Environment International 193 (2024) 109034

Contents lists available at ScienceDirect

Environment International

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

Full length article

Associations between APOE genotypes, urine 8-isoprostane and blood trace elements in middle-aged mothers (CROME study)

Alenka France Štiglic [a,c], Anja Stajnko [b], Alenka Sešek Briški [a], Janja Snoj Tratnik [b], Darja Mazej [b], Aleš Jerin [a,c], Milan Skitek [a,c], Milena Horvat [b,d], Janja Marc [a,c], Ingrid Falnoga [b,*]

[a] Clinical Institute of Clinical Chemistry and Biochemistry, University Medical Centre Ljubljana, Njegoševa 4, 1000 Ljubljana, Slovenia
[b] Department of Environmental Sciences, Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia
[c] University of Ljubljana, Faculty of Pharmacy, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia
[d] Jožef Stefan International Postgraduate School, Jamova cesta 39, 1000 Ljubljana, Slovenia

ARTICLE INFO

Keywords:
APOE alleles
Trace elements
Lipid peroxidation status
Multiple variable linear regression models
Human population

ABSTRACT

Background: There is almost no data on the combined associations between apolipoprotein E gene (*APOE*) genotypes, trace elements (TEs), and lipid peroxidation *in vivo*. The aim of our study was to evaluate the association between *APOE* genotypes and TE levels in blood (B-TEs) and erythrocytes (E-TEs), and 8-isoprostane in urine (U-8-isoprostane) in women with low exposure to potentially toxic TEs and with adequate supply of essential TEs.
Methods: B-TEs, E-TEs and U-8-isoprostane were determined in 172 healthy women of childbearing age (30.1–51.4 years) using ICP-MS and ELISA competitive assay, respectively. All women were divided into three *APOE* genotype groups according to the presence of the ε4 allele, ε2 allele or ε3 homozygotic allele. The associations between B-TEs, E-TE, U-8-isoprostane, and the *APOE* genotype groups were estimated by multiple variable linear regression models with relevant explanatory variables (e.g., age, BMI, and seafood).
Results: All TE and U-8-isoprostane levels were inside the reference ranges for the healthy population. In the multiple variable linear regression models, our results showed that urine 8-isoprostane levels increased by up to 43.3% in the *APOE*4 group compared to the *APOE*3 group and a negligible negative modifying effect for essential TEs. However, the *APOE* genotype groups were associated also with some TEs. A clear positive association was found between the *APOE*2 and *APOE*4 groups (vs. *APOE*3) with B-molybdenum.
Conclusions: Our study suggests that the *APOE*4 genotype played an important role in 8-isoprostane variability in a population with an adequate supply of essential and with low exposure to potentially toxic TEs. Adequate copper, zinc and selenium status seemed to be protective against, while the levels of nonessential TEs were probably too low to play a decisive role in 8-isoprostane formation. The observed impact of the *APOE*2 and *APOE*4 groups on increased B-molybdenum opens a new research topic.

1. Introduction

Apolipoprotein E (protein apoE; gene *APOE*) is a glycoprotein, mainly involved in the regulation of lipid metabolism. Its main function is to maintain lipoproteins' structural integrity and solubilization in the blood (Dose et al., 2016). The human *APOE* gene with two major single nucleotide polymorphisms (SNPs) in exon 4 encodes three major isoforms of the apoE protein—E2, E3 and E4—according to three possible genetic variants—ε2, ε3 and ε4, respectively. Numerous studies have been conducted on the involvement of apoE or the *APOE* gene in lipid and neuronal homeostasis. ApoE isoforms distribute and redistribute lipids among tissues and intracellularly, and they modulate various physiological and pathological processes differently, including normal neurodevelopment and fertility and age-related cardiovascular and neurodegenerative diseases (Dose et al., 2016; Jofre-Monseny et al., 2008; Kacperczyk et al., 2021; Tudorache et al., 2017). Their structural and conformational differences can affect their affinity with various lipid receptors, lipid particles, and essential metals (Ikewaki et al., 2002;

* Corresponding author.
E-mail addresses: alenka.stiglic@kclj.si (A. France Štiglic), anja.stajnko@ijs.si (A. Stajnko), alenka.briski@kclj.si (A. Sešek Briški), janja.tratnik@ijs.si (J. Snoj Tratnik), darja.mazej@ijs.si (D. Mazej), ales.jerin@kclj.si (A. Jerin), milan.skitek@kclj.si (M. Skitek), milena.horvat@ijs.si (M. Horvat), janja.marc@ffa.uni-lj.si (J. Marc), ingrid.falnoga@ijs.si (I. Falnoga).

https://doi.org/10.1016/j.envint.2024.109034
Received 13 June 2024; Received in revised form 21 September 2024; Accepted 23 September 2024
Available online 27 September 2024

Miyata and Smith, 1996; Weisgraber et al., 1982; Xu et al., 2015, 2014), as well as their isoforms' plasma levels and stability (Acharya et al., 2002; Ikewaki et al., 2002; Khan et al., 2013). Consequently, their structural differences are reflected in the intensity of their anti-atherogenic, anti-inflammatory and antioxidative functions, mostly in the order *APOE*2 > *APOE*3 > *APOE*4 (Dose et al., 2016; Jofre-Monseny et al., 2008; Mabile et al., 2003; Miyata and Smith, 1996; Tudorache et al., 2017). The modulation of lipid peroxidation and antioxidant defense mechanisms seem to be a relevant physiological isoform-dependent function of apoE (Egert et al., 2012). However, the interplay and balance between numerous oxidant and antioxidant mechanisms are highly complex and not unambiguous (Sies et al., 2017).

Elevated levels of trace elements (TEs), such as Cu, Cr, V, Co, Mn, Cd, Ni, As, Pb and Hg, are known to be involved in oxidative stress mechanisms through the generation of reactive oxygen species (ROS) and nitrogen species in the body (Nuran Ercal et al., 2001; Valko et al., 2005) leading to damage cellular structures such as lipids in cell membranes, as well as to proteins and DNA (Dröge, 2002). However, essential TEs are commonly involved in the antioxidant defense system (Cu, Zn, Mn, Se, Mo) as part of or by regulating the expression of various biological molecules, such as antioxidant enzymes, glutathione and metallothioneins (MTs) (Barchielli et al., 2022; Graeser et al., 2012; Marreiro et al., 2017; Rixen et al., 2023; Smith and Nordberg, 2015; Valko et al., 2005).

Polyunsaturated fatty acids are major peroxidation targets, especially in conditions in which the balance between the production and elimination of ROS is compromised (Dröge, 2002; Pryor and Stanley, 1975). The non-enzymatic peroxidation of arachidonic acid in membrane phospholipids results in the formation of relatively stable 8-isoprostane (also known as 8-iso prostaglandin F2α, which is excreted in urine (Ito et al., 2019; Le, 2015). As such, it is used as a marker of lipid oxidative status. However, isoprostanes also have biological activity in humans as homeostatic mediators for maintaining physiological functions at low levels and are involved in inflammation and immunity at higher levels (Ahmed et al., 2020; Galano et al., 2017). In several studies, the association of 8-isoprostane has been shown with apoE isoforms and apoE expression (Dietrich et al., 2005; Tangirala et al., 2001; Trares et al., 2020; Yao et al., 2004), and exposure to different metals (Ashrap et al., 2021; Dashner-Titus et al., 2018; Hu et al., 2021). These associations demonstrate the usefulness of 8-isoprostane measurement in the assessment of physiological or pathological oxidative status in relation to apoE and TEs.

TEs metabolism and lipid oxidative status (Dietrich et al., 2005; Dose et al., 2016; Egert et al., 2012; Jofre-Monseny et al., 2008; Miyata and Smith, 1996; Ramassamy et al., 1999) are supposed to differ between the three *APOE* variants. The differences are without adverse consequences for a healthy population, but during disease or specific physiological conditions, such as pregnancy, they can represent a risk or resilient factor for a worse or better outcome, respectively. The data suggest that external and internal factors that affect oxidative stress and its management in women may influence the time taken to conceive and the incidence of spontaneous early termination of pregnancy (Agarwal et al., 2012; Lu et al., 2018). Conception problems are a growing public health concern and identifying factors that can improve fertility and pregnancy outcomes is of great importance. There are nearly no data on the combined association between *APOE* variants, TEs and lipid peroxidation *in vivo*. In the present study, we aim to evaluate the associations between *APOE* genotypes and TEs levels in the blood (B-TEs) and erythrocytes (E-TEs), and 8-isoprostane in the urine (U-8-isoprostane) of women of childbearing age exposed to low concentrations of potentially toxic metals. In this way, we seek to explain differences in (lipid) oxidative status and identify more susceptible or resistant/resilient individuals in the female population of childbearing age, which may impact their general gynecological health, fertility, and pregnancies.

2. Materials and methods

2.1. Study population

The study subjects were 178 non-pregnant healthy women of childbearing age living in the central part of Slovenia. Participants were recruited between May and November 2016 from the EU-funded Cross-Mediterranean Environment and Health Network (CROME-LIFE +) project. All participants previously participated in the EU-funded Public health impact of long-term, low-level, mixed element exposure in susceptible population strata (PHIME-FP6) project. The methods and inclusion criteria were as described by Valent et al. (Valent et al., 2013). We included women who had already participated in the study, so there were no additional inclusion or exclusion criteria. Six participants were excluded from the statistical analyses due to two participants reporting occupational exposure to Cd and/or Hg, two having genotype $\varepsilon 2/\varepsilon 4$ and two having ongoing pregnancy and low hemoglobin, which led to 172 participants being included in the study. All participants signed written consent forms and provided information about their basic personal characteristics (age, weight, and height), living environment, nutritional habits, smoking habits, possible exposure, education, and employment through an interviewer-administered questionnaire.

The National Medical Ethics Committee approved the research protocol (KME 98/05/06 and 65/09/14).

2.2. Samples and sampling data

Non-fasting peripheral blood samples (6 mL K2EDTA BD Vacutainer® tubes for TEs and 3 mL K3EDTA for hemoglobin and hematocrit determination) and spot urine samples (precleaned plastic urine container) were collected during the day by well-trained personnel at appointed visits to the Pediatric clinic at University Medical Centre Ljubljana in the Clinical department for developmental, child and adolescent neurology under supervision of prof. dr. David Neubauer. After collection, the samples were processed according to the standard protocol.

2.3. Analytical methods

2.3.1. Trace elements determination

Trace elements were determined in blood (essential B-Co, B-Cu, B-Mn, B-Mo, B-Se, and B-Zn; nonessential B-Ag, B-Al, B-As, B-Cd, B-Cr, B-Hg, B-Pb, B-Rb, B-Sn, B-Sr and B-V), and erythrocytes (E-Co, E-Cu, E-Mo, E-Mn, E-Se, E-Zn, E-As, E-Cd, E-Hg, E-Pb, E-Rb and E-V) at the Institute of Clinical Chemistry and Biochemistry at the University Medical Centre Ljubljana. Measurements of prepared samples, calibrators and control samples were taken on an ICP-MS with the Octopole Reaction System (7700x, Agilent Technologies, Japan) as previously described in detail (France Štiglic et al., 2024; Stajnko et al., 2019). An aliquot of blood and washed erythrocyte sample, calibrator, or control sample was mixed with ammonium hydroxide solution (containing ethylenediaminetetraacetic acid disodium salt dehydrate, Triton X-100, and 1-butanol) and internal standard solution (Bi, Ge, In, 6Li, Lu, Rh, Sc and Tb). The following isotopes were selected: ^{59}Co, ^{65}Cu, ^{95}Mn, ^{55}Mo, ^{78}Se, ^{66}Zn, ^{107}Ag, ^{27}Al, ^{75}As, ^{111}Cd, ^{53}Cr, ^{201}Hg, ^{85}Rb, ^{18}Sn, ^{88}Sr, and ^{51}V. For Pb, the result was based on the sum of ^{206}Pb, ^{207}Pb and ^{208}Pb isotope measurements. Fourteen points calibration (the range from 0.125 to 7500 μg/L) for blood and 18 points calibration (the range from 0.125 to 50000 μg/L) for erythrocyte samples with three repeated measurements were performed. Very good linearity of calibration curves was achieved ($r > 0.999$). The accuracy of the results was accessed using the reference materials (RM) Seronorm Trace Elements Whole Blood L1 and L2 (Sero). The TE recoveries obtained varied in the range 93.2 − 119 % for RM L1 and 89.4 − 118.7 % for RM L2.Erythrocyte TE values from each sample were normalized by washed erythrocytes hematocrit, as previously described (France Štiglic et al., 2024).

2.4. APOE genotyping

DNA extracts were prepared from of each woman's blood (1–3 mL) following the manufacturer's instructions using FlexiGene® DNA Kit (Qiagen, Hilden, Germany), and in cases where blood samples for extraction were unavailable, extracts were prepared from 0.5 mL saliva using PrepIT-L2P (DNA Genotec Inc.). The isolated DNA concentration and purity were determined spectrophotometrically (NanoDrop 2000c UV–VIS, ThermoFisher Scientific, USA). Samples were stored at −80 °C until genotyping. All SNPs were genotyped using TaqMan® pre-designed SNP Genotyping Assay (Applied Biosystems, Forster City, CA, USA). To identify *APOE* polymorphisms rs429358 (c.334 T > C; Cys112Arg) and rs7412 (c.472C > T; Arg158Cys) LightCycler 480 II (Roche) was used. The methods have previously been described in detail (Palir et al., 2023; Stajnko et al., 2019). The distribution of the APOE genotype frequencies was in Hardy–Weinberg equilibrium ($p > 0.05$; Chi-square test).

2.4.1. 8-isoprostane determination in urine

An ELISA competitive assay was performed for the quantitative determination of 8-isoprostane free fractions in urine according to the manufacturer's instructions (Cayman Chemical, MI, USA).

The measured U-8-isoprostane was corrected in two different ways: with creatinine and with SG. For creatinine correction, U-8-isoprostane concentrations were divided by creatinine concentrations in urine. SG adjustments were performed according to Suwazono et al. (Suwazono et al., 2005) as follows: $c_{corr} = c_s$ x $(SG_{group} - 1)/(SG_s\text{-}1)$, where c_{corr} is the corrected concentration, c_s and SG_s are the concentration and SG measured in the sample, and SG_{group} is the mean SG value of the whole group. For multiple variable linear regression models, the raw urine data and SG_s values were used.

2.4.2. Specific gravity (SG) and creatinine determination in urine

SG was measured using an Atago® PAL-10S Refractometer (Japan).

For creatinine levels in urine, a modified kinetic Jaffe reaction was used according to the manufacturer instructions (Dimension® Clinical Chemistry System, Siemens, USA).

2.4.3. Hematological analyses

Hemoglobin was determined spectrophotometrically using the cyanide-free method, and hematocrit was determined using light scattering (Advia® 2120 Hematology System, Siemens).

2.5. Statistical analysis

For all statistical analyses, both descriptive and inferential, IBM SPSS Statistics 29.0.2.0 (IBM Corporation) was used. The participants were divided into the following three genotype groups according to the presence of $\varepsilon3$, $\varepsilon4$ and $\varepsilon2$ alleles: *APOE*3 (genotype $\varepsilon3/\varepsilon3$), *APOE*4 (genotypes $\varepsilon3/\varepsilon4$ and $\varepsilon4/\varepsilon4$) and *APOE*2 (genotypes $\varepsilon2/\varepsilon2$ and $\varepsilon2/\varepsilon3$). For the detected values were used when values were below the limit of detection (LoD) and the lowest detected values were used when analytes were not detectable. For each variable the normality of the data distribution was assessed using the Kolmogorov-Smirnov test. The independent samples Kruskal–Wallis test and one-way ANOVA test with Bonferroni correction were used to test for differences in TEs and U-8-isoprostane levels between the different *APOE* groups. The Mann–Whitney *U* test and Spearman's rank-order correlation were run to identify differences between the two groups and to determine the relationship between different variables, respectively.

Multiple variable linear regression model diagnostics were performed to estimate associations between *APOE* alleles or genotype groups, B-TEs, E-TEs, and U-8-isoprostane. Linearity was assessed using partial regression plots and a plot of study residuals against predicted values, and the independence of residuals was assessed using the Durbin–Watson statistic. Heteroscedasticity was avoided using normal log-transformation of 8-isoprostane and TE levels, and homoscedasticity was estimated using visual inspection of a plot of studentized residuals versus unstandardized predicted values. All models were checked for collinearity. For regression modeling, U-8-isoprostane and TE levels were log-transformed (ln), and other explanatory variables were not transformed. A 1 % change in the log-transformed independent variable regression coefficient would give an estimated change in the percentage of the U-8-isoprostane level, and for the non-transformed independent variable, a change of 1 unit the regression coefficient multiplied by 100 would provide an estimated change in the percentage of the U-8-isoprostane level, given that all other explanatory variables in the model remained constant. For binary explanatory variables (presence/absence), the regression coefficient multiplied by 100 would provide an estimate of the percentage difference between the two groups.

The explanatory variables used in the models included age, BMI, blood hemoglobin, smoking status, the sum of amalgam fillings and implants, seafood consumption (number of 100 mg portions per day), level of formal education, time of sampling during the day, and *APOE*2 and *APOE*4 versus *APOE*3. In all models, we used specific gravity (SG) as an additional explanatory variable to compensate for differences in personal hydration (Ashraf and Rea, 2017).U-8-isoprostane associations were further tested in models where we added selected TEs as additional explanatory variables.

The coding of binary variables used in the multiple regression was as follows: *APOE*2 = 1, and *APOE*3 = 0; *APOE*4 = 1, and *APOE*3 = 0; currently non-smoking = 0, and smoking = 1; and less than university education = 0 and university or higher = 1. Daily seafood intake was estimated from questionnaire answers on the frequency of seafood intake (every day, several times per week, once per week, 2–3 times per month, once per month, almost never) in terms of the number of 150 g servings of fish consumed per day.

3. Results

3.1. Study group description

The mean age of the 172 participants at the time of collection was 38.9 years (min–max: 30.1–51.4 years). All the women had given birth at least once. Of the included participants, one (0.6 %) was an *APOE* genotype $\varepsilon2/\varepsilon2$ carrier and one (0.6 %) was a genotype $\varepsilon4/\varepsilon4$ carrier, 19 (11.1 %) were genotype $\varepsilon2/\varepsilon3$ carriers, 130 (75.6 %) were genotype $\varepsilon3/\varepsilon3$ carriers, and 21 (12.1 %) were genotype $\varepsilon3/\varepsilon4$ carriers.

As defined in Statistical Analysis section, the participants were divided into the following three genotype groups according to the presence of $\varepsilon3$, $\varepsilon4$ and $\varepsilon2$ alleles: *APOE*3 (genotype $\varepsilon3/\varepsilon3$), *APOE*4 (genotypes $\varepsilon3/\varepsilon4$ and $\varepsilon4/\varepsilon4$) and *APOE*2 (genotypes $\varepsilon2/\varepsilon2$ and $\varepsilon2/\varepsilon3$). Of the participants, 130 (75.6 %) were in the *APOE*3 group, 22 (12.8 %) were in the *APOE*4 group, and 20 (11.6 %) were in the *APOE*2 group. The frequencies matched geographically similar previously reported data (Giau et al., 2015). The groups did not differ significantly in terms of age or body mass index (BMI). According to the self-reported data, there were no significant differences in alcohol consumption, smoking, or number of cigarettes smoked per day between the *APOE* groups. Our population had very low social differences, with 96.5 % of the participants and 96.0 % of their partners being employed and their level of education being high. In our university or higher education group there was lower percentage of smokers (13.4 %) versus our less than university education group (21.3 %) (Mann–Whitney test, $p = 0.168$). The data collected through an interviewer-administered questionnaire stratified by the presence of *APOE* genotype groups are summarized in Table 1.

3.2. Blood TEs, erythrocyte TEs, and urinary 8-isoprostane levels stratified by APOE genotype groups

Table 2 presents B-TEs and E-TEs (normalized by blood hemoglobin) and U-8-isoprostane (normalized by creatinine or SG) stratified by the

Table 1
Selected cohort characteristics stratified by the presence of *APOE* genotype groups.

	All		*APOE*3		*APOE*4		*APOE*2		
	Mean (SD) Median (IQR; range)	N (%)	Mean (SD) Median (IQR; range)	N (%)	Mean (SD) Median (IQR; range)	N (%)	Mean (SD) Median (IQR; range)	N (%)	p
Age (years)	38.9 (3.95) 38.6 (36.1–41.1; 30.1–51.4)	172	39.1 (4.13) 38.7 (35.8–41.2; 30.1–51.4)	130 (75.6)	38.4 (3.49) 38.7 (35.9–40.1; 32.4–47.9)	22 (12.8)	38.2 (3.32) 38.3 (36.3–40.3; 30.5–45.1)	20 (11.6)	0.752[a]
BMI (kg/m^2)	24.9 (5.47) 23.1 (21.0–27.5; 17.6–43.8)	172	24.8 (5.32) 23.1 (20.1–26.0; 17.6–43.8)	130 (75.6)	24.7 (5.93) 22.4 (20.4–22.4; 18.2–39.1)	22 (12.8)	24.7 (5.54) 23.0 (21.3–26.1; 18.9–41.5)	20 (11.6)	0.924[a]
Underweight (<18.5)		3 (1.7)		1 (0.7)		2 (9.1)		0 (0)	0.211[b]
Normal (18.5–24.9)		111 (64.5)		84 (64.6)		13 (59.1)		14 (70)	
Pre-obesity (25.0–29.9)		30 (17.4)		25 (19.7)		3 (13.6)		2 (10)	
Obesity I (30.0–34.9)		16 (9.3)		11 (8.5)		2 (9.1)		3 (15)	
Obesity II (35.0–39.9)		9 (5.2)		7 (5.4)		2 (9.1)		0 (0)	
Obesity III (>40.0)		3 (1.7)		2 (1.5)		0 (0)		1 (5)	
Smoking									
Yes		28 (16.8)		20 (15.4)		6 (22.7)		2 (10.0)	0.290[b]
No		144 (83.2)		110 (84.6)		16 (72.7)		18 (90.0)	
Cigarettes/day	9.8 (6.71) 10.0 (4.75–15.0; 1–20)		9.00 (6.59) 10.0 (30–10.0; 1–20)		12.0 (7.72) 12.5 (4.75–20.0; 1–20)		12.5 (3.54) 12.5 (4.75-N/A; 1–20)		0.522[a]
Years smoking	14.8 (6.9) 15.0 (10.0–20.0; 1.0–30.0)		9.82 (7.78) 15.0 (10.0–20.0; 1–30)		16.8 (4.32) 19 (12.5–20; 10–20)		15.0 (7.07) 19.0 (12.5-N/A; 10–20)		0.494[a]
Ever-smoking									
Yes		41 (23.8)		29 (22.3)		5 (22.7)		7 (35.0)	0.439[b]
No		131 (76.2)		101 (77.7)		17 (77.3)		13 (65.0)	
Education									
Less than university		76 (44.2)		61 (46.9)		7 (31.8)		8 (40.0)	0.386[b]
University or more		96 (55.8)		69 (53.1)		15 (68.2)		12 (60.0)	
Employment									
Yes		166 (96.5)		124 (95.4)		22 (100.0)		20 (100.0)	0.366[b]
No		6 (3.5)		6 (4.6)		0 (0.0)		0 (0.0)	
Seafood intake (portions/day)	0.202 (0.170) 0.143 (0.100–0.143; 0.033–0.500)	172	0.198 (0.166) 0.143 (0.100–0.143; 0.033–0.500)	130	0.248 (0.198) 0.143 (0.100–0.500; 0.033–0.500)		0.181 (0.167) 0.143 (0.100–0.143; 0.033–0.500)		0.409[a]
Amalgam fillings									
Yes		123 (71.3)		98 (75.0)		14 (63.6)		11 (55.0)	0.116[b]
No		49 (28.7)		32 (25.0)		8 (36.4)		9 (45.0)	
Number of fillings	3.63(3.82) 3.0 (0.0–6.0; 0–24)		3.85 (3.65) 3.0 (0.25–6.0; 0–20)		3.00 (5.16) 1.0 (0.0–4.25; 0–24)		2.90 (3.21) 2.0 (0.0–5.0)		0.153[a]
Metal implants									
Yes		25 (14.4)		19 (14.4)		3 (13.6)		3 (15.0)	0.992[b]
No		149 (85.6)		113 (85.6)		19 (86.4)		17 (85.0)	
Number of implants	2.08 (2.51) 1 (1–2; 1–13)		1.79 (1.18) 1 (1–2; 1–5)		1 (0) 1 (1–1; 1–1)		5 (6.93) 1 (1–7/; 1–13)		0.417[a]
Hemoglobin (g/L)	132.6 (10.8) 132 (97–125; 70–167)	172	132.1 (10.9) 132 (125–139; 99–167)		133.8 (11.1) 136 (127–144; 108–148)		131.6 (10.0) 134.5 (123.5–139.5; 113–147)		0.761[c]

(*continued on next page*)

Table 1 (*continued*)

	All		*APOE3*		*APOE4*		*APOE2*		
	Mean (SD) Median (IQR; range)	N (%)	Mean (SD) Median (IQR; range)	N (%)	Mean (SD) Median (IQR; range)	N (%)	Mean (SD) Median (IQR; range)	N (%)	p
Hematocrit	0.385 (0.033) 0.385 (0.365–0.405; 0.265–0.489)	172	0.386 (0.035) 0.384 (0.366–0.407; 0.265–0.489)		0.391 (0.027) 0.399 (0.373–0.409; 0.327–0.432)		0.377 (0.030) 0.384 (0.358–0.400; 0.324–0.422)		0.424[c]
Creatinine in urine (mmol/L)	0.716 (0.645) 0.486 (0.238–1.11; 0.045–3.94)	171	0.713 (0.671) 0.486 (0.215–1.075,0.045–3.94)	129 (75.4)	0.849 (0.610) 0.826 (0.283–1.28; 0.090–2.12)	22 (12.9)	0.585 (0.488) 0.362 (0.260–0.738; 0.158–1.68)	20 (11.7)	0.414[a]
Specific gravity (SG)	1.012 (0.008) 1.010 (1.005–1.109; 1.002–1.032)	171	1.012 (0.008) 1.010 (1.005–1.018; 1.002–1.032)	129 (75.4)	1.013 (0.007) 1.012 (1.008–1.021; 1.004–1.024)	22 (12.9)	1.010 (0.006) 1.008 (1.006–1.013; 1.004–1.023)	20 (11.7)	0.334[a]

APOE3 group: genotype *ε*3/*ε*3; *APOE4* group: genotypes *ε*3/*ε*4 and *ε*4/*ε*4; *APOE2* group: genotypes *ε*2/*ε*2 and *ε*2/*ε*3; p: statistically significant difference between groups according to presence of apo E alleles: a: Independent-Samples Kruskal Wallis Test, b: Chi-square test.; c: Anova with Bonferroni correction for multiple tests; BMI: body mass index.

APOE groups. Some elements were found below LoD in a certain percentage of samples: Mo (3.6 %), Ag (6.1 %), Al (32.7 %), Cd (7.3 %), Cr (83.6 %), Sn (48.4 %), and V (91.5 %) in blood, and Co (9.0 %), Mo (55.1 %), Cd (0.6 %), Hg (0.6 %), and V (70.3 %) in erythrocytes. According to the TE reference intervals for the central Slovenian population (France Štiglic et al., 2024), no major deviations were observed in either group. Exposure to nonessential TEs was low and levels of essential elements were uncompromised. However, there were significant differences between the *APOE* groups for B-Cu, B-Mo, B-Sr, and B-Zn, while no differences were observed for E-TEs (Table 2). In comparison with the *APOE3* group, the *APOE*4 group had higher levels of B-Zn, while the APOE2 group had higher levels of B-Mo and lower levels of B-Cu and B-Sr. The *APOE*2 group also had lower B-Sr levels than the *APOE*4 group. Levels of U-8-isoprostane/creatinine were comparable to those obtained using the immunochemical method on a group of healthy, nonsmoking adult Italians (Chamitava et al., 2018), and no values over their reference intervals were detected. Significant differences in U-8-isoprostane/creatinine or U-8-isoprostane/SG were found between the *APOE*4 group and the *APOE*2 group with higher levels in the former (Table 2). Regardless of the *APOE* genotype, U-8-isoprostane was significantly higher in smokers than in nonsmokers ($p = 0.001$) and had a positive correlation with the number of smoked cigarettes ($r_s = 0.216$, $p = 0.005$). There was no significant correlation of U-8-isoprostane/creatinine levels ($p = 0.758$) and U-8-isoprostane/SG levels ($p = 0.165$) with BMI. In our groups, there were no significant differences in U-8-isoprostane/creatinine ($p = 0.685$) and U-8-isoprostane/creatinine ($p = 0.283$) levels between groups with BMI ≤ 25 ($n = 114$) and BMI > 25 ($n = 58$).

3.3. Associations between B-TEs, E-TE, U-8-isoprostane, and APOE genotype groups (multiple variable linear regression models)

To verify the group differences presented in Table 2, we tried to estimate the associations of the *APOE*2 and/or *APOE*4 groups versus the *APOE*3 group with B-TEs, E-TEs, and U-8-isoprostane adjusted for age, BMI, B-Hb, seafood intake, smoking, amalgams and implants, education, urine SG, and time of sampling. The multiple variable linear regression models with all explanatory variables (age, BMI, B-Hb, seafood intake, smoking, amalgams with implants, education, urine SG and time of sampling) are summarized in Table 3A (blood) and Table 3B (erythrocytes). Presented are models with significant associations with at least one explanatory variable. Estimates without any statistically significant associations are not presented. Statistically significant differences between the APOE groups are also shown in Fig. 1 along with TEs distribution patterns (boxplots). Almost all differences between *APOE* groups that had previously been observed using simple comparison (independent samples Kruskal–Wallis test and one-way ANOVA test with Bonferroni correction for multiple tests) were also found in the multiple variable linear regression models. In addition, the associations of the *APOE* groups with B-Mn, B-Se and E-Rb were also revealed. For the *APOE*2 vs. *APOE3* group, we found (marginally) significant positive associations with B-Mn and B-Mo and negative associations with B-Sr and B-Cu. For the *APOE*4 vs. *APOE*3 groups (marginally) significant positive associations with B-Mo and B-Zn, and negative associations with B-Se and E-Rb were observed. For U-8-isoprostane, a positive association with *APOE*4 was observed. The models explained the 7.7 % (B-Al) to 38.4 % (B-Cd) variability of TE levels in the blood, 8.9 % (E-Rb) to 57.3 % (E-Cd) variability of TE level in erythrocytes and 71.1 % variability of U-8-isoprostane levels in urine.

In the next step, the regression analyses of associations between the *APOE*2 and/or *APOE*4 groups compared to the *APOE*3 group (reference group) and the U-8-isoprostane were repeated using various individual TEs as additional explanatory variables. The results are presented in the supplementary material (Tables A.1 and A.2). The positive association between U-8-isoprostane and the APOE4 group was preserved in all models. In addition, B-Se, B-Zn and E-Cu had negative effects on U-8-

Table 2
The measured B-TEs, E-TEs, and U-8-isoprostane stratified by *APOE* genotype groups.

		*APOE*3				*APOE*4				*APOE*2			
	N	Mean (SD)	Median (IQR)	Min-max	N	Mean (SD)	Median (IQR)	Min-max	N	Mean (SD)	Median (IQR)	Min-max	p
B-Co (μg/L)	124	0.214 (0.157)	0.150 (0.097–0.275)	0.050–0.714	21	0.181 (0.111)	0.139 (0.095–0.139)	0.069–0.432	20	0.210 (0.165)	0.153 (0.083–0.304)	0.069–0.432	0.743
B-Cu (μg/L)	124	652 (150)	**624** (574–691)	467–1569	21	601 (80.9)	603 (545–618)	475–837	20	**576** (96.8)	564 (524–621)	475–837	**0.012**
B-Mo (μg/L)	124	0.521 (0.303)	**0.467** (0.318–0.629)	0.105–1.69	21	0.632 (0.370)	0.580 (0.417–0.672)	0.244–1.67	20	**0.714** (0.438)	0.521 (0.427–0.907)	0.330–2.04	**0.035**
B-Mn (μg/L)	124	8.75 (3.27)	7.78 (6.42–10.6)	2.39–21.6	21	8.94 (2.56)	8.47 (6.63–10.5)	5.75–13.8	20	9.29 (2.87)	8.67 (7.24–11.9)	4.10–14.8	0.503
B-Se (μg/L)	124	106 (13.7)	106 (96–116)	69–142	21	106 (13.5)	105 (96.8–112)	85.5–138	20	106 (9.8)	105 (97.3–114)	92.3–123	0.945
B-Zn (μg/L)	124	5277 (780)	**5358** (4578–5890)	3561–6939	21	5800 (1172)	**5920** (4858–6429)	4548–8582	20	5443 (890)	5357 (4814–6173)	3850–7295	**0.033**[b]
B-Ag (μg/L)	124	0.167 (0.192)	0.127 (0.079–0.172)	0.003–1.38	21	0.139 (0.099)	0.121 (0.584–0.180)	0.04–0.498	20	0.103 (0.079)	0.079 (0.053–0.128)	0.035–0.352	0.057
B-Al (μg/L)	122	4.45 (4.48)	3.48 (0.102–6.08)	0.101–20.4	19	4.71 (3.61)	5.42 (0.900–7.60)	0.101–10.7	20	3.38 (4.23)	2.93 (0.102–4.61)	0.101–17.0	0.325
B-As (μg/L)	124	1.23 (2.12)	0.518 (0.324–1.36)	0.113–19.0	21	1.10 (1.42)	0.655 (0.347–1.14)	0.177–6.59	20	0.879 (0.906)	0.527 (0.353–0.527)	0.158–3.49	0.834
B-Cd (μg/L)	124	0.552 (0.571)	0.387 (0.254–0.543)	0.111–3.84	21	0.724 (0.694)	0.485 (0.199–0.838)	0.099–2.50	20	0.643 (1.10)	0.358 (0.257–0.555)	0.149–5.24	0.784
B-Cr (μg/L)	124	0.364 (0.362)	0.302 (0.194–0.443)	0.055–3.69	21	0.348 (0.239)	0.284 (0.176–0.461)	1.07	20	0.335 (0.147)	0.332 (0.222–0.390)	0.080–0.636	0.964
B-Hg (μg/L)	124	1.58 (1.41)	1.19 (0.678–1.88)	0.107–10.2	21	1.39 (1.14)	1.08 (0.543–2.15)	0.319–4.58	19	1.21 (0.656)	1.35 (0.531–1.64)	0.200–2.84	0.752
B-Pb (μg/L)	124	12.2 (7.49)	10.6 (8.44–13.6)	3.76–69.0	21	13.5 (5.70)	13.1 (9.75–16.2)	5.13–30.2	20	10.6 (4.3)	9.47 (7.23–14.3)	4.57–21.6	0.176
B-Rb (μg/L)	124	2235 (310)	2203 (2041–2434)	1484–3145	21	2222 (396)	2116 (1928–2350)	1778–3132	20	2162 (325)	2171 (1924–2342)	1522–2693	0.620
B-Sn (μg/L)	124	0.175 (0.196)	0.145 (0.090–0.202)	0.001–2.03	21	0.168 (0.156)	0.140 (0.090–0.195)	0.001–0.259	20	0.180 (0.125)	0.161 (0.075–0.224)	0.032–0.509	0.855
B-Sr (μg/L)	124	15.4 (5.64)	13.8 (12.0–17.4)	7.60–40.0	21	14.3 (4.19)	13.7 (11.3–16.6)	7.04–25.8	20	11.3 (2.37)	11.5 (8.99–12.8)	7.80–14.0	**<0.001**
B-V (ng/L)	124	36.6 (24.4)	32.9 (22.0–46.8)	0.02–194	21	40.5 (40.9)	30.4 (20.6–42.0)	4–93.7	20	35.2 (16.9)	37.0 (23.7–48.3)	0.02–62.6	0.768
E-Co[a] (ng/μg)	117	1.22 (0.996)	0.885 (0.478–1.63)	0.003–4.78	19	1.32 (0.973)	1.27 (0.435–1.69)	0.214–4.17	20	1.21 (0.982)	0.910 (0.530–1.43)	0.002–3.52	0.833
E-Cu[a] (ng/μg)	117	5557 (822)	5455 (4995–5918)	4149–8659	19	5534 (708)	5370 (4928–5955)	4687–7207	20	5521 (744)	5518 (4923–6070)	4307–7350	0.982
E-Mo[a] (ng/μg)	117	1.93 (0.103)	1.27 (0.699–2.44)	0.043–29.1	19	1.64 (1.63)	0.943 (0.057–3.22)	0.039–4.57	20	2.59 (2.49)	1.62 (0.146–0.548)	0.214–9.18	0.251
E-Mn[a] (ng/μg)	117	186 (86.4)	163 (130–218)	86.9–521	19	186 (74.0)	180 (134–213)	102–417	20	194 (69.1)	184 (136–237)	101–299	0.597
E-Se[a] (ng/μg)	117	1209 (233)	1202 (1035–1344)	754–2058	19	1154 (220)	1101 (991–1260)	784–1564	20	1240 (197)	1218 (1100–1394)	896–1634	0.448
E-Zn[a] (ng/μg)	117	98.1 (16.6)	96.5 (87.6–106.0)	66.3–153	19	98.6 (15.0)	99.2 (88.1–106.8)	71.8–129	20	99.5 (18.3)	96.8 (86.8–112)	65.7–136	0.945[b]
E-As[a] (ng/μg)	117	16.6 (30.8)	6.06 (3.13–19.1)	0.491–244	19	11.9 (13.4)	5.35 (4.17–14.3)	2.25–55.6	20	13.2 (16.1)	5.83 (3.82–15.7)	2.02–62.2	0.866
E-Cd[a] (ng/μg)	117	11.9 (11.5)	8.90 (5.48–13.7)	1.99–95.4	19	15.5 (18.0)	7.87 (4.73–20.0)	2.40–72.2	20	14.7 (23.7)	7.19 (5.37–14.0)	4.09–112	0.936
E-Hg[a] (ng/μg)	116	27.8 (27.4)	19.7 (10.3–34.4)	0.329–182	19	19.9 (15.6)	14.9 (8.1–31.2)	3.12–57.3	20	26.9 (32.7)	19.6 (10.2–29.3)	2.10–154	0.527
E-Pb[a] (ng/μg)	117	255 (141)	235 (168–303)	80.9–1244	19	259 (107)	224 (161–335)	134–466	20	220 (92.1)	199 (144–270)	114–417	0.438
E-Rb[a] (μg/μg)	117	39.9 (6.58)	38.8 (35.1–43.7)	25.6–68.9	19	36.7 (6.54)	35.2 (31.4–41.4)	27.3–49.2	20	38.7 (5.60)	36.7 (33.7–43.0)	31.9–48.5	0.081
E-V[a] (ng/μg)	117	0.359 (0.328)	0.342 (0.085–0.523)	0.009–1.62	19	0.227 (0.228)	0.150 (0.011–0.369)	0.009–0.731	19	0.398 (0.352)	0.305 (0.146–0.548)	0.010–0.731	0.195
U-8-isoprostane													
(μg/g creatinine)	128	1.02 (0.737)	0.816 (0.603–1.26)	0.110–4.90	22	1.25 (1.04)	**1.04** (0.814–1.39)	0.472–5.64	20	0.767 (0.362)	**0.652** (0.528–0.936)	0.314–1.82	**0.033**
(μg/L SG)	128	0.665 (0.628)	0.498 (0.339–0.703)	0.031–3.851	22	0.894 (1.03)	0.632 (0.425–1.10)	0.163–1.93	20	0.525 (0.397)	0.426 (0.323–0.554)	0.163–1.93	**0.099**

*APOE*3: genotype $\varepsilon3/\varepsilon3$; *APOE*4: genotypes $\varepsilon3/\varepsilon4$ and $\varepsilon4/\varepsilon4$; *APOE*2: genotypes $\varepsilon2/\varepsilon2$ and $\varepsilon2/\varepsilon3$; B: whole blood; E: erythrocyte; IQR: interquartile range; P: plasma; p: statistically significant difference between groups according to presence of *APOE* alleles (for b: Anova, all other: Independent-samples Kruskal-Wallis Test was used. Significance values have been adjusted by the Bonferroni correction for multiple tests. Significant differences ($p < 0.05$) after Bonferroni correction for multiple tests are highlighted in bold.); Min-max: minimal and maximal measured value; SD: standard deviation; a: Erythrocyte TEs values in every sample were normalized by erythrocytes' hematocrit to overcome methodological errors and by blood hemoglobin.; U: urine; 8-isoprostane/creatinine in every urine sample were normalized by creatinine measured in urine to overcome differences in rate of excretion; 8-isoprostane/SG in every urine sample were normalized by SG measured in urine to overcome differences in personal hydration.

Table 3A
The influence of *APOE* genotypes, age, hemoglobin concentration, specific gravity, BMI, smoking, number of amalgam fillings and implants, seafood consumption, education, and daily sampling time on B-TEs (estimated by different multiple variable linear regression models).

Explanatory variables	Ln(B-Co)	Ln(B-Cu)	Ln(B-Mn)	Ln(B-Mo)	Ln(B-Se)	Ln(B-Zn)	Ln(B-Ag)	Ln(B-As)	Ln(B-Al)	Ln(B-Cd)	Ln(B-Hg)	Ln(B-Pb)	Ln(B-Rb)	Ln(B-Sr)
APOE2 vs. ***APOE3***	−0.023 (0.158)	**−0.088***** (−0.149, −0.026)	**0.159**^{o} (−0.004, 0.321)	**0.267*** (0.003, 0.530)	−0.019 (0.027)	0.032 (0.041)	−0.238 (0.152)	−0.141 (0.226)	−0.593 (0.468)	0.141 (0.154)	−0.040 (0.189)	−0.124 (0.103)	−0.034 (0.034)	**−0.262***** (−0.401, −0.123)
APOE4 vs. ***APOE3***	−0.021 (0.158)	−0.038 (0.031)	0.084 (0.080)	**0.248**^{o} (−0.017, 0.512)	**−0.055**^{o} (−0.110, 0.000)	**0.074**^{o} (−0.006, 0.155)	0.120 (0.152)	−0.189 (0.227)	0.462 (0.498)	−0.045 (0.155)	−0.272 (0.187)	0.118 (0.103)	−0.039 (0.035)	−0.008 (0.070)
SG	−3.868 (6.766)	−1.554 (1.328)	0.422 (3.474)	−3.612 (5.727)	0.128 (1.182)	0.548 (1.751)	−5.959 (6.715)	−3.255 (9.705)	−21.452 (20.400)	−9.101 (6.686)	**13.407**^{o} (−2.403, 29.217)	−1.552 (4.426)	−0.096 (1.461)	−3.114 (3.030)
Hb (g/L)	**−0.022***** (−0.032, −0.013)	−0.002 (0.001)	**−0.012***** (−0.017, −0.007)	**−0.009*** (−0.017, −0.002)	**0.002**^{o} (0.000, 0.003)	**0.003*** (0.000, 0.005)	0.001 (0.005)	−0.005 (0.007)	−0.019 (0.014)	−0.003 (0.005)	0.003 (0.006)	0.002 (0.003)	**0.005***** (0.003, 0.007)	**−0.004**^{o} (−0.008, 0.000)
Age (years)	−0.003 (0.012)	0.004 (0.002)	0.007 (0.006)	−0.009 (0.011)	0.002 (0.002)	0.002 (0.003)	**0.024**^{o} (0.000, 0.048)	0.004 (0.018)	0.030 (0.037)	−0.011 (0.012)	0.006 (0.015)	0.008 (0.008)	0.002 (0.003)	0.003 (0.006)
BMI (kg/m2)	−0.011 (0.010)	**0.012***** (0.008, 0.016)	**0.010*** (0.000, 0.020)	0.003 (0.008)	−0.002 (0.002)	0.004 (0.002)	**−0.021*** (−0.040, −0.003)	−0.019 (0.014)	−0.037 (0.029)	0.006 (0.009)	**−0.038***** (−0.060, −0.015)	0.000 (0.006)	−0.001 (0.002)	**−0.010*** (−0.018, −0.001)
Seafood intake (portions/day)	−0.116 (0.299)	0.044 (0.058)	−0.081 (0.152)	0.280 (0.253)	**0.254***** (0.151, 0.356)	0.048 (0.077)	0.382 (0.292)	**1.069*** (0.209, 1.928)	0.939 (0.916)	0.237 (0.294)	**1.313***** (0.613, 2.013)	**0.333**^{o} (−0.054, 0.721)	0.070 (0.065)	0.090 (0.136)
Smoking (yes)	−0.005 (0.138)	**−0.060*** (−0.114, −0.006)	0.020 (0.070)	−0.082 (0.117)	0.006 (0.024)	−0.047 (0.036)	−0.056 (0.133)	0.307 (0.199)	−0.122 (0.415)	**1.148***** (0.880, 1.416)	0.148 (0.163)	**0.188*** (0.008, 0.367)	0.033 (0.030)	−0.002 (0.0621)
Amalgams and implants (number)	0.019 (0.014)	−0.004 (0.003)	0.004 (0.007)	−0.011 (0.012)	−0.003 (0.002)	−0.004 (0.004)	**0.042**** (0.016, 0.069)	−0.013 (0.020)	0.001 (0.042)	0.000 (0.014)	−0.001 (0.016)	0.000 (0.009)	0.000 (0.003)	0.004 (0.006)
Education (≥ university)	0.049 (0.106)	−0.008 (0.021)	0.035 (0.054)	0.006 (0.089)	0.006 (0.018)	0.016 (0.027)	−0.106 (0.104)	**0.355*** (0.053, 0.657)	**−0.584**^{o} (−1.213, 0.044)	−0.033 (0.104)	**0.269*** (0.021, 0.516)	−0.003 (0.069)	−0.019 (0.023)	−0.013 (0.047)
Time of sampling (hour)	−0.027 (0.021)	0.003 (0.004)	−0.016 (0.011)	0.004 (0.018)	**0.010**** (0.002, 0.017)	0.000 (0.006)	−0.014 (0.021)	**0.082**** (0.021, 0.143)	−0.002 (0.065)	**−0.052*** (−0.094, −0.011)	0.018 (0.025)	0.004 (0.014)	−0.002 (0.005)	0.010 (0.010)
Constant	5.730 (6.841)	**7.743***** (5.093, 10.39)	2.941 (3.511)	4.328 (5.790)	**4.128***** (1.771, 6.484)	**7.435***** (3.937, 10.933)	3.440 (6.780)	1.886 (9.812)	25.293 (20.604)	9.534 (6.747)	**−14.113**^{o} (−30.115, 1.888)	3.284 (4.471)	**7.171***** (4.252, 10.089)	**6.289*** (0.233, 12.344)
N	160	155	158	160	158	160	155	159	156	159	159	159	159	157
R^2	0.194	0.310	0.191	0.108	0.224	0.124	0.148	0.169	0.077	0.384	0.211	0.090	0.178	0.159
Adj, R^2	0.135	0.257	0.131	0.042	0.188	0.059	0.083	0.107	0.007	0.339	0.153	0.023	0.117	0.095
F (df)	**3.264***** **(11)**	**5.877***** **(11)**	**3.162***** **(11)**	**1.645**$^{\circ}$ **(11)**	**4.323***** **(11)**	**1.913***** **(11)**	**2.281*** **(11)**	**2.737**** **(11)**	1.102 (11)	**8.402***** **(11)**	**3.604***** **(11)**	1.336 (11)	**2.921**** **(11)**	**2.503**** **(11)**

Summarized are data with statistically significant effects of independent 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; adj. R^2 – percentage of variability of TE or U-8-isoprostane level explained by the model; F – significance of the model; N – number of observations; B: whole blood; E: erythrocyte; statistically significant results are indicated in bold: ^{O} $p < 0.10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 3B
The influence of *APOE* genotypes, age, hemoglobin concentration, specific gravity, BMI, smoking, number of amalgam fillings and implants, seafood consumption, education, and daily sampling time on E-TEs and U-8-isoprostane (estimated by multiple variable linear regression models).

Explanatory variables	Ln(E-Co)	Ln(E-Cu)	Ln(E-Mn)	Ln(E-Se)	Ln(E-Zn)	Ln(E-As)	Ln(E-Cd)	Ln(E-Hg)	Ln(E-Rb)	Ln(U-8-isoprostane)
APOE2 vs. ***APOE3***	0.108	−0.017	0.083	0.016	0.005	−0.009	0.205	−0.003	−0.033	0.049
	(0.192)	(0.023)	(0.084)	(0.039)	(0.031)	(0.285)	(0.126)	(0.224)	(0.033)	(0.158)
APOE4 vs. ***APOE3***	0.139	0.016	0.103	−0.059	0.029	0.020	−0.020	−0.376	**−0.062°**	**0.333***
	(0.191)	(0.024)	(0.086)	(0.041)	(0.032)	(0.291)	(0.131)	(0.229)	(−0.128, 0.005)	(0.033, 0.634)
SG	−8.194	−0.078	0.468	−0.533	1.469	8.615	−5.320	8.370	−0.067	**120.36*****
	(8.137)	(1.020)	(3.632)	(1.710)	(1.348)	(12.333)	(5.570)	(9.691)	(1.438)	(106.8, 133.9)
Hb (g/L)	**−0.022*****	**−0.002***	**−0.014*****	−0.002	**−0.003***	−0.011	−0.003	−0.007	0.001	–
	(−0.033, −0.010)	(−0.003, 0.000)	(−0.019, −0.009)	(0.001)	(−0.005, −0.001)	(0.009)	(0.004)	(0.007)	(0.001)	
Age (years)	0.008	0.003	0.001	0.003	0.001	−0.003	0.004	0.016	−0.001	0.012
	(0.015)	(0.002)	(0.007)	(0.003)	(0.002)	(0.023)	(0.010)	(0.018)	(0.003)	(0.013)
BMI (kg/m2)	−0.006	**0.003*****	0.005	−0.003	0.001	**−0.040***	0.002	−0.020	−0.002	0.009
	(0.012)	(0.000, 0.006)	(0.005)	(0.002)	(0.002)	(−0.074, −0.006)	(0.008)	(0.014)	(0.002)	(0.009)
Seafood intake	−0.347	0.006	−0.158	**0.250****	−0.035	**1.901*****	−0.337	**1.508*****	0.004	0.072
(portions/day)	(0.361)	(0.044)	(0.161)	(0.101, 0.399)	(0.060)	(0.823, 2.979)	(0.245)	(0.661, 2.355)	(0.063)	(0.290)
Smoking (yes)	0.139	−0.029	−0.060	−0.008	**−0.076****	0.118	**1.440*****	0.242	0.020	0.198
	(0.164)	(0.020)	(0.074)	(0.035)	(−0.130, −0.022)	(0.250)	(1.210, 1.669)	(0.196)	(0.029)	(0.136)
Amalgams and	0.023	0.000	0.009	0.001	−0.001	0.014	0.013	−0.013	0.003	0.017
implants (number)	(0.017)	(0.002)	(0.008)	(0.004)	(0.003)	(0.025)	(0.011)	(0.020)	(0.003)	(0.014)
Education	−0.012	−0.015	−0.006	−0.002	−0.020	**0.327°**	−0.121	**0.361***	**−0.048***	−0.112
(≥university)	(0.128)	(0.016)	(0.057)	(0.027)	(0.021)	(−0.056, 0.711)	(0.086)	(0.059, 0.664)	(−0.092, −0.003)	(0.105)
Time of sampling	−0.042	−0.002	−0.016	0.007	−0.001	**0.102****	**−0.056****	0.032	0.000	0.003
(hour)	(0.026)	(0.003)	(0.012)	0.005)	(0.004)	(0.025, 0.180)	(−0.091, −0.022)	(0.031)	(0.005)	(0.021)
Constant	9.427	**6.986*****	4.583	**5.638****	**8.339*****	−8.608	6.508	−7.984	**8.545*****	**−116.5*****
	(8.214)	(4.942, 9.031)	(3.668)	(2.224, 9.052)	(5.648, 11.030)	(12.455)	(5.618)	(9.786)	(5.672, 11.418)	(−130.1, −102.8)
N	150	150	153	152	153	153	149	152	151	165
R^2	0.163	0.109	0.234	0.126	0.137	0.184	0.573	0.183	0.089	0.711
Adj, R^2	0.097	0.038	0.175	0.058	0.071	0.120	0.538	0.119	0.017	0.692
F (df)	**2.458** (11)**	**1.545***** (11)**	**3.949***** (11)**	**1.854°** (11)**	**2.057*** (11)**	**2.905**** (11)**	**16.803***** (11)**	**2.868**** (11)**	1.240 (11)	**38.115***** (10)**

Summarized are data with statistically significant effects of independent 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; adj. R^2 – percentage of variability of TE or U-8-isoprostane level explained by the model; F – significance of the model; N – number of observations; B: whole blood; E: erythrocyte; statistically significant results are indicated in bold: ^{O} $p < 0.10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

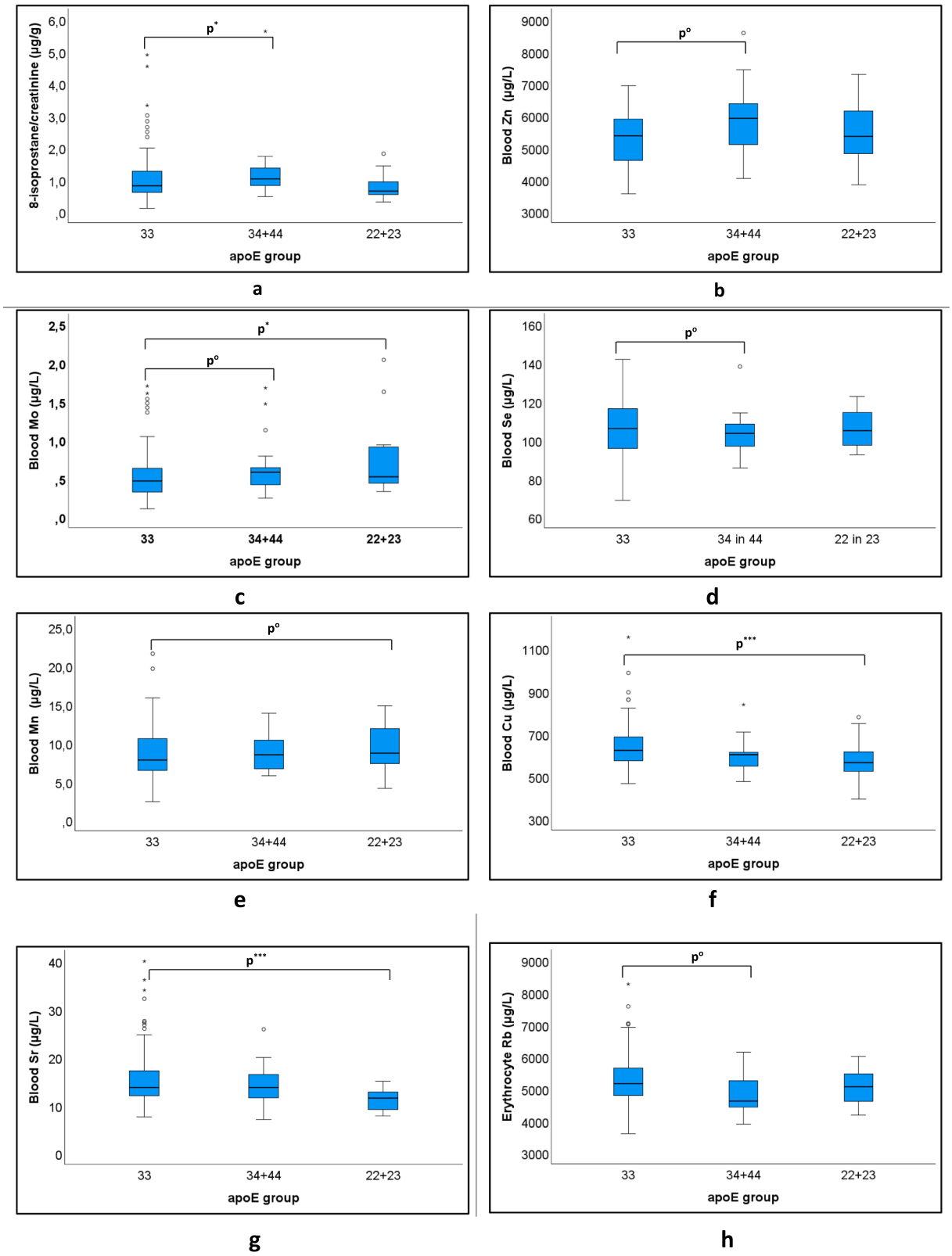

Fig. 1. Differences in U-8-isoprostane (a), blood Zn (b), Mo (c), Se (d), Mn (e), Cu (f), Sr (g), and erythrocyte Rb (h) levels between the *APOE* groups. Boxes represent the median, 25th and 75th percentile. Whiskers mark the minimum and maximum values excluding outliers. Dots represent potential outliers. High extreme values are labelled with an asterisk. Statistically significant differences between *APOE*3 and *APOE*4 or *APOE*2 group estimated by different multiple variable linear regression models (Table 2A and Table 2B) are indicated: ° $p < 0.10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. *APOE*3: genotype $\varepsilon3/\varepsilon3$; *APOE*4: genotypes $\varepsilon3/\varepsilon4$ and $\varepsilon4/\varepsilon4$; *APOE*2: genotypes $\varepsilon2/\varepsilon2$ and $\varepsilon2/\varepsilon3$.

Table 4
The influence of *APOE* genotypes, with multiple TEs, age, hemoglobin concentration, specific gravity, BMI, smoking, number of amalgam fillings and implants, seafood consumption, education, and daily sampling time on U-8-isoprostane (estimated by multiple variable linear regression models).

	Ln(U-8-isoprostane)	
Explanatory variables	**Model 1**	**Model 2**
APOE2 vs. ***APOE3***	0.208 (0.172)	0.014 (0.171)
APOE4 vs. ***APOE3***	**0.433**** (0.123, 0.742)	**0.406*** (0.066, 0.746)
SG	**123.11***** (109.3, 136.9)	**121.99***** (107.3, 136.7)
Hb (g/L)	−0.001 (0.006)	−0.008 (0.006)
Age (years)	0.011 (0.013)	0.007 (0.013)
BMI (kg/m2)	0.015 (0.011)	0.014 (0.010)
Seafood intake (portions/day)	0.357 (0.320)	−0.002 (0.334)
Smoking (yes)	0.181 (0.143)	0.146 (0.150)
Amalgams and implants (number)	0.001 (0.013)	0.014 (0.015)
Education (≥ university)	−0.071 (0.107)	−0.180 (0.114)
Time of sampling (hours)	0.013 (0.022)	0.000 (0.023)
Ln(B-Co)	0.080 (0.091)	–
Ln(B-Cu)	0.306 (0.310)	–
Ln(B-Mn)	−0.217 (0.171)	–
Ln(B-Mo)	−0.069 (0.106)	–
Ln(B-Se)	**−1.007*** (−1.926, −0.088)	–
Ln(B-Zn)	**−0.741*** (−1.411, −0.071)	–
Ln(B-Rb)	0.090 (0.405)	–
Ln(B-Sr)	0.196 (0.175)	–
Ln(B-V)	−0.035 (0.079)	–
Ln(E-Co)	–	−0.026 (0.074)
Ln(E-Cu)	–	**−1.112°** (−2.230, 0.005)
Ln(E-Mo)	–	−0.031 (0.043)
Ln(E-Mn)	–	−0.044 (0.176)
Ln(E-Se)	–	0.506 (0.369)
Ln(E-Zn)	–	−0.194 (0.510)
Ln(E-Rb)	–	−0.050 (0.398)
Ln(E-V)	–	**0.076*** (0.004, 0.147)
constant	**−111.17***** (−127.4, −94.9)	**−109.44***** (−127.2, −91.7)
N	156	146
R^2	0.746	0.740
Adj. R^2	0.708	0.701
F (df)	**19.943***** **(20)**	**19.000***** **(19)**

Summarized are data with statistically significant effects of independent 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; adj. R^2 – percentage of variability of U-8-isoprostane level explained by the model; F – significance of the model; N – number of observations; statistically significant results are indicated in bold: ^{O} $p < 0.10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

isoprostane levels, and E-V had a positive effect.

In the final step, the same association between U-8-isoprostane levels in the *APOE*2 and *APOE*4 groups according to the *APOE*3 group (reference group) was tested by adding a combination of B-TEs or E-TEs (Table 4) instead of a single TE one by one (Tables A.1 and A.2). Combinations were selected based on their (marginally) significant associations with the *APOE*2 and *APOE*4 groups observed in Tables 3A and 3B or with U-8-isoprostane levels presented in Tables A.1 and A.2). Cr, V, Rb, and Mn were added due to their involvement with lipid metabolism according to data from the literature (Amerikanou et al., 2023; Li et al., 2024; Peruzzu et al., 2015; Tinkov et al., 2021).

By adding B-TEs or E-TEs as explanatory variables, the effects of the *APOE*4 group versus the *APOE*3 group on U-8-isoprostane increased from 33.3 % (Table 3B) to 43.3 % and 40.6 %, respectively (Table 4). At the same time, the explained variability (R^2) increased from 71.1 % to 74.6 % with B-TEs and 74.0 % with E-TEs. In comparison with the *APOE*4 group, the influence of TEs on U-8-isoprostane levels was almost negligible, although significant, and was estimated as a 0.7–1.1 % decrease in U-8-isoprostane levels at a 1 % rise in B-Se, B-Zn, or E-Cu. The positive influence of E-V was even smaller (0.07 %).

Except for SG, none of the other explanatory covariables in the presence of *APOE* groups and multiple TEs showed significant associations with U-8-isoprostane in either model. However, among these other variables, we can reveal a nonsignificant positive effect of smoking (18.1 %) and, interestingly, seafood intake (35 %) (Table 4). Both were probably compromised by self-reporting and too low an "intensity" to reach significance in such a low number of participants.

4. Discussion

In the present study, we aimed to evaluate the associations between TEs, U-8-isoprostane and *APOE* genotypes in middle-aged women (aged 40–50 years) with BMI below obesity (BMI < 30) in 90 % of participants. The results suggest that in healthy women of childbearing age with low exposure to potentially toxic TEs and an adequate supply of essential TEs (Table 2), the *APOE*4 genotype plays an important role in 8-isoprostane formation as estimated by adjusted linear regression models (Table 3A, 3B). At the same time (in the same models), the marginal modifying effect of essential TEs on U-8-isoprostane levels was observed for B-Zn, B-Se, E-Cu and nonessential E-V (Table 4). The observed associations of *APOE* groups with some essential blood TEs (B-Mo, B-Mn, B-Se, B-Cu, B-Zn and B-Mn) were less clear than those with U-8-isoprostane. They were mostly weak, and for all of them the adjusted R^2 for the models was much lower than that for U-8-isoprostane (Table 3B). Since their concentrations are known to be influenced by dietary intake and time of sampling, particularly for Zn (Brown et al., 2004; Ceballos-Rasgado et al., 2024), we are rather reserved in interpretating their associations with the *APOE* groups, precisely because of the non-standardized sampling conditions. To some extent, these differences may also be attributed to some other normal physiological processes.

However, TEs metabolism and lipid metabolism (Dietrich et al., 2005; Dose et al., 2016; Egert et al., 2012; Jofre-Monseny et al., 2008; Miyata and Smith, 1996; Ramassamy et al., 1999; Smith et al., 1998) are supposed to be modified by APOE variants. Metal ions such as Cu, Fe, and Zn can bind to apoE (Miyata and Smith, 1996; Xu et al., 2015, 2014); stabilize its structure, as in the case of Zn (Xu et al., 2015); or

affect *APOE* gene expression (Xu et al., 2014) in an isoform-dependent manner. According to data from the literature, all four TEs are implicated in antioxidative defense processes and/or lipid metabolism (Barchielli et al., 2022; Chen et al., 2019; González-Domínguez et al., 2022; Li et al., 2021; Lumsden et al., 2020; Marreiro et al., 2017; Rehder, 2013; Rotter et al., 2015; Suh et al., 2022; Tinkov et al., 2021; Valko et al., 2005; Zhou et al., 2016). Several studies have suggested an association of 8-isoprostane with apoE isoforms and apoE expression (Dietrich et al., 2005; Tangirala et al., 2001; Trares et al., 2020; Yao et al., 2004), and exposure to various metals (Ashrap et al., 2021; Dashner-Titus et al., 2018; Hu et al., 2021), demonstrating the usefulness of urinary 8-isoprostane measurement in assessing (pato)physiological oxidative stress in relation to apoE and TEs. It has been reported that urine, plasma, and arterial isoprostane levels are markedly increased in apoE-deficient mice (Praticò et al., 1998). In a few human studies, increased plasma levels of 8-isoprostane free fractions were found in middle aged ε4 carriers with increased cholesterol levels (Dietrich et al., 2005) and in elderly ε4/ε4 carriers with all-cause dementia (Trares et al., 2020).

The associations between the TEs and U-8-isoprostane (as a marker of lipid peroxidation) levels have previously been described (Ashrap et al., 2021; Dashner-Titus et al., 2018; Hu et al., 2021; Pollack et al., 2012), but very few investigators have addressed the topic of associations among U-8-isoprostane and APOE polymorphism in combination with blood and erythrocyte TEs, especially in low-exposed populations without underlying chronic or acute diseases or specific physiological conditions, such as pregnancy. As mentioned above, in our group of women, only a few TEs were significantly associated with U-8-isoprostane (mostly essential ones), and the associations were marginal (Table 4). This is not surprising, as our population had low exposure to toxic metals, and their levels in blood and erythrocytes were similar to Slovenian population levels (France Štiglic et al., 2024). Pollack et al. (2012) reached similar conclusions, finding no association between U-8-isoprostane and B-Pb, B-Cd, and B-Hg in healthy premenopausal unexposed women (Pollack et al., 2012).

In our group of women, the significant negative association of U-8-isoprostane observed with E-Cu, B-Se, and B-Zn was expected. Zn is well known to be involved in the antioxidant defense system by the regulation of glutathione peroxidase as well as the expression of MTs, and as a cofactor of Cu/Zn superoxide dismutase (Marreiro et al., 2017). Selenium is involved in protection against oxidative stress as a constitutive element of glutathione peroxidases, thioredoxin reductases and selenoprotein P (Barchielli et al., 2022). Copper is involved in antioxidant defense through Cu/Zn superoxide dismutase and ceruloplasmin (Valko et al., 2005) and shares a complex inverted relationship in the periphery (not in brain) with lipid metabolism (Blades et al., 2021). Blades et al (2021) outlined that "Increased cellular copper downregulates lipids and lipogenic genes, and vice versa". Vanadium potentially affects the formation of ROS leading to lipid peroxidation (Rehder, 2013), and our data agreed with this finding because there was a positive connection between E-V and U-8-isoprostane levels. However, the antioxidant action of V has also been reported (Matsubara et al., 1995), and due to its well-known insulin-like properties and its effect on bio-energetic processes and bone formation it has been categorized as an "occasionally beneficial element" (Gupta and Vaswani, 2020). Furthermore, it should be noted that our B-V levels were very low (min–max: 0.02–0.05 μg/L), comparable with previously published levels for populations without occupational V exposure (Agency for Toxic Substances and Disease Registry (ATSDR), 2012) and probably below the threshold level for any serious adverse effect.

Our data show that non-essential TEs in combination with different *APOE* genotypes, did not seem to play a decisive role in 8-isoprostane formation in our population, which had a relatively good supply of protective essential elements and low exposure to potentially toxic elements. Regardless, their levels were clearly and expectedly affected by smoking, education, and seafood intake (Tables 3A and 3B). Among the potentially toxic elements, smoking had a positive effect on B-Cd, B-Pb, and E-Cd, and seafood intake had a positive effect on Hg, Se, and As in blood and erythrocytes and Pb in blood. Less expected and intriguing was the positive association between the *APOE*2 and *APOE*4 groups (vs. *APOE*3) with B-Mo. Mo is an essential element in the form of a Mo cofactor. The five eukaryotic molybdo-enzymes are able to reduce nitrite to NO, a second messenger involved in a multitude of cellular processes (Bender and Schwarz, 2018). In humans, four Mo enzymes are present: cytosolic xanthine oxidase and aldehyde oxidase, mitochondrial sulfite oxidase, and mitochondrial amidoxime-reducing component (mARC). The latter was recently identified as a crucial factor in lipid metabolism, possibly involved in non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) (Clement and Struwe, 2023). Experimental studies have also suggested its regulatory function in hydrogen peroxide and antioxidant metabolism in the outer mitochondrial membrane and peroxisomes (Rixen et al., 2023). Here, it should also be noted that recent studies have highlighted the influence of mitochondria on apolipoprotein E levels in brain cell culture studies (Swerdlow, 2023) and the involvement of *APOE* ε4 allele against NAFLD suggested by human association studies and experimental data (Huebbe et al., 2024).

Previously published *in vivo* studies have shown more pronounced oxidative stress in smokers carrying the ε4 allele than in noncarriers (Graeser et al., 2012), and in another study elevated cholesterol and 8-isoprostane levels were observed in ε4 carriers versus noncarriers (Dietrich et al., 2005). Although differences in U-8-isoprostane levels between smokers and nonsmokers were observed in our study using a simple comparison method (Mann—Whitney *U* test, data not shown), smoking as covariable had no significant association with U-8-isoprostane levels in any multiple variable regression models. In the literature, there is mixed evidence on the impact of age on 8-isoprostane levels (Chamitava et al., 2018; Sakano et al., 2009). The missing data about abdominal obesity (waist circumference) and possibly weight gain, might have given us stronger associations, as there is evidence that U-8-isoprostane correlates with these two parameters (Il'yasova et al., 2012). Higher education, although not significant but persistent in all models, was associated with lower U-8-isoprostane levels. This could be attributed to the impact of education on nutritional habits previously described (Simić et al., 2022) and the lower percentage of smokers in our university or higher education group. The time of sampling was introduced to the regression models, as sampling varied in our study from morning to afternoon. For some TEs, diurnal variation in blood is well known (Grandjean et al., 1992; HONGO et al., 1993), but there is limited data on the daily variation of U-8-isoprostane (Helmersson and Basu, 1999; Kanabrocki et al., 2002). The association of U-8-isoprostane in urine with sampling time was not seen (Tables 3B and 4), which is in line with other researchers' data (Helmersson and Basu, 1999; Kanabrocki et al., 2002).

In sum, our results suggest that TEs have a much smaller impact on U-8-isoprostane levels compared to *APOE* genotypes, and that lipid peroxidation was greater in the presence of the *APOE*4 group than in the *APOE*3 group. However, the interplay and balancing of antioxidant mechanisms are highly complex and not unambiguous. The presented differences in lipid peroxidation processes between *APOE* variants are quite likely without consequences for healthy populations with a good supply of essential elements and low exposure to potentially toxic elements, but they can represent a risk or resilient factor for a worse or better outcome, respectively, during disease. Compared with previous reports, the current study provides a more comprehensive picture of the association between TEs, U-8-isoprostane and *APOE* genotypes. Their modification by total antioxidant status and antioxidant enzymes (GPx1 and catalase activity) for the same study population is in preparation. The observed associations between essential B-TEs and APOE variants are interesting but difficult to interpret. Essential trace elements such as Zn, Cu, Se and Mn are involved in many physiological processes simultaneously and may be influenced by dietary intake, which was not controlled. We only emphasized higher B-Mo in *APOE*2 and *APOE*4 than

in *APOE*3 group because this observation fits well with recent studies related to Mo, *APOE* and NFDL (see above). More puzzling seems to be the lower level of non-essential Sr associated with *APOE*2 group compared to *APOE*3 group. It could be related to its potential antioxidant function (Ru et al., 2024), which can be less needed in *APOE*2 carriers, or to its involvement in bone metabolism. Both Sr, as a known therapeutic anabolic agent (Ru et al., 2024; Zhou et al., 2019), and *APOE*, as a physiological regulator (Dieckmann et al., 2013; Noguchi et al., 2018; Zhang et al., 2022), have been reported to promote osteogenesis in bone and tooth enamel formation. However, although the presence of the homozygous $\varepsilon 2/\varepsilon 2$ genotype in humans suggests a lower bone maintenance effect compared to other genotypes (Dieckmann et al., 2013; Noguchi et al., 2018; Zhang et al., 2014), reports on *APOE* genotype-specific effects on bone mass maintenance and turnover are still inconsistent.

4.1. Study limitations

Due to the relatively small sample, and the small number of *ε2* and *ε4* homozygotic participants (APOE *ε2/ε2* and *ε4/ε4* genotypes), we had to group them with genotypes *ε2/ε3* and *ε3/ε4*, respectively. This might have masked their influence on TEs and U-8-isoprostane. Consequently, the allele influence on TEs and U-8-isoprostane levels which might otherwise be more pronounced in homozygotes, was very likely to have been diminished. As expected, since *APOE*3 is the most common genotype, the *APOE* study groups were not matched in terms of the number of individuals, which is in accordance with the frequencies of the *APOE* genotypes in the Caucasian population. Consequently, these very different sample sizes could have affected the significance of the analyses performed. Our study population encompassed Slovenian middle-aged women of childbearing age, so the results cannot be applied to the general population. Further research is needed to confirm the validity of the results for other groups (e.g., age or gender).

Unfortunately, data on our cohort's acute and chronic physical activity, which could potentially influence U-8-isoprostane levels, were missing, although studies have provided conflicting data on their influence (Il'yasova et al., 2012; Nikolaidis et al., 2011). In addition, we missed data on lipids and abdominal obesity (waist circumference) for some interpretations (to test associations of TEs with lipids). Because the samples were collected as part of a human biomonitoring assessment (with previously defined goals and sampling conditions), we were not able to standardize the preanalytical conditions (such as the participants' preparation and the timing of the collection to obtain morning fasting samples). This may have led to higher variability in the results and, consequently, more difficult interpretation and conclusions.

We are aware that possible cross-reactions in the ELISA method used for the determination of U-8-isoprostane may affect the result to a limited extent.

Given this study's limited sample size and the lack of adequate consideration of confounding factors in the analysis, the results should be interpreted with caution.

5. Conclusion

Our study suggests that in healthy women of childbearing age with low exposure to potentially toxic TEs and an adequate supply of essential TEs, the *APOE*4 genotype may play an important role in 8-isoprostane formation, given that the presence of the *ε4* allele increased U-8-isoprostane levels by up to 43.3 % in comparison with the *APOE*3 group (estimated by multiple variable linear regression models).

At the same time (in the same models), a marginal modifying effect of essential TEs on U-8-isoprostane levels was observed for B-Zn, B-Se, and E-Cu. All three were negatively associated with U-8-isoprostane and therefore possibly related to protective effects against 8-isoprostane formation. On the contrary, nonessential E-V was associated with increased U-8-isoprostane; however, due to its small effect and low concentration levels, the association could be without biological implication.

None of the other explanatory covariables (age, BMI, smoking status, sum of amalgam fillings and implants, seafood consumption, level of formal education, daily sampling time, and blood hemoglobin), except SG, showed significant associations with U-8-isoprostane.

The observed impact of the *APOE*2 and *APOE*4 groups on increased B-Mo and of *APOE*2 on decreased B-Sr have opened the unknown areas.

Further studies are needed to validate the associations detected in this study and to verify our findings in other populations.

CRediT authorship contribution statement

Alenka France Štiglic: Investigation, Formal analysis, Methodology, Data curation, Validation, Conceptualization, Visualization, Writing – original draft. **Anja Stajnko:** Formal analysis, Methodology, Writing – review & editing. **Alenka Sešek Briški:** Data curation, Writing – review & editing. **Janja Snoj Tratnik:** Data curation, Writing – review & editing. **Darja Mazej:** Data curation, Writing – review & editing. **Aleš Jerin:** Formal analysis, Writing – review & editing. **Milan Skitek:** Funding acquisition, Conceptualization, Writing – review & editing. **Milena Horvat:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing. **Janja Marc:** Conceptualization, Methodology, Writing – review & editing. **Ingrid Falnoga:** Conceptualization, Methodology, Supervision, Writing – review & editing.

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.

Acknowledgments

This work was supported by the Slovenian Research Agency, Slovenia (Research program P1 0143 and J3-4527), University Medical Centre Ljubljana, Slovenia (Research program TP 20190117), and European Commission Directorate for Environment through the LIFE+ programme (project CROME, LIFE12 ENV/GR/001040). We would like to thank all co-workers and participants involved in the CROME-LIFE project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2024.109034.

References

Acharya, P., Segall, M.L., Zaiou, M., Morrow, J., Weisgraber, K.H., Phillips, M.C., Lund-Katz, S., Snow, J., 2002. Comparison of the stabilities and unfolding pathways of human apolipoprotein E isoforms by differential scanning calorimetry and circular dichroism. Biochim. Biophys. Acta BBA - Mol. Cell Biol. Lipids 1584, 9–19. https://doi.org/10.1016/S1388-1981(02)00263-9.

Agarwal, A., Aponte-Mellado, A., Premkumar, B.J., Shaman, A., Gupta, S., 2012. The effects of oxidative stress on female reproduction: a review. Reprod. Biol. Endocrinol. 10, 49. https://doi.org/10.1186/1477-7827-10-49.

Agency for Toxic Substances and Disease Registry (ATSDR), 2012. Toxicological profile for Vanadium. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

Ahmed, O.S., Galano, J.-M., Pavlickova, T., Revol-Cavalier, J., Vigor, C., Lee, J.-C.-Y., Oger, C., Durand, T., 2020. Moving forward with isoprostanes, neuroprostanes and phytoprostanes: where are we now? Essays Biochem. 64, 463–484. https://doi.org/10.1042/EBC20190096.

Amerikanou, C., Kleftaki, S.-A., Karavoltsos, S., Tagkouli, D., Sakellari, A., Valsamidou, E., Gioxari, A., Kalogeropoulos, N., Kaliora, A.C., 2023. Vanadium, cobalt, zinc, and rubidium are associated with markers of inflammation and oxidative stress in a Greek population with obesity. Front. Endocrinol. 14. https://doi.org/10.3389/fendo.2023.1265310.

Ashraf, M.M., Rea, R., 2017. Effect of dehydration on blood tests. Pract. Diabetes 34, 169–171. https://doi.org/10.1002/pdi.2111.

Ashrap, P., Watkins, D.J., Milne, G.L., Ferguson, K.K., Loch-Caruso, R., Fernandez, J., Rosario, Z., Vélez-Vega, C.M., Alshawabkeh, A., Cordero, J.F., Meeker, J.D., 2021. Maternal Urinary Metal and Metalloid Concentrations in Association with Oxidative Stress Biomarkers. Antioxidants 10. https://doi.org/10.3390/antiox10010114.

Barchielli, G., Capperucci, A., Tanini, D., 2022. The Role of Selenium in Pathologies: An Updated Review. Antioxidants 11. https://doi.org/10.3390/antiox11020251.

Bender, D., Schwarz, G., 2018. Nitrite-dependent nitric oxide synthesis by molybdenum enzymes. FEBS Lett. 592, 2126–2139. https://doi.org/10.1002/1873-3468.13089.

Blades, B., Ayton, S., Hung, Y.H., Bush, A.I., La Fontaine, S., 2021. Copper and lipid metabolism: A reciprocal relationship. Biochim. Biophys. Acta BBA - Gen. Subj. 1865, 129979. https://doi.org/10.1016/j.bbagen.2021.129979.

Brown, K.H., Rivera, J.A., Bhutta, Z., Gibson, R.S., King, J.C., Lönnerdal, B., Ruel, M.T., Sandtröm, B., Wasantwisut, E., Hotz, C., 2004. International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull 25, S99–S.

Ceballos-Rasgado, M., Brazier, A.K.M., Gupta, S., Moran, V.H., Pierella, E., Fekete, K., Lowe, N.M., 2024. Methods of Assessment of Zinc Status in Humans: An Updated Review and Meta-analysis. Nutr. Rev., nuae072 https://doi.org/10.1093/nutrit/nuae072.

Chamitava, L., Garcia-Larsen, V., Cazzoletti, L., Degan, P., Pasini, A., Bellisario, V., Corsico, A.G., Nicolis, M., Olivieri, M., Pirina, P., Ferrari, M., Stasinopoulos, M.D., Zanolin, M.E., 2018. Determination of adjusted reference intervals of urinary biomarkers of oxidative stress in healthy adults using GAMLSS models. PLOS ONE 13, e0206176. https://doi.org/10.1371/journal.pone.0206176.

Chen, X., Chen, Y., Shen, J., Xu, J., Zhu, L., Gu, X., He, F., Wang, H., 2019. Positive modulation of osteogenesis on a titanium oxide surface incorporating strontium oxide: An in vitro and in vivo study. Mater. Sci. Eng. C 99, 710–718. https://doi.org/10.1016/j.msec.2019.02.013.

Clement, B., Struwe, M.A., 2023. The History of mARC. Molecules 28. https://doi.org/10.3390/molecules28124713.

Dashner-Titus, E.J., Hoover, J., Li, L., Lee, J.-H., Du, R., Liu, K.J., Traber, M.G., Ho, E., Lewis, J., Hudson, L.G., 2018. Metal exposure and oxidative stress markers in pregnant Navajo Birth Cohort Study participants. Free Radic. Biol. Med. 124, 484–492. https://doi.org/10.1016/j.freeradbiomed.2018.04.579.

Dieckmann, M., Beil, F.T., Mueller, B., Bartelt, A., Marshall, R.P., Koehne, T., Amling, M., Ruether, W., Cooper, J.A., Humphries, S.E., Herz, J., Niemeier, A., 2013. Human apolipoprotein E isoforms differentially affect bone mass and turnover in vivo*. J. Bone Miner. Res. 28, 236–245. https://doi.org/10.1002/jbmr.1757.

Dietrich, M., Hu, Y., Block, G., Olano, E., Packer, L., Morrow, J.D., Hudes, M., Abdukeyum, G., Rimbach, G., Minihane, A.M., 2005. Associations between apolipoprotein E genotype and circulating F2-isoprostane levels in humans. Lipids 40, 329–334. https://doi.org/10.1007/s11745-006-1390-4.

Dose, J., Huebbe, P., Nebel, A., Rimbach, G., 2016. APOE genotype and stress response - a mini review. Lipids Health Dis. 15, 121. https://doi.org/10.1186/s12944-016-0288-2.

Dröge, W., 2002. Free Radicals in the Physiological Control of Cell Function. Physiol. Rev. 82, 47–95. https://doi.org/10.1152/physrev.00018.2001.

Egert, S., Rimbach, G., Huebbe, P., 2012. ApoE genotype: from geographic distribution to function and responsiveness to dietary factors. Proc. Nutr. Soc. 71, 410–424. https://doi.org/10.1017/S0029665112000249.

Ercal, N., Gurer-Orhan, H., Aykin-Burns, N., 2001. Toxic Metals and Oxidative Stress Part I: Mechanisms Involved in Me-tal induced Oxidative Damage. Curr. Top. Med. Chem. 1, 529–539. https://doi.org/10.2174/1568026013394831.

France Štiglic, A., Falnoga, I., Briški, A.S., Žavbi, M., Osredkar, J., Skitek, M., Marc, J., 2024. Reference intervals of 24 trace elements in blood, plasma and erythrocytes for the Slovenian adult population. Clinical Chemistry and Laboratory Medicine (CCLM) 62, 946–957. https://doi.org/10.1515/cclm-2023-0731.

Galano, J.-M., Lee, Y.Y., Oger, C., Vigor, C., Vercauteren, J., Durand, T., Giera, M., Lee, J.-C.-Y., 2017. Isoprostanes, neuroprostanes and phytoprostanes: An overview of 25years of research in chemistry and biology. Prog. Lipid Res. 68, 83–108. https://doi.org/10.1016/j.plipres.2017.09.004.

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

González-Domínguez, Á., Millán-Martínez, M., Domínguez-Riscart, J., Mateos, R.M., Lechuga-Sancho, A.M., González-Domínguez, R., 2022. Altered Metal Homeostasis Associates with Inflammation, Oxidative Stress, Impaired Glucose Metabolism, and Dyslipidemia in the Crosstalk between Childhood Obesity and Insulin Resistance. Antioxidants 11. https://doi.org/10.3390/antiox11122439.

Graeser, A.-C., Huebbe, P., Storm, N., Höppner, W., Döring, F., Wagner, A.E., Rimbach, G., 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.

Grandjean, P., Nielsen, G.D., Jørgensen, P.J., Hørder, M., 1992. Reference intervals for trace elements in blood: significance of risk factors. Scand. J. Clin. Lab. Invest. 52, 321–337. https://doi.org/10.1080/00365519209088366.

Gupta, P.K., Vaswani, S., 2020. Basic information about vanadium 'ultra-trace element or occasionally beneficial element' and its various functions in animals: A review article. J. Entomol. Zool. Stud. 8, 645–653.

Helmersson, H., Basu, S., 1999. F2-Isoprostane excretion rate and diurnal variation in human urine. Prostaglandins Leukot. Essent. Fatty Acids 61, 203–205. https://doi.org/10.1054/plef.1999.0091.

Hongo, T., Suzuki, T., Ishida, H., Kabuto, M., Neriishi, K., 1993. Diurnal Variation of Plasma Minerals and Trace Elements in a Group of Japanese Male Adults. J. Nutr. Sci. Vitaminol. (Tokyo) 39, 33–46. https://doi.org/10.3177/jnsv.39.33.

Hu, W., Wang, Y., Wang, T., Ji, Q., Jia, Q., Meng, T., Ma, S., Zhang, Z., Li, Y., Chen, R., Dai, Y., Luan, Y., Sun, Z., Leng, S., Duan, H., Zheng, Y., 2021. Ambient particulate matter compositions and increased oxidative stress: Exposure-response analysis among high-level exposed population. Environ. Int. 147, 106341. https://doi.org/10.1016/j.envint.2020.106341.

Huebbe, P., Bilke, S., Rueter, J., Schloesser, A., Campbel, G., Glüer, C.-C., Lucius, R., Röcken, C., Tholey, A., Rimbach, G., 2024. Human APOE4 Protects High-Fat and High-Sucrose Diet Fed Targeted Replacement Mice against Fatty Liver Disease Compared to APOE3. Aging Dis. 15, 259–281. https://doi.org/10.14336/AD.2023.0530.

Ikewaki, K., Zech, L.A., Brewer Jr, H.B., Rader, D.J., 2002. Comparative in vivo metabolism of apolipoproteins E2 and E4 in heterozygous apoE2/4 subjects. J. Lab. Clin. Med. 140, 369–374. https://doi.org/10.1067/mlc.2002.129066.

Il'yasova, D., Wang, F., Spasojevic, I., Base, K., D'Agostino Jr, R.B., Wagenknecht, L.E., 2012. Urinary F2-Isoprostanes, Obesity, and Weight Gain in the IRAS Cohort. Obesity 20, 1915–1921. https://doi.org/10.1038/oby.2011.292.

Ito, F., Sono, Y., Ito, T., 2019. Measurement and Clinical Significance of Lipid Peroxidation as a Biomarker of Oxidative Stress: Oxidative Stress in Diabetes, Atherosclerosis, and Chronic Inflammation. Antioxidants 8. https://doi.org/10.3390/antiox8030072.

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.

Kacperczyk, M., Kmieciak, A., Kratz, E.M., 2021. The Role of ApoE expression and variability of its glycosylation in human reproductive health in the light of current information. Int. J. Mol. Sci. 22. https://doi.org/10.3390/ijms22137197.

Kanabrocki, E.L., Murray, D., Hermida, R.C., Scott, G.S., Bremner, W.F., Ryan, M.D., Ayala, D.E., Third, J.L.H.C., Shirazi, P., Nemchausky, B.A., Hooper, D.C., 2002. Circadian variation in oxidative stress markers in healthy and type II diabetic men. Chronobiol. Int. 19, 423–439. https://doi.org/10.1081/CBI-120002914.

Khan, T.A., Shah, T., Prieto, D., Zhang, W., Price, J., Fowkes, G.R., Cooper, J., Talmud, P. J., Humphries, S.E., Sundstrom, J., Hubacek, J.A., Ebrahim, S., Lawlor, D.A., Ben-Shlomo, Y., Abdollahi, M.R., Slooter, A.J., Szolnoki, Z., Sandhu, M., Wareham, N., Frikke-Schmidt, R., Tybjærg-Hansen, A., Fillenbaum, G., Heijmans, B.T., Katsuya, T., Gromadzka, G., Singleton, A., Ferrucci, L., Hardy, J., Worrall, B., Rich, S.S., Matarin, M., Whittaker, J., Gaunt, T.R., Whincup, P., Morris, R., Deanfield, J., Donald, A., Davey Smith, G., Kivimaki, M., Kumari, M., Smeeth, L., Khaw, K.-T., Nalls, M., Meschia, J., Sun, K., Hui, R., Day, I., Hingorani, A.D., Casas, J.P., 2013. Apolipoprotein E genotype, cardiovascular biomarkers and risk of stroke: Systematic review and meta-analysis of 14 015 stroke cases and pooled analysis of primary biomarker data from up to 60 883 individuals. Int. J. Epidemiol. 42, 475–492. https://doi.org/10.1093/ije/dyt034.

Le, N.-A., 2015. Lipoprotein-associated oxidative stress: a new twist to the postprandial hypothesis. Int. J. Mol. Sci. 16, 401–419. https://doi.org/10.3390/ijms16010401.

Li, B., Huang, Y., Luo, C., Peng, X., Jiao, Y., Zhou, L., Yin, J., Liu, L., 2021. Inverse association of plasma molybdenum with metabolic syndrome in a chinese adult population: a case-control study. Nutrients 13. https://doi.org/10.3390/nu13124544.

Li, C.-P., Song, Y.-X., Lin, Z.-J., Ma, M.-L., He, L.-P., 2024. Essential trace elements in patients with dyslipidemia: a meta-analysis. Curr. Med. Chem. 31, 1–20. https://doi.org/10.2174/0929867330666230428161653.

Lu, J., Wang, Z., Cao, J., Chen, Y., Dong, Y., 2018. A novel and compact review on the role of oxidative stress in female reproduction. Reprod. Biol. Endocrinol. 16, 80. https://doi.org/10.1186/s12958-018-0391-5.

Lumsden, A.L., Mulugeta, A., Zhou, A., Hyppönen, E., 2020. Apolipoprotein E (APOE) genotype-associated disease risks: a phenome-wide, registry-based, case-control study utilising the UK Biobank. eBioMedicine 59. https://doi.org/10.1016/j.ebiom.2020.102954.

Mabile, L., Lefebvre, C., Lavigne, J., Boulet, L., Davignon, J., Lussier-Cacan, S., Bernier, L., 2003. Secreted apolipoprotein E reduces macrophage-mediated LDL oxidation in an isoform-dependent way. J. Cell. Biochem. 90, 766–776. https://doi.org/10.1002/jcb.10697.

Marreiro, D.D., Cruz, K.J., Morais, J.B., Beserra, J.B., Severo, J.S., De Oliveira, A.R., 2017. Zinc and Oxidative Stress: Current Mechanisms. Antioxidants 6. https://doi.org/10.3390/antiox6020024.

Matsubara, T., Musat-Marcu, S., Misra, H.P., Dhalla, N.S., 1995. Protective effect of vanadate on oxyradical-induced changes in isolated perfused heart. Mol. Cell. Biochem. 153, 79–85. https://doi.org/10.1007/BF01075921.

Miyata, M., Smith, J.D., 1996. Apolipoprotein E allele–specific antioxidant activity and effects on cytotoxicity by oxidative insults and β–amyloid peptides. Nat. Genet. 14, 55–61. https://doi.org/10.1038/ng0996-55.

Nikolaidis, M.G., Kyparos, A., Vrabas, I.S., 2011. F2-isoprostane formation, measurement and interpretation: The role of exercise. Prog. Lipid Res. 50, 89–103. https://doi.org/10.1016/j.plipres.2010.10.002.

Noguchi, T., Ebina, K., Hirao, M., Otsuru, S., Guess, A.J., Kawase, R., Ohama, T., Yamashita, S., Etani, Y., Okamura, G., Yoshikawa, H., 2018. Apolipoprotein E plays crucial roles in maintaining bone mass by promoting osteoblast differentiation via ERK1/2 pathway and by suppressing osteoclast differentiation via c-Fos, NFATc1, and NF-κB pathway. Biochem. Biophys. Res. Commun. 503, 644–650. https://doi.org/10.1016/j.bbrc.2018.06.055.

Palir, N., Stajnko, A., Snoj Tratnik, J., Mazej, D., Briški, A.S., France-Štiglic, A., Rosolen, V., Mariuz, M., Giordani, E., Barbone, F., Horvat, M., Falnoga, I., 2023. ALAD and APOE polymorphisms are associated with lead and mercury levels in Italian pregnant women and their newborns with adequate nutritional status of zinc and selenium. Environ. Res. 220, 115226. https://doi.org/10.1016/j.envres.2023.115226.

Peruzzu, A., Solinas, G., Asara, Y., Forte, G., Bocca, B., Tolu, F., Malaguarnera, L., Montella, A., Madeddu, R., 2015. Association of trace elements with lipid profiles and glycaemic control in patients with type 1 diabetes mellitus in northern Sardinia, Italy: An observational study. Chemosphere 132, 101–107. https://doi.org/10.1016/j.chemosphere.2015.02.052.

Pollack, A.Z., Schisterman, E.F., Goldman, L.R., Mumford, S.L., Perkins, N.J., Bloom, M. S., Rudra, C.B., Browne, R.W., Wactawski-Wende, J., 2012. Relation of Blood Cadmium, Lead, and Mercury Levels to Biomarkers of Lipid Peroxidation in Premenopausal Women. Am. J. Epidemiol. 175, 645–652. https://doi.org/10.1093/aje/kwr375.

Praticò, D., Tangirala, R.K., Rader, D.J., Rokach, J., FitzGerald, G.A., 1998. Vitamin E suppresses isoprostane generation in vivo and reduces atherosclerosis in ApoE-deficient mice. Nat. Med. 4, 1189–1192. https://doi.org/10.1038/2685.

Pryor, W.A., Stanley, J.P., 1975. Suggested mechanism for the production of malonaldehyde during the autoxidation of polyunsaturated fatty acids. Nonenzymic production of prostaglandin endoperoxides during autoxidation. J. Org. Chem. 40, 3615–3617. https://doi.org/10.1021/jo00912a038.

Ramassamy, C., Averill, D., Beffert, U., Bastianetto, S., Theroux, L., Lussier-Cacan, S., Cohn, J.S., Christen, Y., Davignon, J., Quirion, R., Poirier, J., 1999. Oxidative damage and protection by antioxidants in the frontal cortex of Alzheimer's disease is related to the apolipoprotein E genotype. Free Radic. Biol. Med. 27, 544–553. https://doi.org/10.1016/S0891-5849(99)00102-1.

Rehder D., 2013. Vanadium. Its role for humans.. Met Ions Life Sci 139–69. https://doi.org/10.1007/978-94-007-7500-8_5. PMID: 24470091; PMCID: PMC7120733.

Rixen, S., Indorf, P.M., Kubitza, C., Struwe, M.A., Klopp, C., Scheidig, A.J., Kunze, T., Clement, B., 2023. Reduction of Hydrogen Peroxide by Human Mitochondrial Amidoxime Reducing Component Enzymes. Molecules 28. https://doi.org/10.3390/molecules28176384.

Rotter, I., Kosik-Bogacka, D., Dołęgowska, B., Safranow, K., Lubkowska, A., Laszczyńska, M., 2015. Relationship between the Concentrations of Heavy Metals and Bioelements in Aging Men with Metabolic Syndrome. Int. J. Environ. Res. Public. Health 12, 3944–3961. https://doi.org/10.3390/ijerph120403944.

Ru, X., Yang, L., Shen, G., Wang, K., Xu, Z., Bian, W., Zhu, W., Guo, Y., 2024. Microelement strontium and human health: comprehensive analysis of the role in inflammation and non-communicable diseases (NCDs). Front. Chem. 12.

Sakano, N., Takahashi, N., Wang, D.-H., Sauriasari, R., Takemoto, K., Kanbara, S., Sato, Y., Takigawa, T., Takaki, J., Ogino, K., 2009. Plasma 3-nitrotyrosine, urinary 8-isoprostane and 8-OHdG among healthy Japanese people. Free Radic. Res. 43, 183–192. https://doi.org/10.1080/10715760802663124.

Sies, H., Berndt, C., Jones, D.P., 2017. Oxidative Stress. Annu. Rev. Biochem. https://doi.org/10.1146/annurev-biochem-061516-045037.

Simić, A., Hansen, A.F., Syversen, T., Lierhagen, S., Ciesielski, T.M., Romundstad, P.R., Midthjell, K., Åsvold, B.O., Flaten, T.P., 2022. Trace elements in whole blood in the general population in Trøndelag County, Norway: The HUNT3 Survey. Sci. Total Environ. 806, 150875. https://doi.org/10.1016/j.scitotenv.2021.150875.

Smith, D.R., Nordberg, M., 2015. Chapter 2 - General Chemistry, Sampling, Analytical Methods, and Speciation*, in: Nordberg, G.F., Fowler, B.A., Nordberg, M. (Eds.), Handbook on the Toxicology of Metals (Fourth Edition). Academic Press, San Diego, pp. 15–44. https://doi.org/10.1016/B978-0-444-59453-2.00002-0.

Smith, J.D., Miyata, M., Poulin, S.E., Neveux, L.M., Craig, W.Y., 1998. The relationship between apolipoprotein E and serum oxidation-related variables is apolipoprotein E phenotype dependent. Int. J. Clin. Lab. Res. 28, 116–121. https://doi.org/10.1007/s005990050030.

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.

Suh, J.H., Zyba, S.J., Shigenaga, M., McDonald, C.M., King, J.C., 2022. Marginal Zinc Deficiency Alters Essential Fatty Acid Metabolism in Healthy Men. J. Nutr. 152, 671–679. https://doi.org/10.1093/jn/nxab425.

Suwazono, Y., Åkesson, A., Alfvén, T., Järup, L., Vahter, M., 2005. Creatinine versus specific gravity-adjusted urinary cadmium concentrations. Biomarkers 10, 117–126. https://doi.org/10.1080/13547500500159001.

Swerdlow, R.H., 2023. The Alzheimer's Disease Mitochondrial Cascade Hypothesis: A Current Overview. J. Alzheimers Dis. 92, 751–768. https://doi.org/10.3233/JAD-221286.

Tangirala, R.K., Praticó, D., FitzGerald, G.A., Chun, S., Tsukamoto, K., Maugeais, C., Usher, D.C., Puré, E., Rader, D.J., 2001. Reduction of Isoprostanes and Regression of Advanced Atherosclerosis by Apolipoprotein E *. J. Biol. Chem. 276, 261–266. https://doi.org/10.1074/jbc.M003324200.

Tinkov, A.A., Bogdański, P., Skrypnik, D., Skrypnik, K., Skalny, A.V., Aaseth, J., Skalnaya, M.G., Suliburska, J., 2021. Trace Element and Mineral Levels in Serum, Hair, and Urine of Obese Women in Relation to Body Composition, Blood Pressure, Lipid Profile, and Insulin Resistance. Biomolecules 11. https://doi.org/10.3390/biom11050689.

Trares, K., Gào, X., Perna, L., Rujescu, D., Stocker, H., Möllers, T., Beyreuther, K., Brenner, H., Schöttker, B., 2020. Associations of urinary 8-iso-prostaglandin F2α levels with all-cause dementia, Alzheimer's disease, and vascular dementia incidence: results from a prospective cohort study. Alzheimers Dement. 16, 804–813. https://doi.org/10.1002/alz.12081.

Tudorache, I.F., Trusca, V.G., Gafencu, A.V., 2017. Apolipoprotein E - A Multifunctional Protein with Implications in Various Pathologies as a Result of Its Structural Features. Comput. Struct. Biotechnol. J. 15, 359–365. https://doi.org/10.1016/j.csbj.2017.05.003.

Valent, F., Horvat, M., Sofianou-Katsoulis, A., Spiric, Z., Mazej, D., Little, D., Prasouli, A., Mariuz, M., Tamburlini, G., Nakou, S., Barbone, F., 2013. Neurodevelopmental Effects of Low-level Prenatal Mercury Exposure From Maternal Fish Consumption in a Mediterranean Cohort: Study Rationale and Design. J. Epidemiol. 23, 146–152. https://doi.org/10.2188/jea.JE20120030.

Valko, M., Morris, H., Cronin, T.D.M., 2005. Metals, Toxicity and Oxidative Stress. Curr. Med. Chem. 12, 1161–1208. https://doi.org/10.2174/0929867053764635.

Weisgraber, K.H., Innerarity, T.L., Mahley, R.W., 1982. Abnormal lipoprotein receptor-binding activity of the human E apoprotein due to cysteine-arginine interchange at a single site. J. Biol. Chem. 257, 2518–2521. https://doi.org/10.1016/S0021-9258(18)34954-8.

Xu, H., Finkelstein, D.I., Adlard, P.A., 2014. Interactions of metals and Apolipoprotein E in Alzheimer's disease. Front. Aging Neurosci. 6.

Xu, H., Gupta, V.B., Martins, I.J., Martins, R.N., Fowler, C.J., Bush, A.I., Finkelstein, D.I., Adlard, P.A., 2015. Zinc affects the proteolytic stability of Apolipoprotein E in an isoform-dependent way. Met. Neurodegener. 81, 38–48. https://doi.org/10.1016/j.nbd.2015.06.016.

Yao, J., Petanceska, S.S., Montine, T.J., Holtzman, D.M., Schmidt, S.D., Parker, C.A., Callahan, M.J., Lipinski, W.J., Bisgaier, C.L., Turner, B.A., Nixon, R.A., Martins, R.N., Ouimet, C., Smith, J.D., Davies, P., Laska, E., Ehrlich, M.E., Walker, L.C., Mathews, P.M., Gandy, S., 2004. Aging, gender and APOE isotype modulate metabolism of Alzheimer's Aβ peptides and F2-isoprostanes in the absence of detectable amyloid deposits. J. Neurochem. 90, 1011–1018. https://doi.org/10.1111/j.1471-4159.2004.02532.x.

Zhang, J., Sun, B., Zhao, H., Zhang, T., He, D., Lin, J., Chen, F., 2022. Apolipoprotein E is an effective biomarker for orthodontic tooth movement in patients treated with transmission straight wire appliances. Am. J. Orthod. Dentofacial Orthop. 161, 255–262.e1. https://doi.org/10.1016/j.ajodo.2020.08.020.

Zhang, S.Q., Zhang, W.Y., Ye, W.Q., Zhang, L.J., Fan, F., 2014. Apolipoprotein E gene E2/E2 genotype is a genetic risk factor for vertebral fractures in humans: a large-scale study. Int. Orthop. 38, 1665–1669. https://doi.org/10.1007/s00264-014-2380-4.

Zhou, C., Chen, Y.Q., Zhu, Y.H., Lin, G.F., Zhang, L.F., Liu, X.C., He, F.M., 2019. Antiadipogenesis and Osseointegration of Strontium-Doped Implant Surfaces. J. Dent. Res. 98, 795–802. https://doi.org/10.1177/0022034519850574.

Zhou, B., Su, X., Su, D., Zeng, F., Wang, M.H., Huang, L., Huang, E., Zhu, Y., Zhao, D., He, D., Zhu, X., Yeoh, E., Zhang, R., Ding, G., 2016. Dietary intake of manganese and the risk of the metabolic syndrome in a Chinese population. Br. J. Nutr. 116, 853–863. https://doi.org/10.1017/S0007114516002580.