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Maternal *APOE* ε2 as a possible risk factor for elevated prenatal Pb levels☆

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ABSTRACT

Lead (Pb) is a global contaminant associated with multiple adverse health effects. Humans are especially vulnerable during critical developmental stages. During pregnancy, exposure to Pb can occur through diet and release from maternal bones. Apolipoprotein E gene (*APOE*) variants (ε2, ε3, ε4 alleles) may influence sex steroid hormones, bone metabolism, and Pb kinetics.

We examined the interplay among maternal *APOE* (m*APOE*) genotypes, fetal sex, parity, and Pb in maternal and cord blood (mB-Pb, CB-Pb) using linear regression models. Our study involved 817 pregnant women and 772 newborns with measured adequate levels of zinc and selenium. We compared carriers of the ε2 and ε4 alleles to those with the ε3/ε3 genotype.

The geometric means (range) of mB-Pb and CB-Pb were 11.1 (3.58–87.6) and 9.31 (1.82–47.0) ng/g, respectively. In cases with female fetuses, the maternal m*APOE* ε2 allele was associated with higher, while the m*APOE* ε4 allele was associated with lower mB-Pb and CB-Pb levels. *Nulliparity* increased the strength of the observed associations. These findings highlight the significance of m*APOE* genetics, fetal sex, and parity in prenatal Pb kinetics. Notably, the maternal ε2 allele may increase the risk of Pb exposure.

Research dealt with human subject; therefore, this paragraph is included in the article

In Italy, the protocol was approved by the Ethics Committees of the University of Udine and the Institute for Maternal and Child Health, IRCCS Burlo Garofolo in Trieste. The Ethics Committee of the University Hospital Centre Rijeka approved the protocol for the Croatian participants; in Slovenia, the Ethics Committee of the University Medical Centre of Ljubljana, and in Greece, the Ethics Committee of the Institute of Child Health of Athens. Research was conducted in accordance with the Declaration of Helsinki, and all participants signed an informed consent form.

1. Introduction

In recent decades, the general population has been exposed to relatively low levels of lead (Pb), primarily through diet and internally from bones, which have accumulated Pb from lifelong past exposure. In

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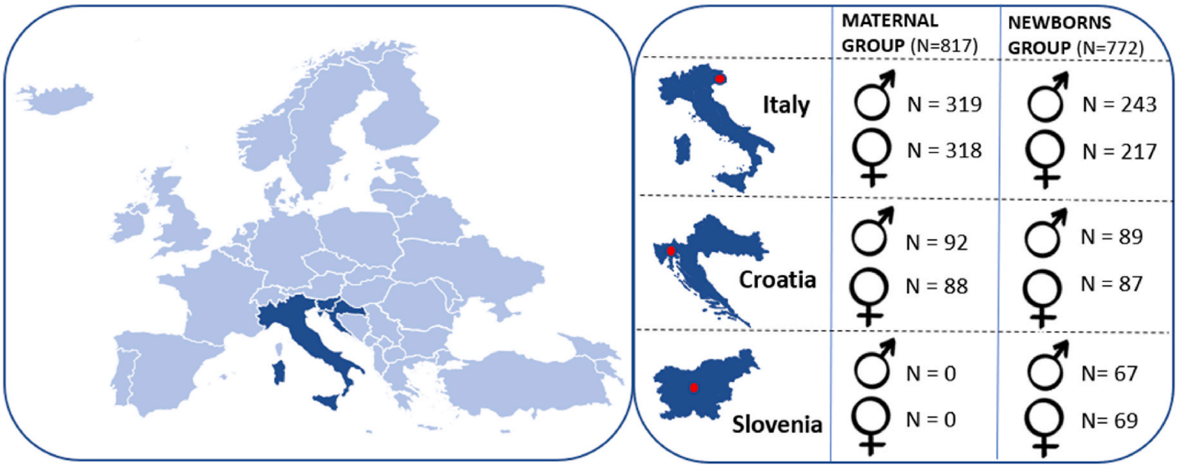

Fig. 1. Study area and study design: Participants stratified by country of residence and fetal/newborn sex.

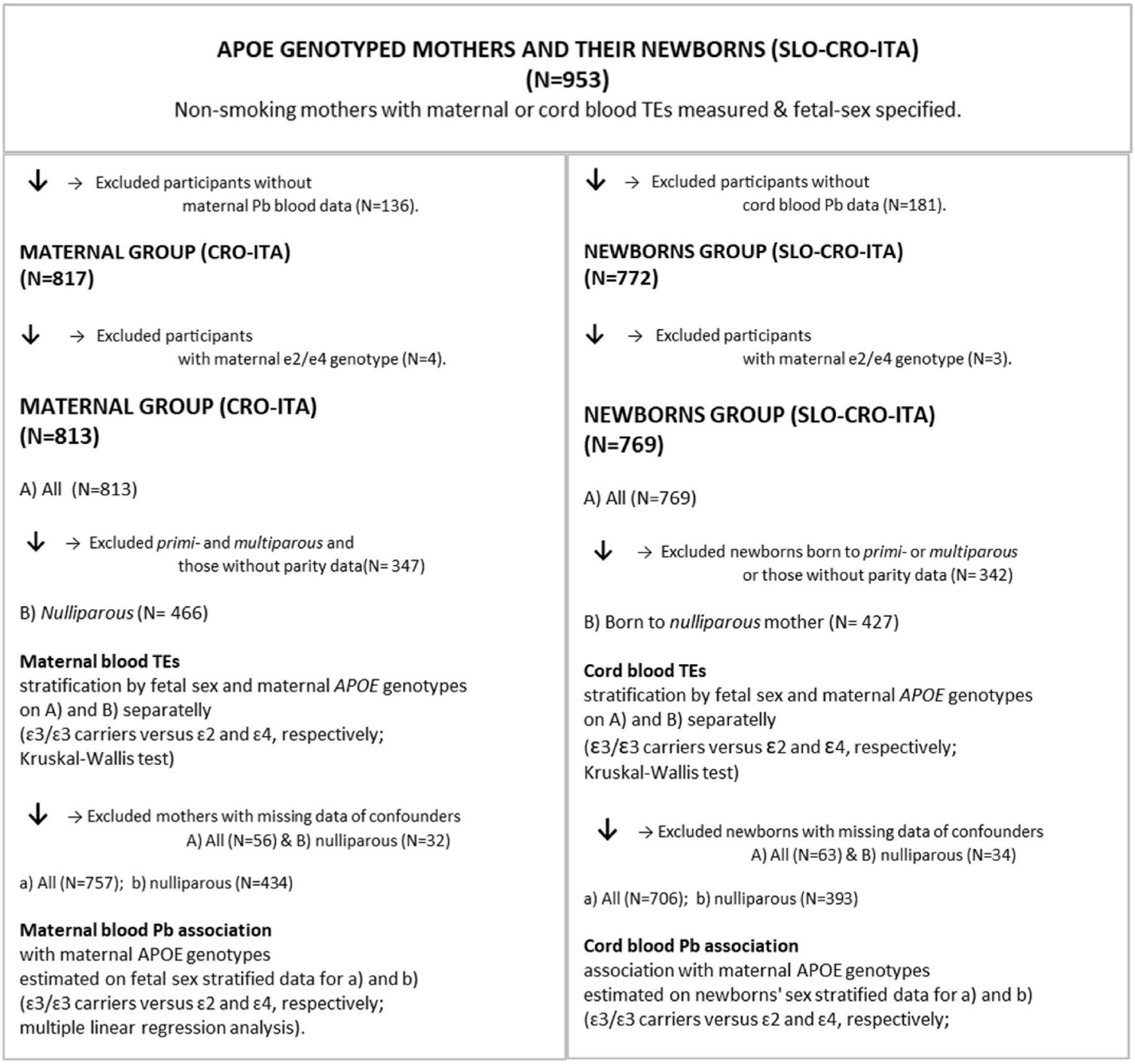

Fig. 2. Flowchart depicting the selection of participants for statistical analyses.

adults, the majority (94%) of absorbed Pb is distributed to the bones, where it can be retained for decades, with slow desorption representing internal Pb exposure (ATSDR, 2020; Bergdahl and Skerfving, 2022). To illustrate the extent of endogenous exposure, bone remodeling and modeling replace about 20–25% of trabecular and 10% of cortical bone annually (Kovacs, 2020). The degree of Pb intake through the

Table 1
m*APOE* genotype and allele frequencies in **Maternal group** and **Newborns group,** stratified by fetal/newborn sex.

Maternal group	Frequency N (%)	Frequency N (%) ♂	Frequency N (%) ♀	p
Genotype	817 (100)	411 (100)	406 (100)	0.138
ε2/ε2	5 (0.61)	4 (0.97)	1 (0.25)	
ε2/ε3	77 (9.42)	41 (9.98)	36 (8.87)	
ε2/ε4*	4 (0.49)	3 (0.73)	1 (0.25)	
ε3/ε3	607 (74.3)	306 (74.5)	301 (74.1)	
ε3/ε4	119 (14.6)	57 (13.9)	62 (15.3)	
ε4/ε4	5 (0.61)	0 (0)	5 (1.23)	
Allele	1634 (100)	822 (100)	812 (100)	0.208
ε2	91 (5.57)	52 (6.33)	39 (4.80)	
ε3	1410 (86.3)	710 (86.4)	700 (86.2)	
ε4	133 (8.14)	60 (7.30)	73 (8.99)	
Newborns group				
Genotype	772 (100)	399 (100)	373 (100)	0.112
ε2/ε2	5 (0.65)	4 (1.00)	1 (0.27)	
ε2/ε3	68 (8.81)	36 (9.02)	32 (8.58)	
ε2/ε4*	3 (0.39)	3 (0.75)	0 (0)	
ε3/ε3	583 (75.5)	302 (75.7)	281 (75.3)	
ε3/ε4	109 (14.1)	54 (13.5)	55 (14.8)	
ε4/ε4	4 (0.52)	0 (0)	4 (1.07)	
Allele	1544 (100)	798 (100)	746 (100)	0.342
ε2	81 (5.25)	47 (5.89)	34 (4.56)	
ε3	1343 (87.0)	694 (87.0)	649 (87.0)	
ε4	120 (7.77)	57 (7.14)	63 (8.45)	

N – number of observations; ♂ – fetal sex is male; ♀ – fetal sex is female; * – participants with ε2/ε4 were not included in statistical analysis as its function can resemble that of ε3/ε3.

gastrointestinal tract is affected by many factors (Mushak, 1991), including age, pregnancy, and nutrient-Pb interactions, such as competition with calcium (Ca) and iron (Fe) for absorption. During pregnancy, the proportion of Ca absorbed from the intestines into the bloodstream doubles by the 12th week of gestation, and this heightened absorption rate is maintained until term. The positive Ca balance is initially deposited in the maternal skeleton until fetal demand rises, leading to increased Ca release from maternal bones in the second part of pregnancy. This release peaks during the third trimester when 80% of fetal skeleton's mineral content is accreted (Ryan and Kovacs, 2021; Arnold et al., 2021). Dietary Pb and previously stored maternal bone Pb can follow the same pathways as Ca in a competitive manner due to its affinity to similar ligands, potentially contributing to elevated Pb levels in maternal blood (mB-Pb) during pregnancy (Gulson et al., 2016; Téllez-Rojo et al., 2004). mB-Pb can then readily pass through the placenta, resulting in prenatal Pb exposure (Skerfving and Bergdhal, 2015; ATSDR, 2020; Bergdahl and Skerfving, 2022). As Pb from bone can add to the mB-Pb burden, it is vital to understand and study gene polymorphisms that could also influence Pb mobilization from the bone. One such gene is the apolipoprotein E gene (*APOE*), which has been studied in relation to bone metabolism (bone turnover/remodeling) including bone mineral density (BMD) and bone fractures (Niemeier et al., 2012; Dieckmann et al., 2014; Zhang et al., 2014; Noguchi et al., 2018), as well as its effect on bone in combination with bone-lipids (Wang et al., 2023) and hormones like estrogen (Qi et al., 2023).

Apolipoprotein E (APOE) is a multifunctional lipid-binding glycoprotein expressed in various tissues and cells, including osteoblasts. It plays an important role in general and neuronal lipid metabolism (Tudorache et al., 2017). Moreover, emerging evidence suggests its role in preserving bone mass, presumably by delivering lipids and vitamin K to osteoblasts (Dieckmann et al., 2014; Niemeier et al., 2012) and influencing cholesterol, Ca, and vitamin D levels (Huebbe et al., 2011). The *APOE* gene is polymorphic, with two single nucleotide polymorphisms (SNPs; rs429358 and rs7421) forming three distinct protein isoforms: APOE2, APOE3, and APOE4, encoded by the ε2, ε3, and ε4 alleles, respectively. These isoforms have different binding affinities for lipids, receptors, oxidants, and some metals, leading to different functional properties and disease risks (Kara et al., 2017; Tudorache et al., 2017; Lumsden et al., 2020). The isoallelic ε2 genotype potentially has the lowest impact on maintaining bone mass (Dieckmann et al., 2014) and protection against bone fracture (Zhang et al., 2014). Accordingly, the ε2 allele has been identified as a potential genetic risk factor for skeletal disorders. However, most research on *APOE* SNPs focusses on ε4 allele, often overlooking the effect of ε2 (Niemeier et al., 2012).

Another frequently overlooked aspect in *APOE* studies is the impact of sex, despite the well-documented sex-related effects of *APOE* and its interaction with sex hormones (Belloy et al., 2019; Gamache et al., 2020). Equally important is the influence of fetal sex on fetal and maternal physiology, the functioning of the placenta, and the maternal-placental-fetal response to environmental toxicants (Al-qaraghouli et al., 2017; Enninga et al., 2015; O'Tierney-Ginn, 2020; Clifton, 2010). The influence of fetal sex on associations between maternal *APOE* genotypes and Pb levels in cord blood (CB–Pb) was also evident in our recent research on pregnant Italian women (2nd to 3rd trimester) and their newborns participating in the PHIME (Public Health Impact of Long-Term, Low-Level Mixed Element Exposure in Susceptibility Population Strata) study (Palir et al., 2023). We noted that girls born to ε2 allele carriers had higher CB-Pb levels than girls born to mothers without this allele.

In the present study, we extended our previous work by including participants from three countries (i.e., Slovenia, Italy, and Croatia) which were all part of the PHIME project and followed the same study protocol. This substantially increased the sample size, allowing us to exclude smoking participants and stratify both mothers and newborns by fetal/newborn sex. We aim to estimate whether the maternal ε2 allele, presumably associated with higher bone remodeling (Dieckmann et al., 2014), is linked to elevated Pb levels in blood during the second half of pregnancy when the fetal Ca accretion is elevated, although increased skeletal resorption of minerals predominates during lactation period (Ryan and Kovacs, 2021). The associations of maternal *APOE* (m*APOE*) genotypes with mB-Pb and CB-Pb were tested separately according to fetal/newborn sex. To eliminate potential confounding effects of parity on placental detoxification functioning (Prior et al., 2014), we also conducted separate analyses on *nulliparous* women and their newborns. Zinc (Zn) and selenium (Se) levels were as well followed to assess the nutritional status of pregnant women, as Zn and Se deficiency can impact gastrointestinal absorption of Pb (Ahamed and Siddiqui, 2007) and the integrity of the skeleton (Zofkova et al., 2017). Additionally, given that the PHIME study was designed to assess mercury (Hg) levels in this Mediterranean cohort with expected seafood consumption, and considering our prior findings of associations between Hg and *APOE* (Snoj Tratnik et al., 2017; Trdin et al., 2020; Palir et al., 2023), we conducted stratified association analysis on Hg as well. This can serve as an important indicator, considering Hg does not accumulate in the bone.

2. Material and methods

2.1. Study population

During the years 2006–2009, pregnant women from four Mediterranean countries - Italy, Croatia, Slovenia, and Greece - were recruited as participants in the PHIME study (Miklavčič et al., 2013; Valent et al., 2013). In Italy, the protocol was approved by the Ethics Committees of the University of Udine and the Institute for Maternal and Child Health, IRCCS Burlo Garofolo in Trieste. The Ethics Committee of the University Hospital Centre Rijeka approved the protocol for the Croatian participants; in Slovenia, the Ethics Committee of the University Medical Centre of Ljubljana, and in Greece, the Ethics Committee of the Institute of Child Health of Athens. The research was conducted in accordance with the Declaration of Helsinki, and all participants signed an informed consent form. The recruitment process, detailed study protocol, and the

Table 2a
Maternal group general characteristics and trace elements stratified by fetal sex.

Maternal group participants	All		♂		♀		**p**
	AM ± SD (min-max)	**N (%)**	**AM ± SD (min-max)**	**N (%)**	**AM ± SD (min-max)**	**N (%)**	
MOTHERS (m)		817		411		406	
mAge (years)	32.2 ± 4.57 (18–44)	813	32.1 ± 4.88 (18–44)	410	32.2 ± 4.25 (20–44)	403	0.979
mPre-pregnancy BMI (kg/m^2)	22.7 ± 3.86 (15.6–46.7)	815 (100)	22.7 ± 3.95 (15.6–42.4)	409 (100)	22.7 ± 3.76 (16.9–46.7)	406 (100)	0.873
Underweight (<18.5)		58 (7.12)		35 (8.56)		23 (5.66)	0.539
Normal (18.5 – < 25)		596 (73.1)		289 (70.7)		307 (75.6)	
Overweight (25 – < 30)		113 (13.9)		59 (14.4)		54 (13.3)	
Obesity (30 – < 40)		44 (5.40)		24 (5.87)		20 (4.93)	
Severe obesity (≥40)		4 (0.49)		2 (0.49)		2 (0.49)	
mParity	0.52 ± 0.68 (0–4)	815 (100)	0.51 ± 0.68 (0–4)	411 (100)	0.52 ± 0.68 (0–4)	404 (100)	0.621
nulliparous		467 (57.3)		239 (58.2)		228 (56.4)	0.336
primiparous-1		287 (35.2)		137 (33.3)		150 (37.1)	
multiparous-2		52 (6.38)		32 (7.79)		20 (4.95)	
multiparous-3		6 (0.74)		2 (0.49)		4 (0.99)	
multiparous-4		3 (0.37)		1 (0.24)		2 (0.50)	
mEducation		810 (100)		410 (100)		400 (100)	0.530
Elem. or high school		523 (64.6)		269 (65.6)		254 (63.5)	
University or higher		287 (35.4)		141 (34.4)		146 (36.5)	
mSeafood intake frequency (150 g portion/day)	0.36 ± 0.26 (0–2.21)	809	0.36 ± 0.27 (0–2.21)	407	0.37 ± 0.26 (0–1.56)	402	0.651
mEGW (weeks)		770		384		386	
2nd trimester (14–26)	20.5 ± 0.58 (19–24)	509 (66.1)	20.5 ± 0.58 (19–23)	262 (68.2)	20.6 ± 0.58 (19–24)	247 (64.0)	0.816
3rd trimester (27–40)	34.2 ± 3.43 (28–41)	261 (33.9)	34.2 ± 3.47 (28–41)	122 (31.8)	34.2 ± 3.42 (28–41)	139 (36.0)	0.991
mTrace elements	**AM ± SD (min-max) GM (95%CI)**	**N (%)**	**AM ± SD (min-max) GM (95% CI)** ♂	**N (%)** ♂	**AM ± SD (min-max) GM (95%CI)** ♀	**N (%)** ♀	
mB-Pb (ng/g)	12.3 ± 6.99 (3.58–87.6) 11.1 (10.7–11.4)	817	12.2 ± 6.13 (3.86–55.5) 11.1 (10.6, 11.5)	411	12.4 ± 7.77 (3.58–87.6) 11.0 (10.6, 11.5)	406	0.558
mB-Hg (ng/g)	3.13 ± 3.34 (0.11–39.6) 2.17 (2.04, 2.30)	815	2.98 ± 3.00 (0.11, 22.0) 2.06 (1.89, 2.25)	411	3.28 ± 3.65 (0.12, 39.6) 2.28 (2.10, 2.48)	404	0.228
mB-Zn (μg/g)	5.56 ± 1.14 (2.92–11.0) 5.45 (5.38, 5.52)	817	5.54 ± 1.08 (2.92–10.8) 5.44 (5.34, 5.54)	411	5.57 ± 1.19 (3.08–11.0) 5.46 (5.36, 5.57)	406	0.893
mP-Se (ng/mL)	73.9 ± 15.3 (33–118) 72.2 (71.1, 73.4)	786	73.5 ± 15.3 (35–115) 71.8 (70.2, 73.4)	397	74.4 ± 15.3 (33–118) 72.7 (71.1, 72.6)	389	0.427
mP-Zn (μg/mL)	0.73 ± 0.09 (0.46–1.08) 0.73 (0.72, 0.73)	784	0.74 ± 0.10 (0.46, 1.08) 0.73 (0.72, 0.74)	396	0.73 ± 0.09 (0.49, 1.05) 0.73 (0.72, 0.74	388	0.653

AM – arithmetic mean; SD – standard deviation; min – minimum; max – maximum; m – maternal; B – blood; P – plasma; GM – geometric mean; CI – confidence interval; ♂ – male fetal sex; ♀ – female fetal sex; p – values indicate statistically significant difference between the fetal sexes; EGW – estimated gestation week of pregnancy at maternal blood sampling.

number of participants from each country have been described elsewhere (Miklavčič et al., 2013; Valent et al., 2013).

The main inclusion criteria for mothers were a singleton, low-risk pregnancy, aged 18 years or older, and residency in the same region for at least 2 years. Recruitment and sampling took place at local maternity hospitals. Maternal fasting peripheral venous blood samples (whole blood, plasma, and serum) were collected during the prenatal period in Italy and Croatia only. Of those, 65% were taken during the second trimester (19–24 weeks), with 99% of those collected in the second half of the second trimester (20–24 weeks), and the remaining 35% during the third trimester of pregnancy (28–41 weeks). At delivery, mixed cord blood (whole blood, plasma, and serum) and cord tissue were collected in all countries.

In the present study, we introduced additional exclusion criteria and included only non-smoking participants with data on the m*APOE* genotype, fetal sex, and Pb concentrations in either maternal or cord blood. Accordingly, our study population comprised 953 mothers and 772 newborns from three countries: Italy, Croatia, and Slovenia. As previously mentioned, blood Pb levels were not available for Slovenian mothers. Therefore, the maternal group included 817 blood samples from mothers in Italy and Croatia, while the newborns group consisted of 772 cord blood samples from Italy, Croatia, and Slovenia. Detailed information on the number of participants with measured Pb levels in blood or cord blood, stratified by country of residence and fetal/newborn sex, is shown in Fig. 1. Participants' demographic, lifestyle, and personal information were obtained through interviews with the mothers conducted during the study.

2.2. *Determination of trace elements*

Measurements of Pb, Hg, and Zn in maternal blood and cord blood were performed at the Jožef Stefan Institute, Ljubljana, Slovenia, and Zn and Se in plasma at the University Medical Centre Ljubljana, Institute of Clinical Chemistry and Biochemistry, Ljubljana, Slovenia.

Concentrations of Pb and Zn in blood were determined via inductively coupled plasma mass spectrometry (ICP-MS), following the method described by Jagodic et al. (2017). The LOD for Pb was 1.30 ng/g, and for Zn 20.0 ng/g.

Blood Hg concentrations were determined via atomic absorption spectrometry using a direct mercury analyzer (Milestone, USA) (Miklavčič et al., 2013) and the LOD was 0.02 ng/g.

Zeeman electrothermal atomic absorption spectroscopy (ET-AAS) (Varian SpektrAA-800 ETAA spectrometer) was used to measure Se concentration in plasma (mP-Se) (Kobal et al., 2004), while flame AAS (Varian SpekrtAA-250 Plus FAAS) was used to measure Zn concentration (mP-Zn) (Tsalov and Zaprianov, 1983).

For all measurements, strict quality control procedures were followed, and blank samples, control samples, and reference materials were measured together with the samples on a daily basis, as described in the above-listed references.

2.3. *DNA isolation and genotyping*

DNA for *APOE* genotyping was isolated from maternal peripheral venous blood in the Croatian (Trdin et al., 2020) and Italian (Palir et al.,

Table 2b
Newborns group general characteristics and trace elements of match-paired mothers and newborns stratified by newborn sex.

Newborns group participants	All		♂		♀		
	AM ± SD (min-max)	**N (%)**	**AM ± SD (min-max)**	**N (%)**	**AM ± SD (min-max)**	**N (%)**	**p**
MOTHERS (m)		772		399		373	
mAge (years)	31.8 ± 4.49 (18–44)	767	31.8 ± 4.80 (18–44)	398	31.9 ± 4.13 (20–44)	369	0.709
mPre-pregnancy BMI (kg/m^2)	22.3 ± 4.09 (13.4–46.7)	770 (100)	22.3 ± 4.12 (15.6–42.4)	397 (100)	22.3 ± 4.07 (13.4–46.7)	373 (100)	0.947
Underweight (<18.5)		106 (13.8)		60 (15.1)		46 (12.3)	0.725
Normal (18.5 – < 25)		518 (67.3)		261 (65.7)		257 (68.9)	
Overweight (25 – < 30)		101 (13.1)		52 (13.1)		49 (13.1)	
Obesity (30 – < 40)		42 (5.45)		22 (5.54)		20 (5.36)	
Severe obesity (≥40)		3 (0.39)		2 (0.50)		1 (0.27)	
mEducation		766 (100)		398 (100)		368 (100)	0.332
Elem. or high school		455 (59.4)		243 (61.2)		212 (57.6)	
University or higher		311 (40.6)		155 (38.9)		156 (42.4)	
mParity	0.55 ± 0.70 (0–4)	770 (100)	0.54 ± 0.69 (0–3)	399 (100)	0.56 ± 0.72 (0–4)	371 (100)	0.640
nulliparous		428 (55.6)		225 (56.4)		203 (54.7)	0.363
primiparous-1		276 (35.8)		139 (34.6)		137 (37.0)	
multiparous-2		56 (7.28)		32 (8.02)		24 (6.49)	
multiparous-3		7 (0.91)		4 (1.00)		3 (0.81)	
multiparous-4		3 (0.39)		0 (0)		3 (0.81)	
mSeafood intake frequency (150 g portion/day)	0.35 ± 0.26 (0–2.21)	764	0.35 ± 0.27 (0–2.21)	396	0.35 ± 0.25 (0–1.56)	368	0.846
NEWBORNS (c)		772		399		373	
cLength (cm)	50.7 ± 2.26 (42–58)	760	51.1 ± 2.26 (43–58)	393	50.2 ± 2.17 (42–55)	367	**0.000**
cWeight (g)	3452 ± 482 (1450–5140)	763	3519 ± 472 (1790–4930)	396	3380 ± 483 (1450–5140)	367	**0.001**
cEGA (weeks)	39.5 ± 1.43 (28–42)	727 (100)	39.5 ± 1.37 (35–42)	375 (100)	39.4 ± 1.50 (28–42)	352 (100)	0.351
Pre-term (<37)		16 (2.20)		5 (1.33)		11 (3.13)	0.110
Full term (37–42)		711 (97.8)		370 (98.7)		341 (96.9)	
Post-term (> 42)		–		–		–	
cTrace elements	**AM ± SD (min-max)** **GM (95%CI)**	**N (%)**	**AM ± SD (min-max)** **GM (95% CI)** ♂	**N (%)** ♂	**AM ± SD (min-max)** **GM (95%CI)** ♀	**N (%)** ♀	
CB-Pb (ng/g)	10.4 ± 5.28 (1.82–47.0) 9.31 (9.01, 9.63)	772	10.3 ± 4.88 (1.82–34.1) 9.33 (8.92, 9.75)	399	10.5 ± 5.67 (2.63–47.0) 9.30 (8.85, 9.77)	373	0.785
CB-Hg (ng/g)	4.56 ± 4.40 (0.12, 32.8) 3.05 (2.85, 3.26)	766	4.70 ± 4.66 (0.12, 32.8) 3.10 (2.81, 3.41)	395	4.42 ± 4.10 (0.14, 26.3) 3.00 (2.73, 3.30)	371	0.727
CB-Zn (μg/g)	2.36 ± 0.60 (1.16–7.24) 2.29 (2.26, 2.33)	771	2.33 ± 0.56 (1.18–4.76) 2.23 (2.22, 2.32)	399	2.39 ± 0.65 (1.16–7.24) 2.32 (2.26, 2.37)	372	0.403

AM – arithmetic mean; SD – standard deviation; min – minimum; max – maximum; m – maternal; c – child; CB –cord blood; GM – geometric mean; CI – confidence interval; ♂ – male newborn sex; ♀ – female newborn sex; p – values indicate statistically significant difference between the fetal sexes; Length – newborn length at birth; Weight – newborn weight at birth; EGA – estimated gestational age at delivery.

2023) cohorts of the PHIME study, whereas for the Slovenian cohort, blood for DNA isolation was obtained during their recruitment in the PHIME follow-up study within the CROME-LIFE + project (Cross-Mediterranean Environment and Health Network) (2013–2017) (Stajnko et al., 2019).DNA extraction was performed using the FlexiGene® DNA kit (Qiagen, Hilden, Germany), following the manufacturer's protocols. The quantity and quality of the isolated DNA were determined using an ultraviolet–visible (UV-VIS) spectrophotometer NanoDrop 2000c (Thermo Fisher Scientific, USA).

Single nucleotide polymorphism (SNP) genotyping for *APOE* (rs7412 and rs429358) was performed using predesigned TaqMan SNP Genotyping Assays (Applied Biosystems, USA), as described in previous studies (Snoj Tratnik et al., 2017; Trdin et al., 2020; Palir et al., 2023). SNP frequencies were tested using Pearson's chi-squared test for deviation from the Hardy–Weinberg equilibrium (HWE) ($p > 0.05$). The basic SNP characteristics are given in the appendix (Table A1).

2.4. Statistics

Descriptive statistics were employed to evaluate participant characteristics derived from questionnaires (maternal age, pre-pregnancy body mass index [BMI], parity, education, daily seafood consumption, estimated gestation week [EGW] at venous blood sampling, newborn estimated gestation age [EGA] at delivery, newborn sex, length, and weight). Continuous variables were expressed as arithmetic means ± standard deviation (AM ± SD) with minimum and maximum values, while continuous variables were presented as frequency and percentage distributions. Additionally, concentrations of trace elements (TEs) were presented as AM ± SD and as geometric means with a 95% confidence interval (GM, 95% CI).

General characteristics were stratified by fetal/newborn sex, and differences between sexes were assessed using Chi-squared test for categorical variables and the Mann-Whitney *U* test for continuous variables. Parity was defined as the number of pregnancies reaching a gestation period of 20 weeks or more, with *nulliparity* indicating the first pregnancy surpassing 20 weeks and *multiparity* indicating 1 to 4 previous pregnancies extending beyond 20 weeks. Seafood intake frequency was determined based on participant responses regarding the consumption of 150 g of various seafood types at different frequencies (never, <1 × /month, 1–3 × /month, 1 × /week, 2–4 × /week, 5–6 × /week, 1 × /day, 2–3 × /day or > 3 × /day) (Miklavčič et al., 2013; Valent et al., 2013). EGW at blood sampling and EGA at delivery were calculated based on menstrual history.

Concentrations of Pb were further expressed as GM with 95% CI, stratified by fetal/newborn sex, with the statistical comparison between m*APOE* genotypes conducted using the Kruskal-Wallis test.

The associations between m*APOE* genotype and mB-Pb and CB-Pb as well as mB-Hg and CB-Hg were estimated using fetal/newborn-sex-stratified linear regression models with an adjustment for different confounding variables introduced in our previous work (Palir et al., 2023). Maternal blood zinc (mB-Zn) and cord blood zinc (CB–Zn) values were utilized as approximate substitutes for unmeasured hemoglobin and iron values, according to Gibson et al. (2008) and Houghton et al. (2016). Carriers of the ε2 (genotype: ε2/ε2 and ε2/ε3) and ε4 (genotype:

Table 3
mB-Pb and CB-Pb levels (GM, 95% CI) stratified by fetal sex and m*APOE* genotype.

	GM (95% CI) N			p
	ε3/ε3	**ε2/ε2, ε2/ε3**	**ε3/ε4, ε4/ε4**	
Maternal group				
♂				
All, N=408				
mB-Pb ng/g	11.2 (10.6, 11.8) 306	11.4 (10.0, 13.1) 45	10.2 (9.38, 11.1) 57	0.404
Nulliparous, N=238				
mB-Pb ng/g	11.2 (10.5, 12.0) 178	11.2 (9.56, 13.2) 26	9.82 (8.77, 11.0) 34	0.311
♀				
All, N=405				
mB-Pb ng/g	*10.9 (10.4, 11.4) 301*	*12.2 (10.6, 14.0) 37*	*10.9 (9.66, 12.3) 67*	*0.102*
Nulliparous, N=228				
mB-Pb ng/g	***10.8 (10.1, 11.5) 178***	***13.2 (11.1, 15.8) 20***	***10.6 (8.61, 12.9) 30***	***0.062***
Newborns group				
♂				
All, N=396				
CB-Pb ng/g	9.28 (8.80, 9.79) 302	9.23 (8.05, 10.6) 40	9.75 (8.83, 10.8) 54	0.659
Nulliparous, N=224				
CB-Pb ng/g	9.35 (8.71, 10.0) 172	9.37 (8.02, 11.0) 20	10.1 (9.01, 11.4) 32	0.366
♀				
All, N=373				
CB-Pb ng/g	9.35 (8.82, 9.91) 281	10.1 (8.46, 12.0) 33	8.65 (7.69, 9.73) 59	0.349
Nulliparous, N=203				
CB-Pb ng/g	**9.49 (8.75, 10.3) 156**	**12.2 (10.3, 14.5) 18**	**8.81 (7.37, 10.5) 29**	**0.046**

♂ – fetal/newborn sex is male; ♀ – fetal/newborn sex is female; GM – geometric mean; CI – confidence interval; m – maternal; B – blood; CB – cord blood; N – number of observations; p – statistically significant difference between any of the three groups; significant results ($p \leq 0.05$) are in bold, and marginally significant results ($p > 0.5$ and < 0.10) are in bold italics.

ε3/ε4 and ε4/ε4) allele were compared to the common m*APOE* genotype (ε3/ε3), and additional comparisons were made between ε4 carriers and ε2 carriers. Participants with ε2/ε4 were not included in statistical analysis as its function can resemble that of ε3/ε3.

The effect of m*APOE* genotypes on mB-Pb and mB-Hg concentrations was studied while controlling for mother's age, pre-pregnancy BMI, parity (*nulliparous/multiparous*), education (high school or lower/university or higher), EGW, seafood intake frequency, country of residence, and mB-Zn levels **(Model 1)**. To eliminate the influence of parity, which has been reported to affect blood Pb levels (Lewin et al., 2017; Bocca et al., 2020), the same model was run including only *nulliparous* women **(Model 2)**. The effect of m*APOE* on CB-Pb and CB-Hg was tested in a model adjusted for mother's age, pre-pregnancy BMI, parity (*nulliparous/multiparous*), education (high school or lower/university or higher), seafood frequency intake, country of residence, CB-Zn levels, newborn length and weight, and EGA **(Model 3)**. Again, to exclude the influence of parity, the model including only the newborns born to *nulliparous* mothers was run **(Model 4)**. All models were run separately based on fetal/newborn sex (♂ – male, ♀ – female). A flowchart depicting the selection of participants for statistical analyses is shown in Fig. 2 and statistical models' formulas are provided in the appendix (Equations A1 - A4).

Statistical analyses were conducted using **STATA12/SE** and R version 3.6.0 with **RStudio version February 1, 1335**. Natural log-transformation was applied to dependent variables (mB-Pb, CB-Pb, mB-Hg, and CB-Hg) to approximate a normal distribution. The estimation coefficients (b) of the independent variables were exponentiated (exp(b)) for easier interpretation. If the independent variables were log-transformed, their effects were presented with estimation coefficients (b), as in the case of mB-Zn and CB-Zn. Statistical significance (p-value) was set at a p-value of ≤ 0.05, with marginal significance defined as p-values between > 0.05 and ≤ 0.1.

3. Results and discussion

3.1. mAPOE genotype and allele frequencies

The frequencies of m*APOE* genotypes and alleles for the Maternal and Newborns groups are listed in Table 1. We did not observe significant differences in allele frequencies between the groups or between the fetal sexes. The frequencies were geographically consistent with those reported in the literature (Giau et al., 2015). Although the frequencies of *APOE* genotypes can vary even between neighboring countries, our study locations were geographically close and showed similar percentages of each genotype (data not shown).

The presence of the isoallelic genotypes ε2/ε2 and ε4/ε4 was exceptionally rare, accounting for less than or equal to 1% across all groups examined. Notably, the ε2/ε2 genotype was observed in only one mother with a female offspring and in four mothers with male offspring, while no instances of the ε4/ε4 genotype were detected among mothers with male offspring and only five instances among those with female offspring. Overall, there were no statistically significant differences in m*APOE* genotypes based on fetal/newborn sex (Table 1).

3.2. General characteristics and trace elements levels

Tables 2a and 2b presents the general characteristics and TEs concentrations of the Maternal and Newborns groups, respectively. These tables include data for all participants within the groups and are further stratified by fetal/newborn sex. Notably, no significant differences in general characteristics between the groups based on fetal/newborn sex were observed, with the expected exception of newborns' weight and length ($p < 0.001$, Table 2b).

As previously reported (Barbone et al., 2019; Miklavčič et al., 2013; Palir et al., 2023; Snoj Tratnik et al., 2017; Trdin et al., 2020), the participants exhibited very low to moderate levels of potentially toxic TEs, including Pb and Hg, in their maternal blood and cord blood samples. In this study, participants consistently showed low levels of Pb, with GM (95%CI) in maternal blood being 11.1 (10.7, 11.4) ng/g and in cord blood 9.31 (9.01, 9.63) ng/g. Only four women exceeded the recommended blood Pb threshold for pregnant women, which is set at 50 ng/mL (CDC, 2010; Taylor et al., 2014), and 13 women exceeded the recently updated CDC threshold of 35 ng/mL (Ruckart et al., 2021). None of the newborns' CB-Pb levels exceeded these thresholds, which is particularly noteworthy given the higher volume of erythrocytes in cord blood compared to venous blood. The observed Pb levels are

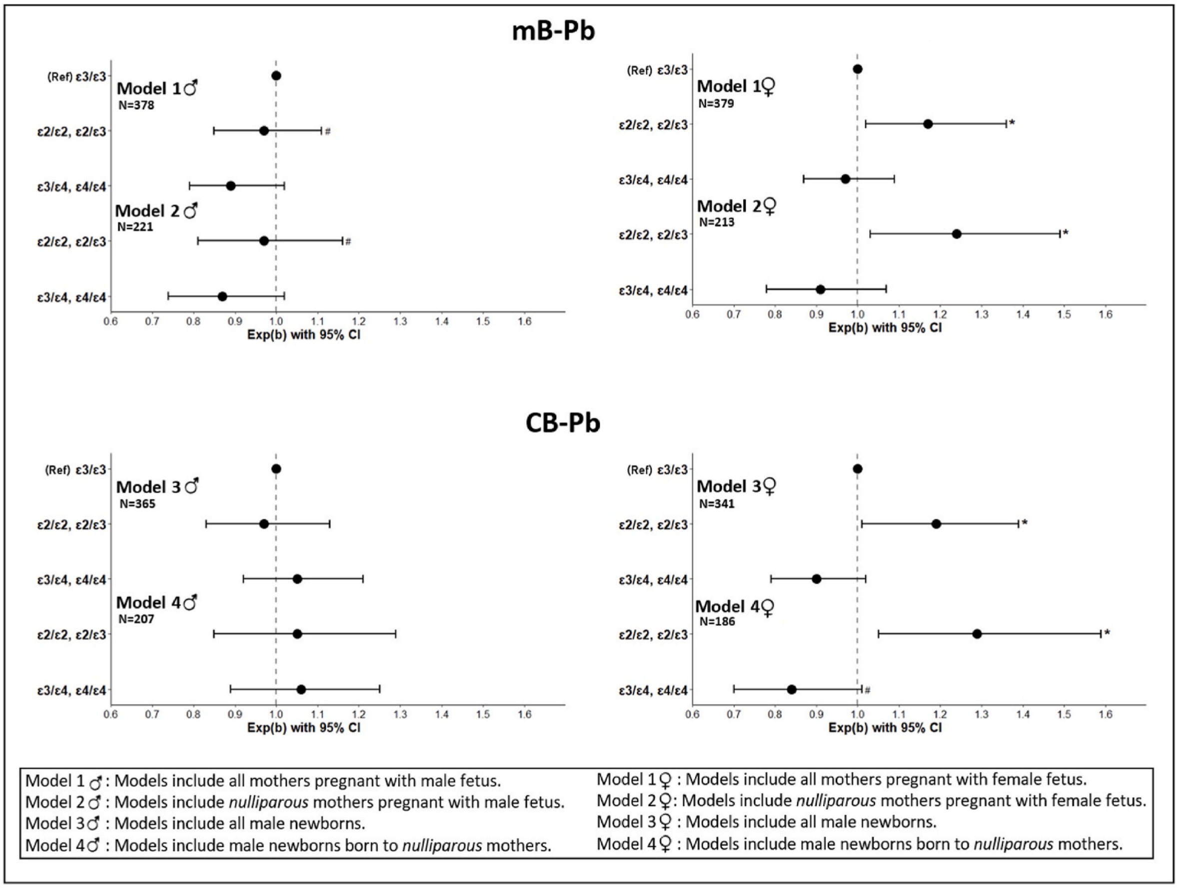

Fig. 3. The influence of *APOE* genotypes on mB-Pb and CB-Pb levels based on linear regression models, comparing the **ε2/ε2, ε2/ε3 and ε3/ε4, ε4/ε4 genotypes to the ε3/ε3 (reference genotype).**

♂ – fetal/newborn sex is male; ♀ – fetal/newborn sex is female; m – maternal; B – blood; CB – cord blood; exp(b) – exponentiation of the B coefficient; #p ≤ 0.1, *≤0.05. Models were adjusted for.

Model 1: mAge, mBMI, mParity (nulliparity/multiparity), mEducation (high school or lower/university or higher), mSeafood consumption, mEGW, mCountry (ITA/CRO), mB_Zn_log; m – maternal; c – child.

Model 2: as for Model 1 but without mParity.

Model 3: mAge, mBMI, mParity (nulliparity/multiparity); mEducation (high school or lower/university or higher), mSeafood consumption, mCountry (ITA/CRO/SLO), CB_Zn_log, cEGA, cWeight, cLength; m – maternal; c – child.

Model 4: as for Model 4 but without mParity.

Number of observations (N), statistical significance (p), the percentage of variability of Pb level explained by the model (R^2) and estimates of above listed variables for each model are given in tables A1 and A2 (Appendix).

representative of the post-industrial countries with an early ban of leaded gasoline (Poropat et al., 2018). In recent decades, environmental exposure to Pb has been gradually decreasing, largely due to the elimination of leaded gasoline and lead pipes used in plumbing (Bergdahl and Skerfving, 2022). Leaded gasoline was banned in Italy in 2002 (OECD, 2003), in Croatia in 2006 (Zorana et al., 2016), and in Slovenia in 2001 (OECD, 2012). Consequently, all participants were born significantly before the phase-out of leaded gasoline and could have potentially been exposed to it for 11–41 years until their inclusion in the PHIME project (2006–2009). Therefore, mB-Pb and CB-Pb in our study reflect both current external exposure and past internally accumulated exposure.

Consistent with prior reports on Italian and Croatian participants (Palir et al., 2023; Trdin et al., 2020), adequate levels of mP-Se and mP-Zn were also observed among the participants, with levels of 72.2 (71.1, 73.4) ng/mL and 0.73 (0.72, 0.73) μg/mL, respectively (Table 2a). These finding indicate good micronutrient status (Abbassi-Ghanavati et al., 2009; Thomson, 2004; Varsi et al., 2017), with no significant differences among groups stratified by fetal sex (Table 2a).

3.3. *Lead concentrations in maternal blood and newborns' cord blood stratified by mAPOE genotype and fetal sex (Bivariate analysis)*

We conducted a simple comparison of mB-Pb and CB-Pb among m*APOE* genotypes, stratified by fetal/newborn sex, including all mothers or only *nulliparous*, and all newborns or only newborns born to *nulliparous* mothers (Table 3). Statistically significant or marginally significant differences in mB-Pb and CB-Pb concentrations between m*APOE* genotypes were observed only for *nulliparous* women with female offspring and in female newborns. In both cases, ε2 genotypes were associated with higher Pb levels. A similar trend was noted for mB-Pb in mothers carrying female fetuses, regardless of parity status (referred to as All). However, since blood Pb levels can be influenced by various factors (age, education, seafood intake, country of residence, EGW, etc.), particularly when concentrations are low, we accounted for these potential confounding variables using multiple linear regression models.

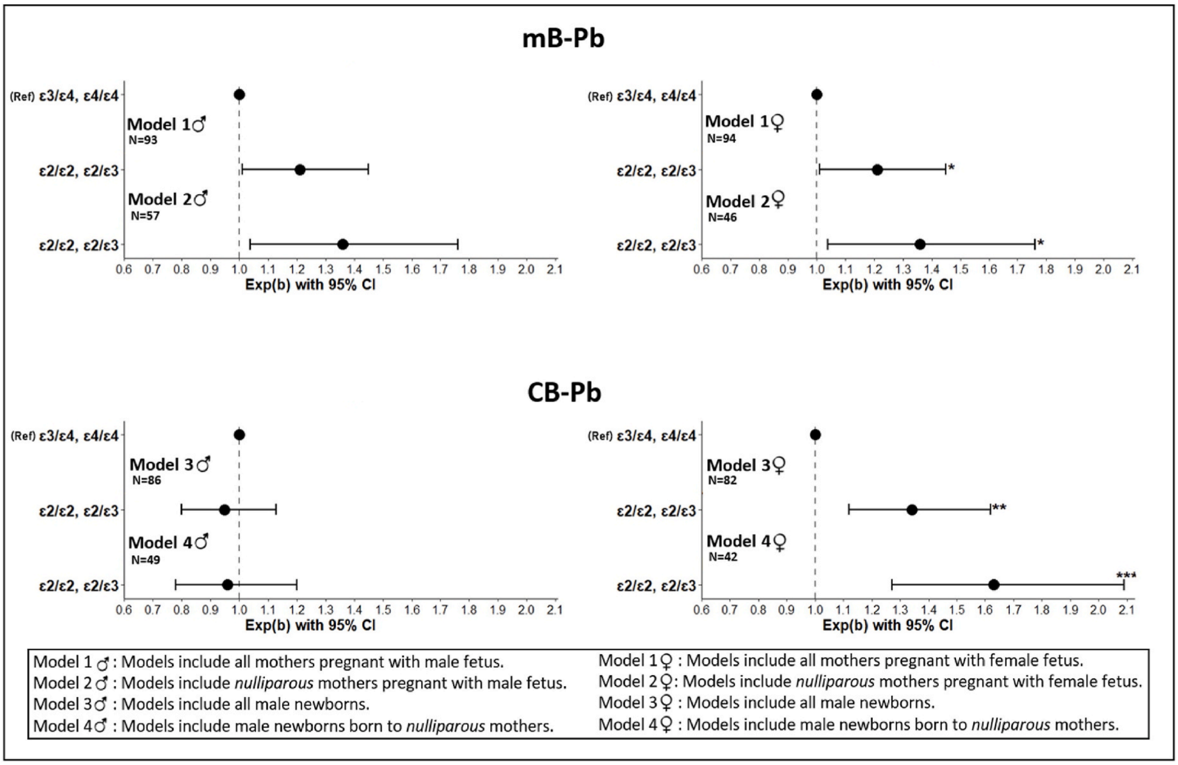

Fig. 4. The influence of *APOE* genotypes on mB-Pb and CB-Pb levels based on linear regression models, comparing the ε2/ε2, ε2/ε3 genotype to the ε3/ε4, ε4/ε4 (reference genotype).

♂ – fetal/newborn sex is male; ♀ – fetal/newborn sex is female; m – maternal; B – blood; CB – cord blood; exp(b) – exponentiation of the B coefficient; $*p \leq 0.05$, $**p \leq 0.01$, $**p \leq 0.001$. Models were adjusted for.

Model 1: mAge, mBMI, mParity (nulliparity/multiparity), mEducation (high school or lower/university or higher), mSeafood consumption, mEGW, mCountry (ITA/CRO), mB_Zn_log; m – maternal; c – child.

Model 2: as for Model 1 but without mParity.

Model 3: mAge, mBMI, mParity (nulliparity/multiparity); mEducation (high school or lower/university or higher), mSeafood consumption, mCountry (ITA/CRO/SLO), CB_Zn_log, cEGA, cWeight, cLength; m – maternal; c – child.

Model 4: as for Model 4 but without mParity.

Number of observations (N), statistical significance (p), the percentage of variability of Pb level explained by the model (R^2) and estimates of above listed variables for each model are given in tables A3 and A4 (Appendix).

3.4. Associations between mAPOE genotypes and Pb (Hg) levels in maternal (cord)blood stratified by fetal/newborn sex and parity (multiple linear regression models)

We employed fetal/newborn-sex-stratified multiple linear regression models to estimate the associations between m*APOE* genotypes and mB-Pb and CB-Pb (Fig. 3 – 4, Table A2-A5). Firstly, we ran the models with all participants (Model 1, Model 3) and then focused on *nulliparous* women and their newborns (Model 2, Model 4). We tested the impact of the ε2 or ε4 genotypes versus the "neutral" ε3 homozygotes, as well as ε4 compared to ε2. Fig. 3 – 4 present the estimates for Pb, while the estimates for additional explanatory variables are presented in the Appendix (Tables A2 – A5).

The results confirmed the association between the presence of the ε2 allele and higher concentrations of mB-Pb and CB-Pb, but only when the fetal/newborn sex was female. Mothers carrying the ε2 allele had 17% (95%CI 2%, 36%) higher mean mB-Pb levels than mothers with the ε3/ε3 genotype (Fig. 3, Model 1♀, Table A2). In cord blood, we observed a slightly higher increase of 19% (95CI 1%, 39%) in mean Pb levels if m*APOE* genotype was ε2/ε2 or ε2/ε3 in comparison to ε3/ε3 (Fig. 3, Model 3♀, Table A3). As aforementioned in the introduction, several studies suggest that *APOE* is involved in bone homeostasis, including maintaining bone mass by regulating bone metabolism, including turnover (Dieckmann et al., 2014; Noguchi et al., 2018; Wang et al., 2023). Some studies highlight the isoallelic ε2 genotype as having the weakest effect on maintaining bone mass (Dieckmann et al., 2014; Niemeier et al., 2012; **S. Q.** Zhang et al., 2014). This aligns with our findings; if bone turnover is indeed higher among ε2 carriers (Dieckmann et al., 2014), it could influence blood Pb concentrations. Notably, the majority of accumulated Pb, both historically and during the first half of pregnancy, is stored in bones (ATSDR, 2020) and can be released along with Ca during the second half of pregnancy when sampling was performed. Furthermore, our models reveal modest yet statistically significant positive associations between age and mB-Pb, as well as CB-Pb (which were consistently slightly higher when the fetal/newborn sex was female) (Table A3 - A4). This suggests that part of the Pb measured in maternal and cord blood originates from historically accumulated bone-Pb, as demonstrated by isotopic measurements in previous studies (Gulson et al., 1997; Kovacs, 2016).

In our research, when fetal/newborn sex was male, no association was found between mB-Pb and CB-Pb levels when comparing carriers of the ε2 allele to ε3/ε3 (Fig. 3, Model 1♂, Model 3♂, Table A2 – A3). We believe these sex-based differences can be at least partially attributed to sex hormones. Jasienska et al. (2015) reported interactions between sex hormones and *APOE* genotypes in fertile females, and Glynn et al. (2016) observed higher levels of serum sex hormones in mothers carrying female fetuses. Moreover, in an ongoing study involving mothers in the third trimester and their newborns from Kyrgyzstan ($N = 91$), we observed significantly higher levels of testosterone, progesterone, estradiol, and cholesterol in mothers pregnant with female fetus

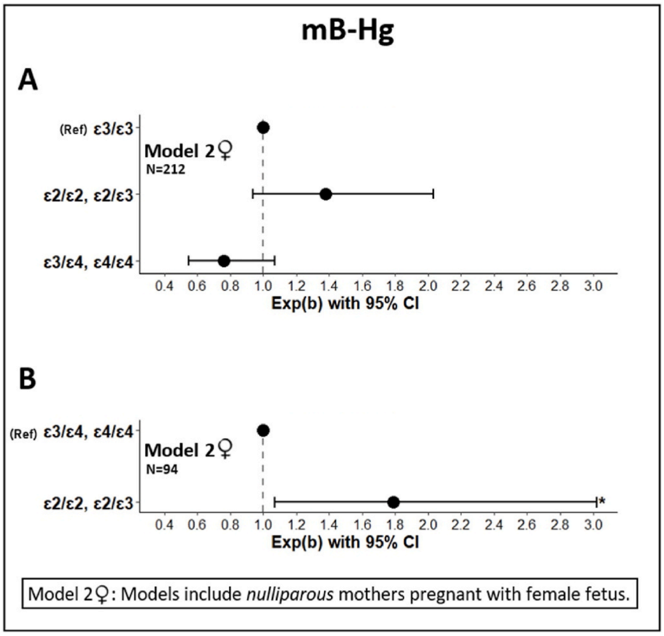

Fig. 5. The influence of *APOE* genotypes on mB-Hg levels based on linear regression models, comparing (A) the *ε*2/*ε*2, *ε*2/*ε*3 and *ε*3/*ε*4, *ε*4/*ε*4 genotypes to the *ε*3/*ε*3 (reference genotype) and (B) comparing the *ε*2/*ε*2, *ε*2/*ε*3 genotype to the *ε*3/*ε*4, *ε*4/*ε*4 (reference genotype). ♂ – fetal/newborn sex is male; ♀ – fetal/newborn sex is female; m – maternal; B – blood; CB – cord blood; exp(b) – exponentiation of the B coefficient; *p ≤ 0.05. Models were adjusted for.
Model 2: mAge, mBMI, mEducation (high school or lower/university or higher), mSeafood consumption, mEGW, mCountry (ITA/CRO), mB_Zn_log; m – maternal; c – child.Number of observations (N), statistical significance (p), the percentage of variability of Hg level explained by the model (R^2) and estimates of above listed variables for each model are given in tables A6 and A7 (Appendix).

compared to those pregnant with a male; the difference was even higher if the mothers carried the ε4 allele (Tursunova et al., 2023; unpublished data). In the elderly population, increasing evidence suggests the involvement of sex hormones (particularly estradiol but also progesterone) in APOE effects related to AD, menopause-associated bone loss, dyslipidemia, and the risk of cardiovascular disease (Belloy et al., 2019; Gamache et al., 2020, Valencia-Olvera et al., 2022). It is evident that various sex-based hormonal-APOE interactions occur in (patho)physiological, metabolic processes during early life and later, with different effects at different life stages and health conditions (Valencia-Olvera et al., 2022; Jasienska et al., 2015). The absence of associations between mB-Pb and CB-Pb levels with *APOE* genotypes could also be attributed to the larger size, higher bone mineral density, and therefore presumed increased mineral flux into the skeletal system of male babies. This enhanced mineral flux might obscure any association between the low concentrations of Pb observed in this study and *APOE* genotype.

The influence of the ε2 allele on mB-Pb and CB-Pb became even more pronounced when only *nulliparous* mothers and their newborns were included. The difference in Pb concentrations between ε3/ε3 and ε2/ε2, ε2/ε3 increased to 24% (95%CI 3%, 49%) in blood (Fig. 3, Model 2♀, Table A2) and to 29% (95%CI 5%, 59%) in cord blood (Fig. 3, Model 4♀, Table A3) when nulliparity was considered as an inclusion factor. It is important to note, that in the Maternal group, the average age was 32.2 years for all participants **(Model 1)** and 31.2 years for *nulliparous* women **(Model 2)** and in the Newborns group, the corresponding values were 31.8 **(Model 3)** and 30.9 years **(Model 4)**. As the age distribution of mothers was similar between the 'All' and '*Nulliparous*' groups, our findings may indicate the modulating effects of the *ε*2 allele and *nulliparity* on Pb kinetics, leading to an increased transfer of Pb from maternal blood to cord blood. The protective effect of parity, or parity as a negative predictor for Pb, Cd and/or Hg exposure in maternal and cord blood, has already been reported (Lewin et al., 2017; Bocca et al., 2020; Trdin et al., 2020). Lewin et al. (2017) measured several toxicants and found that chemical concentrations are generally higher in *nulliparous* women than in *uniparous* or *multiparous* women, likely due to the enhanced placenta's ability to detoxify toxic elements. Parity can affect metal kinetics through various processes (Gundacker and Hengstschläger, 2012), and *nulliparous* women are generally more vulnerable due to less effective placental detoxification functioning (Prior et al., 2014), whereas *multiparity* (however below five or more pregnancies), signifies a protective metabolic adaptation of the placenta. A recent study by Punshon et al. (2019) reported that "parity was positively associated with placental weight, efficiency increased for every previous pregnancy and placental disc symmetry was greater among women with higher number of previous pregnancies". It should also be noted that in our study, only 1% of the participants had three or four previous pregnancies, and none had five or more (Tables 2a and **2b**), which could signify a higher-risk pregnancy (Bai et al., 2002).

When conducting the statistical analysis, we also identified an association between the m*APOE* ε4 allele and mB-Pb concentrations. The m*APOE* ε4 allele showed a protective effect against Pb exposure, although the associations did not reach statistical significance in all groups (Fig. 3, Table A2 – A3). Among mothers pregnant with a male fetus, ε4 carriers had mean mB-Pb levels that were 11% (95%CI -21%, +2%) or 13% (95%CI -26%, +2%) lower than those in the ε3/ε3 group, depending on whether all participants were included or only *nulliparous* women (Fig. 3, Model 1♂, Model 2♂, Table A2). Furthermore, in female newborns, a trend towards lower CB-Pb in the ε4 allele group compared to the ε3/ε3 group was found (p = 0.104) (Fig. 3, Model 3♀, Table A3). This trend became marginally significant (p = 0.063) when only newborns born to *nulliparous* women were included (−16%, 95%CI -30%, +1%) (Fig. 3, Model 4♀, Table A3). These observations could indicate the protective function of maternal ε4 for pregnant women and their newborns. Despite the ε4 allele being considered a risk factor for age-related diseases like late-onset AD, it has been proposed to have beneficial effects in early life and on fertility (Tudorache et al., 2017; Jasienska et al., 2015; Oria et al., 2020; Trdin et al., 2020). Huebbe et al. (2011) demonstrated, in targeted replacement mice and humans, that the *APOE* ε4 allele is associated with higher levels of cholesterol and vitamin D, and more efficient intestinal Ca absorption, leading to higher Ca levels.

To further examine the impact of the ε2 allele, we conducted an additional step by directly comparing the effects of the ε2 and ε4 alleles, which in previous steps displayed opposite effects on mB-Pb and CB-Pb. As expected, significant differences in Pb levels were only observed when the fetal/newborn sex was female. Carriers of the *ε*2 allele demonstrated 21% (95%CI 1%, 45%) higher mean mB-Pb levels than ε4 when considering all participants regardless of parity (Fig. 4, Model 1♀, Table A4). When focusing specifically on *nulliparous* women, this difference increased to 36% (95%CI 4%, 76%) (Fig. 4, Model 2♀, Table A4). In cord blood, if m*APOE* genotype included *ε*2, mean Pb levels were 34% (95%CI 12%, 62%) higher compared to *ε*4 genotypes when analyzing all newborns (Fig. 4, Model 3♀, Table A5) and 63% (95%CI 27%, 109%) when analyzing only those born to *nulliparous* women (Fig. 4, Model 4♀, Table A5). These results support the possible impact of the ε2 allele on increased (cord)blood Pb levels, which could be driven by various multifactorial mechanisms, including increased bone remodeling.

In our study, we also tested associations between m*APOE* genotype and Hg (cord)blood levels using the same models. Similar to the findings for mB-Pb, we observed a 38% (95%CI -6%, 103%) higher mean mB-Hg in ε2 carriers in comparison to ε3/ε3 genotype when fetal sex was female and mothers were *nulliparous*; at the same time, ε4 carriers were associated with lower mB-Hg levels (Fig. 5A, Table A6, Model 2♀). Hence, significant associations emerged when comparing ε2 allele carriers to ε4 carriers, demonstrating that ε2 carriers exhibited 79% (95%CI 7%, 202%) higher mB-Hg levels (Fig. 5B–Table A7, Model 2♀). However, in other models analyzing m*APOE* genotypes and mB-Hg or CB-Hg, no associations were found (data not shown). Knowing that Hg does not readily store in the bones, this finding suggests that *ε*2 may be acting through various pathways. Experimental data on different interactions such as APOE/selenoprotein P (Jin et al., 2020), selenoprotein P/Pb (Bi et al., 2019), APOE/metallothioneins (Augsten et al., 2011; Graeser et al., 2012), and metallothioneins/Pb (He et al., 2014) suggest that APOE has a complex impact on Pb on various levels. Thus, our results point to the influence of the m*APOE ε*2 allele on increased mB-Pb and CB-Pb levels; however, determining the primary mechanisms behind this influence remains a challenge, particularly at low exposure levels where accurately estimating external dietary or environmental Pb exposure and distinguishing it from internal bone Pb exposure can be difficult. Future mechanistic studies are necessary to ascertain the significance of the *APOE ε*2 allele.

3.5. Study strengths and limitations

Our main strength is the relatively large sample size, which enabled us to compare ε2 allele carriers with ε4 allele carriers and those with the 3/3 genotype. Many studies involving *APOE* often diminish the significance of the ε2 allele by combining it with ε3/ε3 or excluding ε2 carriers altogether. Another notable strength is our stratification of all statistical analyses based on fetal/newborn sex, whereas in many studies, sex is merely added as a confounder. Furthermore, our study's robustness extends to the inclusion of additional trace elements such as Zn and Se, offering insights into nutritional status and their potential impact on gestational Pb absorption and bone integrity. Additionally, we investigated the influence of m*APOE* on Hg, recognizing that Hg in contrast with Pb, is not stored in the bone.

However. there are some limitations in the study that increase uncertainty in data and limit the refinement of statistical associations. The first is a common issue in epidemiological and human biomonitoring studies: reliance on self-reported data on smoking status, dietary habits, etc. Another limitation is the lack of data on hemoglobin/hematocrit levels, which we partly compensated for by including mB-Zn and CB-Zn concentrations in the statistical models. Additionally, another challenge of the study is the sampling of mixed CB instead of arterial or venous CB, resulting in random ratios of arterial and venous CB in samples. Hemoglobin values vary between arterial and venous CB (Masoumi et al., 2017), which affects the concentration of erythrocytes-accumulating elements like Pb. It is also known that fetal demand for TEs and other nutrients varies greatly depending on the week of gestation; therefore, it would be extremely beneficial to sample women for prenatal blood analysis in the same week of pregnancy. This could eliminate another bias due to changing lipid status during pregnancy (Zheng et al., 2017). Having data on hormonal and lipid status would also improve the observed associations.

4. Conclusions

We discovered fetal/newborn-sex-based associations between m*APOE* genotypes and blood Pb levels in pregnant women and newborns, who had low blood Pb levels and adequate plasma Zn and Se levels. The presence of the maternal *ε*2 allele was significantly associated with higher mean mB-Pb and CB-Pb levels when the fetal/newborn sex was female. This may be linked to heightened bone turnover among *ε*2 carriers, a factor potentially amplified by the unique calcium dynamics of pregnancy and modifying effects between sex hormones and *APOE* genotypes. Conversely, the ε4 allele was linked with lower mB-Pb regardless of fetus sex and lower CB-Pb in female newborns, suggesting a protective effect of ε4 against Pb exposure and proposing beneficial effects of *APOE* ε4 early in life. Notably, genotype-based observations were contingent on fetus/newborn sex and were more pronounced in the case of *nulliparity*. These findings highlight the importance of considering sex, genetic polymorphisms, and parity as influencing factors in future studies.

CRediT authorship contribution statement

Neža Palir: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Anja Stajnko:** Writing – review & editing, Visualization, Investigation, Formal analysis, Data curation. **Darja Mazej:** Writing – review & editing, Formal analysis, Data curation. **Alenka France Štiglic:** Data curation. **Valentina Rosolen:** Writing – review & editing, Resources, Data curation. **Marika Mariuz:** Resources. **Luca Ronfani:** Resources. **Janja Snoj Tratnik:** Writing – review & editing, Methodology. **Agneta Annika Runkel:** Writing – review & editing, Methodology. **Veronika Tursunova:** Resources. **Janja Marc:** Resources, Methodology. **Igor Prpić:** Writing – review & editing, Resources, Methodology. **Zdravko Špirić:** Writing – review & editing, Project administration, Methodology. **Fabio Barbone:** Writing – review & editing, Resources, Project administration, Methodology. **Milena Horvat:** Writing – review & editing, Resources, Project administration, Methodology, Funding acquisition, Formal analysis. **Ingrid Falnoga:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.119583.

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