ng0996-55.
Močenić, I., Kolić, I., Radić Nišević, J., Belančić, A., Snoj Tratnik, J., Mazej, D., Falnoga, I., Vlašić-Cicvarić, I., Štimac, T., Špirić, Z., Horvat, M., Prpić, I., 2019. Prenatal selenium status, neonatal cerebellum measures and child nevrodevelopment at the age of 18 months. Env. Res. 176. https://doi.org/10.1016/j.envres.2019.108529.
Morris, M.C., Brockman, J., Schneider, J.A., Wang, Y., Beneett, D.D., Tangney, C.C., van de Rest, O., 2016. Association of Seafood consumption, brain mercury levels, and APO ε4 status with brain neuropathology in older adults. JAMA 315, 489–497. https://doi.org/10.1001/jama.2015.19451.
Mutter, J., Naumann, J., Sadaghiani, C., Schneider, R., Walach, H., 2004. Alzheimer disease: Mercury as pathogenetic factor and apolipoprotein E as a moderator. Neuro. Endocrinol. Let. 25, 331–339.
Nehls, M., 2016. Unified theory of Alzheimer's disease (UTAD): implications for prevention and curative therapy. Mol. Psychiatry 15, 1–52. https://doi.org/10.1186/s40303-016-0018-8.
Ng, S., Lin, C.-C., Hwang, Y.-H., Hsieh, W.-S., Liao, H.-F., Chen, P.-C., 2013. Mercury, APOE, and children's neurodevelopment. Neurotoxicology 37, 85–92. https://doi.org/10.1016/j.neuro.2013.03.012.
Pieczyńska, J., Grajeta, H., 2015. The role of selenium in human conception and pregnancy. J. Trace Elem. Med Biol. 29, 31–38. https://doi.org/10.1016/j.jtemb.2014.07.003.
Prior, T., Mullins, E., Bennet, E., Kumar, S., 2014. Influence of parity on fetal hemodynamics and amniotic fluid volume at term. Ultrasound Obs. Gynecol. 44, 688–692. https://doi.org/10.1002/uog.13332.
Prpić, I., Milardović, A., Vlašić-Cicvarić, I., Špirić, Z., Radić Nišević, J., Vukelić, P., Snoj Tratnik, J., Mazej, D., Horvat, M., 2017. Prenatal exposure to low-level methylmercury alters the child's fine motor skills at the age of 18 months. Environ. Res. 152, 369–374. https://doi.org/10.1016/j.envres.2016.10.011.
Rayman, M.P., 2012. Selenium and human health. Lancet 379, 1256–1268. https://doi.org/10.1016/S0140-6736(11)61452-9.
Rayman, M.R., 2016. Is adequate selenium important for healthy human pregnancy? In: Hatfield, D.L., Schweizer, U., Tsuji, P.A., Gladyshe, V.N. (Eds.), Selenium: Its Molecular Biology and Role in Human Health. Springer Science, NY, USA, pp. 353–364.
Rea, I.M., Dellet, M., Mills, K.I., 2016. ACUME2 Project. Living long and ageing well: is epigenomics the missing link between nature and nurture? Biogerontology 17, 33–54. https://doi.org/10.1007/s10522-015-9589-5.
Santonen, T., Aitio, A., Fowler, B., Nordberg, M., 2015. Biological Monitoring and Biomarkers. In: Nordberg, G.A., Fowler, B.A., Nordberg, M. (Eds.), Handbook on the Toxicology of Metals. Elsevier, London, UK, pp. 155–171.
Smith, J.S., Ashford, J.W., Perffeti, T.A., 2019. Putative survival advantageous in young apolipoprotein e4 carriers are associated with increased neuronal stress. J. Alzheimers Dis. 885–923. https://doi.org/10.3233/JAD-181089.
Snoj Tratnik, J., Falnoga, I., Trdin, A., Mazej, D., Fajon, V., Miklavčič, A., Kobal, A.B., Sešek Briški, A., Krsnik, M., Neubauer, D., Kodrič, J., Stropnik, S., Gosar, D., Lešnik Musek, P., Marc, J., Jurkovič Mlakar, S., Petrović, O., Vlašič Cicvarić, I., Prpič, I., Milardovič, A., Radić Nišević, J., Vuković, D., Fišić, E., Špirić, Z., Horvat, M., 2017. Prenatal mercury exposure, neurodevelopment and apolipoprotein E genetic polymorphism. Environ. Res. 152, 375–385. https://doi.org/10.1016/j.envres.2016.08.035.
Stajnko, A., Šlejkovec, Z., Mazej, D., France-Štiglic, A., Briški, A.S., Prpić, I., Špirić, Z., Horvat, M., Falnoga, I., 2019. Arsenic metabolites; selenium; and AS3MT, MTHFR, AQP4, AQP9, SELENOP, INMT, and MT2A polymorphisms in Croatian-Slovenian population from PHIME-CROME study. Environ. Res. 170, 301–319. https://doi.org/10.1016/j.envres.2018.11.045.
Storm, T., Christensen, E.I., Christensen, J.N., Kjaergaard, T., Uldbjerg, N., Larsen, A., Honoré, B., Madsen, M., 2016. Megalin is predominantly observed in vesicular structures in first and third trimester cytotrophoblasts of the human placenta journal of histochemistry and cytochemistry. J. Histochem. Cytochem. 64. https://doi.org/10.1369/0022155416672210.
Taylor, C.M., Golding, J., Edmond, A.D., 2014. Lead, cadmium and mercury levels in pregnancy: the need for international concern. J. Dev. Orig. Heal. Dis 5, 16–30. https://doi.org/10.1017/S2040174413000500.
Taylor, V., Goodale, B., Raab, A., Schwertdle, T., Reimer, K., Conklin, S., Karagas, M.R., Francesconi, K.A., 2017. Human exposure to organic arsenic species through seafood. Sci. Tot. Env. 580, 266–282. https://doi.org/10.1016/j.scitotenv.2016.12.113.
Team, R.C., 2017. R: A language and environment for statistical computing. [WWW Document]. R Found. Stat. Comput. Vienna, Austria.
Thomson, C.D., 2004. Assessment of requirements for selenium and adequacy of selenium status: a review. Eur. J. Clin. Nutr. 58, 391–402. https://doi.org/10.1038/sj.ejcn.1601800.
Tsalev, D.L., Zaprianov, Z.K.Z., 1983. Determination of individual Elements. In: Atomic Absorption Spectrometry in Occupational and Environmental Health Practice. Florida CRC Press, Florida, pp. 219–221.
Tuminello, E.R., Han, S.D., 2011. The apolipoprotein E antagonistic pleiotropy hypothesis: Review and recommendations. Int. J. Alzheimer's Dis. 2011, 1–12. https://doi.org/10.4061/2011/726197.
Ufer, C., Wang, C.C., 2011. The roles of glutathione peroxidases during embryo development. Front. Mol. Neurosci. 4. https://doi.org/10.3389/fnmol.2011.00012.
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. 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., Carrozzi, 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. https://doi.org/10.2188/jea.JE20120168.
Vitek, M.P., Brown, C.M., Colton, C.A., 2009. APOE genotype-specific differences in the innate immune response. Neurobiol. Aging 30, 1350–1360. https://doi.org/10.1016/j.neurobiolaging.2007.11.014.
Witsch-Baumgartner, M., Gruber, M., Kraft, H.G., Rosi, M., Clayton, P., Giros, M., Haas, D., Kelley, R.I., Krajewska-Walasek, M., Utermann, G., 2004. Maternal apo E genotype is a modifier of the Smith-Lemli-Opitz syndrome. J. Med. Genet. 41, 577–584. https://doi.org/10.1136/jmg.2004.018085.
Witsch-Baumgartner, M., Lanthaler, B., 2015. Birthday of a syndrome: 50 years anniversary of Smith–Lemli–Opitz Syndrome. Eur. J. Hum. Genet. 23, 277–278. https://doi.org/10.1038/ejhg.2014.87.
Wright, R.O., Hu, H., Silverman, E.K., Tsaih, S.W., Schwartz, J., Bellinger, D., Palazuelos, E., Weiss, S.T., Hernandez-Avila, M., 2003. Apolipoprotein E genotype predicts 24-month Bayley Scales Infant Development Score. Pediatr. Res. 54, 819–825. https://doi.org/10.1203/01.PDR.0000090927.53818.DE.
Xu, H., Finkelstein, D.I., Adlard, P., 2014. Interactions of metals and apolipoprotein e in Alzheimer's disease. Front. Aging Neurosci. 6, 1–7. https://doi.org/10.3389/fnagi.2014.00121.
Zeng, H., Uthus, E.O., Combs, G.F.J., 2005. Mechanistic aspects of the interaction between selenium and arsenic. Inorg. Biochem. 99, 1269–1274. https://doi.org/10.1016/j.jinorgbio.2005.03.006.
Zhang, H., Feng, X., Chan, H.M., Larssen, T., 2014. New insights into traditional health risk assessments of mercury exposure: Implications of selenium. Env. Sci. Technol. 48, 1206–1212. https://doi.org/10.1021/es4051082.