



Smoking is associated with altered serum and hair essential metal and metalloid levels in women

Anatoly V. Skalny^{a,b}, Eugeny P. Serebryansky^c, Tatiana V. Korobeinikova^{a,b},
Aristidis Tsatsakis^{a,d}, Constantine Vardavas^{d,e}, Monica M.B. Paoliello^f, Tatiana I. Sotnikova^{a,g},
Michael Aschner^f, Alexey A. Tinkov^{a,h,*}

^a IM Sechenov First Moscow State Medical University (Sechenov University), 119435, Moscow, Russia

^b Peoples' Friendship University of Russia (RUDN University), 117198, Moscow, Russia

^c Micronutrients Ltd, 105082, Moscow, Russia

^d Laboratory of Toxicology, Medical School, University of Crete, Voutes, 700 13, Heraklion, Crete, Greece

^e Center for Global Tobacco Control, Harvard School of Public Health, 02115, Boston, United States

^f Department of Molecular Pharmacology, Albert Einstein College of Medicine, 10461, Bronx, NY, USA

^g City Clinical Hospital n. a. S.P. Botkin of the Moscow City Health Department, 125284, Moscow, Russia

^h Laboratory of Ecobiomonitoring and Quality Control, Yaroslavl State University, 150003, Yaroslavl, Russia

ARTICLE INFO

Handling Editor: Dr. Jose Luis Domingo

Keywords:
Tobacco
Cigarettes
Chromium
Selenium
Zinc

ABSTRACT

The objective of the study was to evaluate the association between smoking and essential metal (Co, Cr, Cu, Fe, Mn, V, Zn) and metalloid (Se) levels in hair and serum of adult women using inductively coupled plasma-mass spectrometry (ICP-MS). In this cross-sectional study, a total of 344 women 20–70 years old including 199 smokers and 145 non-smoking women were enrolled. Serum Cu, Fe, and Zn levels in smoking women were found to be 6%, 8%, and 3% lower of levels in non-smokers, respectively. In contrast, circulating Mn, V, and especially Cr concentrations in smoking women exceeded the respective values in non-smoking women by 5%, 14%, and 54%. Hair Fe and Se levels in smoking women were 17% and 23% lower as compared to non-smoking controls, respectively. In multiple regression models, smoking severity was inversely associated with serum and hair Se concentrations, whereas the relationship to serum and hair Cr was positive. In addition, serum Zn and hair Fe levels were found to be inversely associated with the number of cigarettes per day. These findings hypothesize that health hazards of smoking may be at least in part be mediated by alteration in essential metal and metalloid metabolism.

1. Introduction

The prevalence of smoking worldwide may be as high as 20% according to World Health Organization estimates (World Health Organization, 2018). Despite a significant reduction in overall smoking prevalence, the total number of smokers has increased (GBD 2019 Tobacco Collaborators, 2021). Although men are commonly characterized by higher smoking rates than women (Allen et al., 2014), the prevalence of smoking in men and women may be nearly similar in certain populations (PCASRM, 2018).

Despite the well-known hazardous effects of smoking, sex may significantly affect vulnerability to tobacco-induced diseases with an increase in the risk of stroke and early death in women (Haghani et al.,

2020). Smoking has been shown to have a significant adverse effect on female fertility, as well as pregnancy and fetal development (PCASRM, 2018). Specifically, maternal smoking is associated with intrauterine growth retardation, low birth weight, congenital malformations (Berlin and Oncken, 2018). Despite a significant decrease in the prevalence of smoking in pregnancy the number remains high, especially in young women (Skalis et al., 2021). Therefore, female smoking is considered as a significant population health hazard.

Adverse effects of smoking are mediated by a large number of toxic substances in tobacco smoke (Talhout et al., 2011), hence the risk assessment of chemical mixtures – such as those in tobacco-presents a valuable occasion for a comparative law approach (Vinceti and Filippini, 2021). Toxic metals, including cadmium and lead, originating from

* Corresponding author. Laboratory of Ecobiomonitoring and Quality Control, Yaroslavl State University, 150003, Yaroslavl, Russia.

E-mail address: tinkov.a.a@gmail.com (A.A. Tinkov).

<https://doi.org/10.1016/j.fct.2022.113249>

Received 3 May 2022; Received in revised form 9 June 2022; Accepted 16 June 2022

Available online 18 June 2022

0278-6915/© 2022 Elsevier Ltd. All rights reserved.

tobacco smoke were shown to contribute significantly to its inflammatory (Milnerowicz et al., 2015) and carcinogenic effect (Stavrides, 2006). In addition to these non-essential toxic metals, tobacco products also contain essential metals such iron, copper, zinc, among others (Majewska et al., 2018). Although recent studies clearly demonstrated an association between smoking and toxic metal accumulation in the organism (Mortada et al., 2004; Shakeri et al., 2021), the existing data on the impact of smoking on body burden of essential metals and trace elements are insufficient (Kocuyigit et al., 2001; Ates Alkan et al., 2019) and sometimes contradictory (Meltzer et al., 2016).

Therefore, the objective of the study was to evaluate the association between smoking and essential metal and metalloid levels in hair and serum of adult women.

2. Materials and methods

The present study was performed in agreement with the ethical principles set by the Declaration of Helsinki (1964) and its later amendments (World Medical Association, 2013). The protocol of the present study was approved by the Yaroslavl State University (Yaroslavl, Russia) Faculty of Biology and Ecology Ethics Committee (No. 5, February 17, 2021).

2.1. Study design and setting

In this cross sectional study, a total of 344 women aged 20–70 years old were enrolled in the study. One hundred and ninety-nine women at interviewing reported smoking, whereas 145 non-smoking women were included into the control group. Self-reported smoking duration (years) and the number of cigarettes per day were also recorded along with body weight, height, and age. Body mass index (BMI) was calculated according to the standard formula. The key inclusion criterion was cigarette smoking, but not waterpipe and e-cigarette use. Exclusion criteria included acute and chronic inflammatory diseases, and hormone replacement therapy. In order to exclude the impact of side factors on systemic metal levels additional exclusion criteria included occupational exposure to metals, metallic implants including dental amalgams, specific dietary patterns (vegetarians, raw food diet, etc.), as well as the use of metal-containing supplements.

2.2. Sample collection

Assessment of essential metal and metalloid status was performed through analysis of serum and hair samples. Blood samples were collected in the morning after overnight fasting via cubital vein puncture with 9-ml Vacuette® tubes (Greiner Bio-One International AG, Austria). The obtained whole blood samples were subjected to centrifugation at 1600g for 10 min to obtain serum. Only hemolysis-free samples were used for subsequent analysis. Serum samples were transferred to Eppendorf tubes and stored at $-40\text{ }^{\circ}\text{C}$ until analysis. Directly prior analysis thawed serum samples were diluted at a volume ratio of 1:15 with an acidified ($\text{pH} = 2.0$) solution consisting of 1% 1-Butanol (Merck KGaA, Darmstadt, Germany), 0.1% Triton X-100 (Sigma-Aldrich, Co., St. Louis, MO USA), 0.07% HNO_3 (Sigma-Aldrich, Co., St. Louis, MO USA) diluted in 18.2 M Ω cm distilled deionized water.

Hair samples are also used as the substrate for metal and trace element body burden assessment, being indicative of long-term changes in metal status (Chojnacka and Mikulewicz, 2018), a substrate used in assessing tobacco exposures (Go et al., 2021). Hair samples were collected from the occipital region of the head using ethanol-pre-cleaned stainless-steel scissors. Subsequently, only proximal 1–2 cm the hair strands in a quantity of 0.05–0.1 g were excised and stored in paper envelopes at ambient conditions until analysis. Directly prior analysis hair samples were washed with acetone and rinsed thrice with 18.2 M Ω cm deionized water (Labconco Corp., Kansas City, MO, USA) for removal of dust, dirt, and other external contaminants. After washing the samples

were dried at $60\text{ }^{\circ}\text{C}$ under exhaust ventilation to a stable weight. 50 mg of the washed hair samples were entered into Teflon tubes containing 5 ml of 65% HNO_3 (Sigma-Aldrich Co., St. Louis, MO, USA) and subjected to decomposition in Berghof SpeedWave-4 DAP-40 microwave system (Berghof Products + Instruments GmbH, 72800 Eningen, Germany) at $170\text{--}180\text{ }^{\circ}\text{C}$ for 20 min. The obtained solutions were adjusted with distilled deionized water to a final volume of 15 ml and used for analysis.

2.3. Analytical procedures

Assessment of cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), selenium (Se), vanadium (V), and zinc (Zn) levels in serum and hair of the examined women was performed with inductively-coupled plasma mass-spectrometry at NexION 300D (PerkinElmer Inc., Shelton, CT, USA) with 7-port FAST valve and ESI SC-2 DX4 autosampler (Elemental Scientific Inc., Omaha, NE, USA). System calibration was carried out using stock solutions from Universal Data Acquisition Standards Kits (PerkinElmer Inc., Shelton, CT 06484, USA). In addition, internal on-line standardization was performed using 10 $\mu\text{g/l}$ solutions of Yttrium (Y) and Rhodium (Rh) prepared from Pure Single-Element Standard Kits (PerkinElmer Inc., Shelton, CT 06484, USA) in order to account for incomplete acidity and viscosity matching between the studied samples and calibration standards.

Laboratory quality control was performed permanently using the certified reference materials (CRMs) of human plasma ClinChek® Plasma Control (RECIPE Chemicals + Instruments GmbH, Germany) and hair (GBW09101, Shanghai Institute of Nuclear Research, Shanghai, China). The recovery rates for human plasma and hair were 92–108% and 89–110% for all studied metal(loid)s, respectively. The analytical laboratory of the Center for Biotic Medicine (Moscow, Russia) is also a participant of the Occupational and Environmental Laboratory Medicine External Quality Assessment Schemes (OELM EQAS).

2.4. Statistical analyses

Statistical analysis was performed with Statistica 10.0 (Statsoft, OK, USA). Due to skewed distribution, median and the respective interquartile range boundaries were used as descriptive statistics. For further processing the raw data were log-transformed. Due to high variability of age and anthropometric parameters, group comparisons were performed using analysis of covariance (ANCOVA) with adjustment for age and BMI using Bonferroni post-hoc. Spearman coefficient was used for correlation analysis. Multiple regression models were composed for the evaluation of the independent association of metal levels in serum or hair with the number of cigarettes per day. All tests were considered significant at $p < 0.05$.

3. Results

Analysis of smoking habits in the examined women demonstrated that the number of cigarettes per day and smoking duration varied from 1 to 45 and from 1 to 40 years, respectively (Table 1). No significant difference in age, height, weight, and BMI values was observed between

Table 1
Anthropometric characteristics and smoking habits of the examined women.

Metal(loid)	Non-smokers (n = 145)	Smokers (n = 199)	P value
Age, years old	43.9 \pm 13.4	40.0 \pm 12.4	0.131
Height, cm	165.2 \pm 5.8	166.0 \pm 5.9	0.307
Weight, kg	64.5 \pm 13.3	64.5 \pm 13.8	0.759
BMI, kg/m ²	23.7 \pm 5.0	23.5 \pm 5.0	0.412
Years of smoking	–	9.2 \pm 7.3	–
Number of cigarettes per day	–	13.4 \pm 8.6	–

Data expressed as Mean \pm SD.

the non smoking controls and smokers, respectively. Smoking duration in years was shown to be significantly correlated with both age ($r = 0.643$; $p < 0.001$) and BMI ($r = 0.181$; $p = 0.011$), whereas the number of cigarettes smoked per day was not significantly associated with these parameters.

Smoking was also associated with altered serum essential metal and metalloid levels (Table 2). Specifically, serum Cu, Fe, and Zn levels in smoking women were found to be 6%, 8%, and 3% lower as compared to non-smokers, respectively. In contrast, circulating Mn, V, and especially Cr concentrations in smoking women exceeded the respective control values by 5%, 14%, and 54%.

In hair (Table 3), a significant decrease in Fe and Se content was observed. Specifically, hair Fe and Se levels in smoking women were 17% and 23% lower as compared to non-smoking controls, respectively. In contrast, hair Zn content tended to be 3% higher in smokers, although this difference was only nearly significant.

In agreement with the between group comparisons (smokers vs. non smokers), correlation analysis (Table 4) demonstrated that serum Fe and Zn levels were significantly and inversely correlated with years of smoking, whereas serum Cr levels was positively correlated with the latter. Serum Zn levels also negatively correlated with the number of cigarettes per day. It is also notable that serum Cr and Mn concentrations trended toward a positive association with the number of cigarettes per day.

Correspondingly, hair Cr was characterized by a significant and a nearly-significant positive correlation with smoking duration in years and the number of cigarettes per day, respectively. In addition, hair Se content was significantly inversely associated with both smoking duration and the number of cigarettes smoked per day. Correlations between the number of cigarettes per day and the level of other metals and metalloids in hair were not significant.

In order to identify independent associations between essential metal and metalloid levels in hair and serum with the number of cigarettes per day after adjustment for variability in age and BMI, multiple linear regression analysis was performed (Table 5).

Years of smoking as well as the number of cigarettes per day were inversely associated with serum Zn and Se concentrations. Serum Se levels were also characterized by a nearly significant inverse association with years of smoking. In contrast, Cr levels were characterized by a positive relationship with years of smoking. Serum Cr levels also tended to be directly associated with the number of cigarettes per day, although this association did not reach the level of significance. Finally, the number of cigarettes per day was found to be inversely associated with age.

Although both models based on serum metal and metalloid levels were significantly associated with smoking severity, the predictive capacity of such models was lower than 10%.

Select patterns were shown for hair metal and metalloid levels (Table 6). Specifically, hair Cr levels were considered significant positive predictors of smoking duration and the number of cigarettes per day. The number of cigarettes per day was also inversely associated with the age of the subjects, whereas no relationship with age was noted for the

years of smoking. Despite the lack of significant group differences, hair Mn content was characterized by a positive association with the number of cigarettes per day. Analogous to serum, predictive potential of the models based on hair metal levels was low despite being significant.

4. Discussion

The findings described herein demonstrate that smoking is associated with a significant reduction in systemic Cu, Fe, Se, and Zn levels. In contrast, smokers were characterized by a significant increase in hair and serum Cr concentrations, whereas a significant increase in Mn level was detected only in blood serum. These findings may be indicative of the potential role of smoking in the deficiency of certain essential metals and metalloids, as well as an additional source of Cr and Mn exposure.

Generally, the data corroborate trends noted in other studies that aimed to assess circulating metal levels in smokers. For example, Kocyigit et al. (2001) detected significantly lower Se and higher Cu levels in plasma of young Turkish adults (Kocyigit et al., 2001). Similar findings were obtained in another study from Czech Republic (Benes et al., 2005). Ates Alkan et al. (2019) also showed a significant reduction in Se concentrations, as well as an increase in serum Mn levels in association with altered blood rheology in Turkish smokers (Ates Alkan et al., 2019). Examination of smokers and non-smokers from Birjand (Iran) demonstrated that smoking is associated with significantly lower Fe and Zn levels in blood serum, whereas the concentrations of Cu and Mn appeared to be elevated (Shakeri et al., 2021). At the same time, no associations between smoking and blood Co or Mn levels were revealed in Norwegian women (Meltzer et al., 2016).

Hair analysis performed in Poland also demonstrated significantly lower Se, Fe, Cr levels in smokers, whereas hair Zn content was higher in the smoker group (Chojnacka et al., 2006). Another study demonstrated higher hair Cu and Zn levels along with reduction of Fe content in smokers (Massadeh et al., 2011).

Several studies addressed the impact of tobacco smoking on Se status and metabolism. Smoking was shown to be inversely associated with serum (Kafai and Ganji, 2003) Se levels in Korean and American populational studies, respectively. In addition, a study in Norway revealed significantly lower whole blood and serum Se levels, as well as decreased selenoprotein glutathione peroxidase activity in smokers (Ellingsen et al., 2009). Vinceti et al. (2015) also demonstrated reduced serum Se levels due to a decrease in selenoprotein P and glutathione peroxidase levels, whereas inorganic Se fraction was increased due to elevation of Se(IV). On the contrary, no significant impact of smoking on plasma selenoprotein distribution was demonstrated (Letsiou et al., 2014). Moreover, in heavy smokers increased serum Se levels were found to be associated with higher risk of lung cancer mortality (Sua-dicani et al., 2012). At the same time, the present study used serum Se levels for assessment of Se status that may be affected by systemic inflammation in contrast to erythrocyte Se levels, being considered as a more effective marker (Stefanowicz et al., 2013).

We also demonstrated that serum Zn concentration was inversely associated with smoking and its severity in women, being in agreement

Table 2

Comparison of the serum metal and metalloid concentrations in smoking women and non-smoking controls.

Metal(loid)	Non-smokers (n = 145)	Smokers (n = 199)	p value	Reference values		
				Alimonti et al. (2005) (Italy)	Heitland and Köster, 2006 (Germany)	Liu et al. (2017) (China)
Co, ng/ml	0.67 (0.551–0.899)	0.625 (0.562–0.784)	0.200	0.06–0.42	0.05–0.41	0.057–1.130
Cr, ng/ml	1.078 (0.734–1.844)	1.66 (1.091–2.394)	<0.001	0.07–0.28	–	0.2198–4.2877
Cu, µg/ml	1.191 (1.063–1.371)	1.116 (0.94–1.262)	0.001	0.648–1.301	0.804–1.620	1.07–3.624
Fe, µg/ml	1.52 (1.177–1.768)	1.399 (0.99–1.817)	0.018	0.886–2.455	–	0.558–2.65
Mn, ng/ml	2.042 (1.798–2.463)	2.147 (1.768–2.909)	0.028	0.31–1.02	5.7–14.6	1.2–8.4
Se, µg/ml	0.104 (0.098–0.114)	0.103 (0.09–0.119)	0.747	–	0.105–0.164	0.0399–0.1116
V, ng/ml	6.141 (4.994–6.997)	7.045 (5.631–8.943)	0.009	0.03–0.11	0.021–0.103	0.0236–2.3231
Zn, µg/ml	0.919 (0.864–0.98)	0.897 (0.816–0.985)	0.016	0.597–1.028	–	0.51.8–1.113

Data are expressed as median (IQR); p values are provided according to ANCOVA with Bonferroni adjustment at the level of significance of $p < 0.05$.

Table 3
Comparison of hair ($\mu\text{g/g}$) metal levels in female smokers and non-smokers.

Metal(loid)	Non-smokers (n = 145)	Smokers (n = 199)	p value	Reference values		
				Dongarrà et al. (2011) (Italy)	Luo et al. (2014) (China)	Skalny et al. (2015) (Russia)
Co	0.012 (0.007–0.022)	0.012 (0.006–0.022)	0.953	0.01–0.85	LOQ–0.028	0.011–0.085
Cr	0.067 (0.045–0.13)	0.076 (0.042–0.165)	0.285	0.001–0.28	0.053–0.87	0.06–0.40
Cu	13.66 (11.15–21.77)	14.66 (11.54–20.25)	0.654	11.4–60.7	6.25–11.78	12.1–44.5
Fe	11.59 (8.27–18.34)	9.67 (7.55–14.19)	0.016	–	LOQ–27.50	8.9–25.6
Mn	0.421 (0.272–0.917)	0.471 (0.263–0.947)	0.902	0.002–0.95	0.070–2.85	0.32–2.05
Se	0.426 (0.351–0.509)	0.326 (0.231–0.393)	<0.001	0.05–0.75	0.42–0.88	0.094–0.504
V	0.011 (0.007–0.019)	0.011 (0.006–0.02)	0.678	0.022–0.17	LOQ–0.066	0.010–0.056
Zn	201.13 (163.45–253.72)	207.86 (179.94–243.04)	0.073	128.34–347.40	121.20–228.02	140.0–315.1

Data are expressed as median (IQR); p values are provided according to ANCOVA with Bonferroni adjustment at the level of significance of $p < 0.05$.

Table 4
Correlation between metal levels in hair and serum, years of smoking and number of cigarettes per day.

Metal	Years of Smoking				Number of Cigarettes per day			
	r	–95%CI	+95%CI	p	R	–95%CI	+95%CI	p
Hair								
Co	0.053	–0.053	0.158	0.328	–0.009	–0.115	0.097	0.866
Cr	0.158	0.053	0.260	0.003	0.069	–0.037	0.173	0.202
Cu	0.025	–0.081	0.130	0.646	0.001	–0.104	0.107	0.980
Fe	0.068	–0.038	0.172	0.210	–0.061	–0.166	0.045	0.259
Mn	0.095	–0.011	0.198	0.080	0.135	0.029	0.237	0.012
Se	–0.074	–0.179	0.032	0.168	–0.042	–0.147	0.064	0.436
V	0.038	–0.068	0.144	0.478	0.001	–0.104	0.107	0.979
Zn	–0.040	–0.145	0.066	0.462	0.104	–0.002	0.207	0.055
Serum								
Co	–0.008	–0.114	0.098	0.880	–0.036	–0.141	0.070	0.511
Cr	0.194	0.078	0.305	0.001	0.108	–0.010	0.223	0.073
Cu	–0.061	–0.166	0.045	0.257	–0.062	–0.166	0.045	0.255
Fe	–0.070	–0.175	0.036	0.194	–0.026	–0.132	0.080	0.632
Mn	0.108	0.002	0.211	0.046	0.117	0.011	0.220	0.030
Se	0.073	–0.033	0.177	0.179	0.019	–0.087	0.124	0.732
V	0.153	0.036	0.267	0.011	0.098	–0.020	0.214	0.104
Zn	–0.125	–0.228	–0.019	0.021	–0.112	–0.216	–0.006	0.038

Data expressed as correlation coefficient (r), 95% confidence interval limits, and the respective p value; correlation is significant at $p < 0.05$.

with earlier observations. In particular, smokers were also characterized by higher rate of insufficient dietary Zn intake that may occur at least partially due to smoking-induced Cd exposure (Kim et al., 2019). A study in Shenzhen also demonstrated twofold lower hair Zn content in smokers as compared to non-smokers (Qin et al., 2021).

It is noteworthy that in parallel with reduced hair and blood Zn levels, smoker hypertensive patients were also characterized by increased urinary Zn concentration, being indicative of excessive metal excretion (Afridi et al., 2010). Correspondingly, smoking was shown to reduce serum/urine Zn ratio in Spanish populations (Suarez-Varela et al., 2015). In view of the existing data, it is proposed that Zn may possess protective effects in smoking-associated lung disorders through its antagonism with Cd (Lin et al., 2014). However certain studies failed to reveal any significant impact of tobacco smoking on serum Zn levels (Galan et al., 2005).

Both hair and serum Fe levels were found to be reduced in smoking women, thus being indicative of a potential association between cigarette smoking and the risk of iron deficiency. Smoking pregnant women were characterized by reduced serum hepcidin, iron, and hemoglobin levels, whereas erythropoietin concentration was elevated (Chetchowska et al., 2016) due to higher carbon monoxide exposure derived from cigarette smoke (Leifert, 2008). Despite conflicting evidence on iron status of smoking pregnant women exist, it has been demonstrated that maternal tobacco smoking significantly decreases newborn iron stores (Pateva et al., 2015).

In contrast, smoking was found to be positively associated with circulating ferritin levels among adult Koreans (Lee et al., 2016). However, in view of smoking-induced systemic inflammation characterized by increased CRP and IL-6 levels (Elisia et al., 2020), the

observed elevation of ferritin levels in smokers may be also mediated by inflammatory signals. Moreover, systemic inflammation is known to induce iron sequestration leading to anemia of chronic disease (Fraenkel, 2017), that may at least partially mediate the observed decreased of systemic iron in female smokers. In addition, smoking was shown to induce hemolysis (Masilamani et al., 2016).

In parallel with Se, Zn, and Fe, we also revealed a significant reduction of serum Cu levels in smoking women. However, this observation is inconsistent with the majority of previous studies, demonstrating an association between smoking and increased Cu levels. Specifically, plasma Cu levels were also characterized by a positive correlation with the number of cigarettes smoked (Lapenna et al., 1995). Moreover, smoking was shown to increase susceptibility to menopausal hormone therapy-induced elevation of serum Cu levels (Jasińska-S-tarczewska et al., 2017). In agreement, urinary Cu levels were found to be significantly reduced in smokers, thus being indicative of higher risk of copper overaccumulation (Kulikowska-Karpińska et al., 2017). Being an acute-phase protein responding to inflammation, circulating ceruloplasmin levels were also found to be increased in smokers in association with complement C3 fragment (Tungtrongchitr et al., 2002).

In contrast to the previously discussed metals, our study also revealed positive association between cigarette smoking and increased Mn, V, and especially Cr body burden. Increased smoking-associated Cr exposure may result from high metal content in tobacco (Lisboa et al., 2020) as well as cigarette smoke (Pappas, 2011). Correspondingly, smoking was shown to be associated with increased blood (Khelifi et al., 2013) and urinary (Chen et al., 2008) Cr levels. Similarly, smoking was shown to result in increased serum Cr levels in Jordanian adults (Banihani et al., 2019). The findings of increased Cr accumulation in lung

Table 5

Multiple linear regression analysis of the association between serum metal and metalloid levels and smoking severity in women.

Parameter	Years of Smoking			Number of Cigarettes per day		
	B	±95%CI	p-value	β	±95%CI	p-value
Age	0.108	-0.024; 0.240	0.108	-0.153	-0.285; -0.022	0.023
BMI	-0.006	-0.139; 0.126	0.925	0.066	-0.066; 0.199	0.325
Co	0.037	-0.083; 0.156	0.548	0.032	-0.088; 0.152	0.599
Cr	0.162	0.025; 0.299	0.021	0.110	-0.027; 0.247	0.117
Cu	-0.026	-0.149; 0.097	0.680	-0.043	-0.167; 0.080	0.489
Fe	-0.059	-0.181; 0.063	0.340	-0.042	-0.164; 0.080	0.499
Mn	-0.085	-0.206; 0.036	0.166	-0.043	-0.164; 0.078	0.486
Se	-0.120	-0.242; 0.002	0.055	-0.126	-0.248; -0.003	0.044
V	0.080	-0.059; 0.220	0.258	0.024	-0.115; 0.164	0.731
Zn	-0.121	-0.239; -0.003	0.044	-0.144	-0.262; -0.026	0.017
Multiple R	0.315			0.312		
Multiple R ²	0.099			0.097		
Adjusted R ²	0.065			0.063		
p for a model	0.002			0.002		

Data are expressed as regression coefficients (β) and the respective p values; association is significant at p < 0.05.

Table 6

Analysis of the association between daily smoking severity and duration of smoking and hair metal and metalloid content in women.

Parameter	Years of Smoking			Number of Cigarettes per day		
	B	±95%CI	p-value	β	±95%CI	p-value
Age	0.047	-0.074; 0.167	0.444	-0.189	-0.308; -0.07	0.002
BMI	0.054	-0.063; 0.172	0.364	0.122	0.005; 0.238	0.040
Co	0.032	-0.106; 0.17	0.649	-0.084	-0.22; 0.053	0.228
Cr	0.130	0.02; 0.241	0.021	0.092	-0.017; 0.202	0.098
Cu	-0.002	-0.134; 0.13	0.976	0.039	-0.091; 0.169	0.555
Fe	0.042	-0.066; 0.149	0.449	-0.062	-0.168; 0.044	0.253
Mn	0.062	-0.052; 0.177	0.283	0.165	0.053; 0.278	0.004
Se	-0.072	-0.181; 0.036	0.190	-0.023	-0.13; 0.084	0.670
V	0.023	-0.089; 0.136	0.684	-0.022	-0.133; 0.09	0.703
Zn	-0.026	-0.139; 0.088	0.657	0.080	-0.032; 0.192	0.162
Multiple R	0.217			0.269		
Multiple R ²	0.047			0.072		
Adjusted R ²	0.018			0.044		
p for a model	0.094			0.005		

Data are expressed as regression coefficients (β) and the respective p values; association is significant at p < 0.05.

cancer tissue may also provide an additional link between smoking and lung carcinogenesis (Kuo et al., 2006).

In parallel with elevated Cr levels, we also showed higher serum V concentration in female smokers as compared to non-smokers. Despite the presence of certain indications of high V content in tobacco, as well as its transfer into tobacco smoke (Adachi et al., 1998), studies addressing the impact of smoking on vanadium metabolism in humans are lacking.

Smoking women were also characterized by higher serum Mn levels when compared to non-smokers, although this association was not significant after adjustment for other metals in multiple regression models. Tobacco was shown to contain significant and higher amounts of Mn compared to other metals (Fresquez et al., 2013), although Mn transfer from tobacco to cigarette smoke is one of the lowest (Pinto et al., 2017). Generally, the existing data on Mn status in smokers are contradictory. On one hand, in Mexican adults both dyslipidemia and smoking were found to be associated with increased serum Mn levels (Rivera-Mancía et al., 2017). On the other hand, certain studies failed to demonstrate an association between smoking and Mn body burden (Jain and Choi, 2015; Filippini et al., 2017).

Moreover, examination of Canadian pregnant women demonstrated that smokers were characterized by significantly reduced blood Mn levels in the third trimester (Takser et al., 2004). Despite these contradictions, the existing data underline the potential risks of smoking-associated disturbances in Mn body burden that may culminate in adverse health effects. Particularly, Mn exposure synergistically with smoking causes a decline in pulmonary ventilation function (Wang et al., 2015). Smoking has also been shown to aggravate Mn-induced tremor (Bast-Pettersen et al., 2004).

The observed increase in Mn, Cr, and V levels in smokers may be mediated by metal content in tobacco and tobacco smoke. Specifically, analysis of tobacco smoke revealed significant levels of Mn in commercial brands of cigarettes (Schneider and Krivna, 1993). Despite rather low and sometimes undetectable Cr levels in cigarette smoke aerosol (Krivan et al., 1994), tobacco smoke was shown to increase Cr content of indoor particles along with toxic As, Cd, Pb, and Ni, but not Mn or V (Slezakova et al., 2009). However, the results of the present study as well as findings from a previous analysis demonstrate that despite indications of significant amounts of Cu, Zn or Se in tobacco smoke, its exposure either does not affect or decreases systemic metal levels (Bernhard et al., 2005). Therefore, the circulating levels of certain essential metals may be not considered as a function of its contents in cigarette smoke, but rather antagonistic effects with toxic substances or other mechanisms.

It is also important to note that the obtained data on hair metal levels generally correspond to the earlier estimated Russian reference values for hair trace element content (Skalny et al., 2015). These values were also in agreement with the reference values obtained for environmentally unexposed Chinese subjects (Luo et al., 2014), whereas hair V content in the examined women was lower than the reference ranges reported by Dongarrà et al. (2011). No reference values of serum metal levels in the Russian population are available for comparison. The obtained values of serum Cu, Fe, and Zn levels were in agreement with the reference ranges obtained by Alimonti et al. (2005), whereas Co, Cr, Mn, and V concentrations significantly exceeded these values. Comparison with data from another European study demonstrated that serum Mn level was even lower than that in inhabitants of Northern Germany (Heitland and Köster, 2006). Oppositely, serum Se levels in the examined subjects appeared to be slightly lower than the respective values in the examined smokers and non-smokers. At the same time, the obtained serum metal and metalloid levels were more similar to the values obtained in China (Liu et al., 2017). As the values obtained for both smokers and non-smokers were generally within the reported reference ranges, the revealed difference may be considered as statistical, rather than biological. However, in view of adverse health effects of marginal Cu, Zn, and Fe deficiency (Hamilton et al., 2000; Lozoff and Georgieff,

2006; Song et al., 2010), increased smoking-associated risk of altered metal homeostasis may hypothetically significantly to smoking-induced diseases.

This current study is not without limitations as due to its cross-sectional design only associations can be assessed and not causality. Moreover, it is possible that other environmental sources may have contributed to the serum and hair metal concentrations, factors which we were not able to take into account in the analysis. Despite the above, the current results confirm the association between smoking status and certain metal concentrations.

5. Conclusions

Taken together, the novel findings reported herein demonstrate that in adult women smoking may be associated with lower systemic Fe, Cu, Se, and Zn levels, as well as higher rate of Cr, V, and Mn accumulation. Further studies are required to better characterize whether disruption of essential metal(loid) metabolism is mediated by interference with antagonistic toxic metals or other mechanisms and to assess if smoking-related health hazards may be at least partially mediated by alteration of essential metal and metalloids metabolism, as well as increased body burden of certain metals with toxic properties upon overaccumulation.

CRediT authorship contribution statement

Anatoly V. Skalny: Conceptualization, Supervision, Writing – review & editing. **Eugeny P. Serebryansky:** Data curation, Formal analysis, Investigation, Writing – original draft. **Tatiana V. Korobeinikova:** Data curation, Formal analysis, Investigation, Writing – original draft. **Aristidis Tsatsakis:** Supervision, Writing – review & editing. **Constantine Vardavas:** Supervision, Writing – review & editing. **Monica M.B. Paoliello:** Data curation, Formal analysis, Investigation, Writing – original draft. **Tatiana I. Sotnikova:** Data curation, Formal analysis, Investigation, Writing – original draft. **Michael Aschner:** Supervision, Writing – review & editing. **Alexey A. Tinkov:** Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft.

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.

Acknowledgements

The study was performed with the support of the Russian Ministry of Science and Higher Education, Project № 0856-2020-0008.

References

- Adachi, A., Asai, K., Koyama, Y., Matsumoto, Y., Okano, T., 1998. Determination of vanadium in cigarettes by atomic absorption spectrophotometry. *Anal Lett* 31, 1769–1776. <https://doi.org/10.1080/00032719808005258>.
- Afridi, H.I., Kazi, T.G., Kazi, N.G., Jamali, M.K., Arain, M.B., Sirajuddin, Baig, J.A., Kandhro, G.A., Wadhwa, S.K., Shah, A.Q., 2010. Evaluation of cadmium, lead, nickel and zinc status in biological samples of smokers and nonsmokers hypertensive patients. *J Hum Hypertens* 24, 34–43. <https://doi.org/10.1038/jhh.2009.39>.
- Alimonti, A., Bocca, B., Mannella, E., Petrucci, F., Zennaro, F., Cotichini, R., D'Ippolito, C., Agresti, A., Caimi, S., Forte, G., 2005. Assessment of reference values for selected elements in a healthy urban population. *Ann Ist Super Sanita* 41, 181–187.
- Allen, A.M., Oncken, C., Hatsukami, D., 2014. Women and smoking: the effect of gender on the Epidemiology, health effects, and cessation of smoking. *Curr Addict Rep* 1, 53–60. <https://doi.org/10.1007/s40429-013-0003-6>.
- Ates Alkan, F., Karis, D., Cakmak, G., Ercan, A.M., 2019. Analysis of the relationship between hemorheologic parameters, aluminum, manganese, and selenium in smokers. *Biol Trace Elem Res* 187, 22–31. <https://doi.org/10.1007/s12011-018-1352-8>.
- Banihani, S.A., Jaradat, S.A., Khader, Y.S., 2019. Serum chromium level is increased in Jordanian smokers, decreased in Jordanians with prediabetes and type 2 diabetes,

- but not altered in Jordanians with hypertension, with obesity, or with family history of diabetes. *Int J Prev Med* 10, 145. https://doi.org/10.4103/ijpvm.IJPVM_137_18.
- Bast-Pettersen, R., Ellingsen, D.G., Hetland, S.M., Thomassen, Y., 2004. Neuropsychological function in manganese alloy plant workers. *Int Arch Occup Environ Health* 77, 277–287. <https://doi.org/10.1007/s00420-003-0491-0>.
- Benes, B., Speváčková, V., Smíd, J., Batářiáová, A., Cejchanová, M., Zítková, L., 2005. Effects of age, BMI, smoking and contraception on levels of Cu, Se and Zn in the blood of the population in the Czech Republic. *Cent Eur J Public Health* 13 (4), 202–207.
- Berlin, I., Oncken, C., 2018. Maternal smoking during pregnancy and negative health outcomes in the offspring. *Nicotine Tob Res* 20 (6), 663–664. <https://doi.org/10.1093/ntr/nty035>.
- Bernhard, D., Rossmann, A., Wick, G., 2005. Metals in cigarette smoke. *IUBMB life* 57, 805–809. <https://doi.org/10.1080/15216540500459667>.
- Chełchowska, M., Ambroszkiewicz, J., Gajewska, J., Jabłońska-Głąb, E., Maciejewski, T. M., Oltarzewski, M., 2016. Hepcidin and iron metabolism in pregnancy: correlation with smoking and birth weight and length. *Biol Trace Elem Res* 173 (1), 14–20. <https://doi.org/10.1007/s12011-016-0621-7>.
- Chen, C.J., Shih, T.S., Chang, H.Y., Yu, H.S., Wu, J.D., Sheu, S.C., Wu, C.E., Chou, T.C., 2008. The total body burden of chromium associated with skin disease and smoking among cement workers. *Sci Total Environ* 391 (1), 76–81. <https://doi.org/10.1016/j.scitotenv.2007.11.011>.
- Chojnacka, K., Mikulewicz, M., 2018. Biomarkers of trace element status. In: Chojnacka, K., Saeid, A. (Eds.), *Recent Advances in Trace Elements*. Wiley, Oxford, pp. 457–467. <https://doi.org/10.1002/9781119133780.ch22>.
- Chojnacka, K., Górecka, H., Górecki, H., 2006. The effect of age, sex, smoking habit and hair color on the composition of hair. *Environ Toxicol Pharmacol* 22 (1), 52–57. <https://doi.org/10.1016/j.etap.2005.11.006>.
- Dongarrà, G., Lombardo, M., Tamburo, E., Varrica, D., Cibella, F., Cuttitta, G., 2011. Concentration and reference interval of trace elements in human hair from students living in Palermo, Sicily (Italy). *Environ Toxicol Pharmacol* 32, 27–34. <https://doi.org/10.1016/j.etap.2011.03.003>.
- Elisia, I., Lam, V., Cho, B., Hay, M., Li, M.Y., Yeung, M., Bu, L., Jia, W., Norton, N., Lam, S., Krystal, G., 2020. The effect of smoking on chronic inflammation, immune function and blood cell composition. *Sci Rep* 10 (1), 19480. <https://doi.org/10.1038/s41598-020-76556-7>.
- Ellingsen, D.G., Thomassen, Y., Rustad, P., Molander, P., Aaseth, J., 2009. The time-trend and the relation between smoking and circulating selenium concentrations in Norway. *J Trace Elem Med Biol* 23 (2), 107–115. <https://doi.org/10.1016/j.jtemb.2009.01.004>.
- Filippini, T., Michalke, B., Grill, P., Malagoli, C., Malavolti, M., Vescovi, L., Sieri, S., Krogh, V., Cherubini, A., Maffei, G., Lucchini, R., Ferrante, M., Vinceti, M., 2017. Determinants of serum manganese levels in an Italian population. *Mol Med Rep* 15, 3340–3349. <https://doi.org/10.3892/mmr.2017.6379>.
- Fraenkel, P.G., 2017. Anemia of inflammation: a review. *Med Clin N* 101 (2), 285–296. <https://doi.org/10.1016/j.mcna.2016.09.005>.
- Fresquez, M.R., Pappas, R.S., Watson, C.H., 2013. Establishment of toxic metal reference range in tobacco from US cigarettes. *J Anal Toxicol* 37 (5), 298–304. <https://doi.org/10.1093/jat/bkt021>.
- Galan, P., Viteri, F.E., Bertrais, S., Czernichow, S., Faure, H., Arnaud, J., Ruffieux, D., Chenal, S., Arnault, N., Favier, A., Roussel, A.M., Hercberg, S., 2005. Serum concentrations of beta-carotene, vitamins C and E, zinc and selenium are influenced by sex, age, diet, smoking status, alcohol consumption and corpulence in a general French adult population. *Eur. J. Clin. Nutr.* 59 (10), 1181–1190. <https://doi.org/10.1038/sj.ejcn.1602230>.
- GBD 2019 Tobacco Collaborators, 2021. Spatial, temporal, and demographic patterns in prevalence of smoking tobacco use and attributable disease burden in 204 countries and territories, 1990–2019: a systematic analysis from the Global Burden of Disease Study 2019. *Lancet* 397 (10292), 2337–2360. [https://doi.org/10.1016/S0140-6736\(21\)01169-7](https://doi.org/10.1016/S0140-6736(21)01169-7).
- Go, M.D., Al-Delaimy, W.K., Schilling, D., Vuylsteke, B., Mehees, S., Spindel, E.R., McEvoy, C.T., 2021. Hair and nail nicotine levels of mothers and their infants as valid biomarkers of exposure to in utero tobacco smoke. *Tob Induc Dis* 19, 100. <https://doi.org/10.18332/tid/143209>.
- Haghani, A., Arpawong, T.E., Kim, J.K., Lewinger, J.P., Finch, C.E., Crimmins, E., 2020. Female vulnerability to the effects of smoking on health outcomes in older people. *PLoS one* 15 (6), e0234015. <https://doi.org/10.1371/journal.pone.0234015>.
- Hamilton, I.M., Gilmore, W.S., Strain, J.J., 2000. Marginal copper deficiency and atherosclerosis. *Biol Trace Elem Res* 78, 179–189.
- Heitland, P., Köster, H.D., 2006. Biomonitoring of 37 trace elements in blood samples from inhabitants of northern Germany by ICP-MS. *J Trace Elem Med Biol* 20, 253–262. <https://doi.org/10.1016/j.jtemb.2006.08.001>.
- Jain, R.B., Choi, Y.S., 2015. Normal reference ranges for and variability in the levels of blood manganese and selenium by gender, age, and race/ethnicity for general U.S. population. *J Trace Elem Med Biol* 30, 142–152. <https://doi.org/10.1016/j.jtemb.2014.12.004>.
- Jasińska-Starzczyńska, M., Szydłowska, I., Mroczek, B., Laszczyńska, M., Chłubek, D., Kemicer-Chmielewska, E., Chelstowski, K., Karakiewicz, B., Cieciewicz, S., Starzczyński, A., 2017. The influence of cigarette smoke exposure on the copper concentration in the serum depending on the use of menopausal hormone therapy. *BioMed Res Int*, 5732380. <https://doi.org/10.1155/2017/5732380>, 2017.
- Kafai, M.R., Ganji, V., 2003. Sex, age, geographical location, smoking, and alcohol consumption influence serum selenium concentrations in the USA: third National Health and Nutrition Examination Survey, 1988–1994. *J Trace Elem Med Biol* 17 (1), 13–18. [https://doi.org/10.1016/S0946-672X\(03\)00040-8](https://doi.org/10.1016/S0946-672X(03)00040-8).

- Khlifi, R., Olmedo, P., Gil, F., Feki-Tounsi, M., Chakroun, A., Rebai, A., Hamza-Chaffai, A., 2013. Blood nickel and chromium levels in association with smoking and occupational exposure among head and neck cancer patients in Tunisia. *Environ Sci Pollut Res* 20 (11), 8282–8294. <https://doi.org/10.1007/s11356-013-1466-7>.
- Kim, K., Melough, M.M., Vance, T.M., Kim, D., Noh, H., Koo, S.I., Chun, O.K., 2019. The relationship between zinc intake and cadmium burden is influenced by smoking status. *Food Chem Toxicol* 125, 210–216. <https://doi.org/10.1016/j.fct.2019.01.004>.
- Kocyyigit, A., Erel, O., Gur, S., 2001. Effects of tobacco smoking on plasma selenium, zinc, copper and iron concentrations and related antioxidative enzyme activities. *Clin Biochem* 34 (8), 629–633. [https://doi.org/10.1016/s0009-9120\(01\)00271-5](https://doi.org/10.1016/s0009-9120(01)00271-5).
- Krivan, V., Schneider, G., Baumann, H., Reus, U., 1994. Multi-element characterization of tobacco smoke condensate. *Fresenius J Anal Chem* 348, 218–225.
- Kulikowska-Karpińska, E., Zdanowicz, M., Gałazyn-Sidorczuk, M., 2017. *Wiad Lek* 70 (4), 697–702.
- Kuo, C.Y., Wong, R.H., Lin, J.Y., Lai, J.C., Lee, H., 2006. Accumulation of chromium and nickel metals in lung tumors from lung cancer patients in Taiwan. *J Toxicol Environ Health A* 69 (14), 1337–1344. <https://doi.org/10.1080/15287390500360398>.
- Lapenna, D., Mezzetti, A., de Gioia, S., Pierdomenico, S.D., Daniele, F., Cuccurullo, F., 1995. Plasma copper and lipid peroxidation in cigarette smokers. *Free Radic Biol Med* 19 (6), 849–852. [https://doi.org/10.1016/0891-5849\(95\)00056-4](https://doi.org/10.1016/0891-5849(95)00056-4).
- Lee, C.H., Goag, E.K., Lee, S.H., Chung, K.S., Jung, J.Y., Park, M.S., Kim, Y.S., Kim, S.K., Chang, J., Song, J.H., 2016. Association of serum ferritin levels with smoking and lung function in the Korean adult population: analysis of the fourth and fifth Korean National Health and Nutrition Examination Survey. *Int J Chron Obstruct Pulmon Dis* 11, 3001–3006. <https://doi.org/10.2147/COPD.S116982>.
- Leifert, J.A., 2008. Anemia and cigarette smoking. *Int J Lab Hematol* 30 (3), 177–184. <https://doi.org/10.1111/j.1751-553X.2008.01067.x>.
- Letsiou, S., Nomikos, T., Panagiotakos, D.B., Pergantis, S.A., Fragopoulou, E., Pitsavos, C., Stefanadis, C., Antonopoulou, S., 2014. Gender-specific distribution of selenium to serum selenoproteins: associations with total selenium levels, age, smoking, body mass index, and physical activity. *BioFactors* 40 (5), 524–535. <https://doi.org/10.1002/biof.1176>.
- Lin, Y.S., Ho, W.C., Caffrey, J.L., Sonawane, B., 2014. Low serum zinc is associated with elevated risk of cadmium nephrotoxicity. *Environ. Res.* 134, 33–38. <https://doi.org/10.1016/j.envres.2014.06.013>.
- Lisboa, T.P., Mimura, A., da Silva, J., de Sousa, R.A., 2020. Chromium levels in tobacco, filter and ash of illicit brands cigarettes marketed in Brazil. *J Anal Toxicol* 44 (5), 514–520. <https://doi.org/10.1093/jat/bkz106>.
- Liu, X., Zhang, Y., Piao, J., Mao, D., Li, Y., Li, W., Yang, L., Yang, X., 2017. Reference values of 14 serum trace elements for pregnant Chinese women: a cross-sectional study in the China nutrition and health survey 2010–2012. *Nutrients* 9, 309. <https://doi.org/10.3390/nu9030309>. PMID: 28335545; PMCID: PMC5372972.
- Lozoff, B., Georgieff, M.K., 2006. Iron deficiency and brain development. *Semin Pediatr Neurol* 13, 158–165.
- Luo, R., Zhuo, X., Ma, D., 2014. Determination of 33 elements in scalp hair samples from inhabitants of a mountain village of Tonglu city, China. *Ecotoxicol Environ Saf* 104, 215–219. <https://doi.org/10.1016/j.ecoenv.2014.03.006>.
- Majewska, U., Piotrowska, M., Sychowska, I., Banas, D., Kubala-Kukus, A., Wudarczyk-Mocko, J., Stabrawa, I., Gózdź, S., 2018. Multielemental analysis of tobacco plant and tobacco products by TXRF. *J Anal Toxicol* 42 (6), 409–416. <https://doi.org/10.1093/jat/bky016>.
- Masilamani, V., AlZahrani, K., Devanesan, S., AlQahtani, H., AlSalhi, M.S., 2016. Smoking induced hemolysis: spectral and microscopic investigations. *Sci Rep* 6, 21095. <https://doi.org/10.1038/srep21095>.
- Massadeh, A., El-Rjoob, A.W., Smadi, H., 2011. Lead, cadmium, copper, zinc, iron, and calcium in human hair as a function of gender, age, smoking, and hair dyeing. *Environ Toxicol Chem* 93 (3), 494–503. <https://doi.org/10.1080/02722248.2010.532797>.
- Meltzer, H.M., Alexander, J., Brantsæter, A.L., Borch-Johnsen, B., Ellingsen, D.G., Thomassen, Y., Holmen, J., Ydersbond, T.A., 2016. The impact of iron status and smoking on blood divalent metal concentrations in Norwegian women in the HUNT2 Study. *J Trace Elem Med Biol* 38, 165–173. <https://doi.org/10.1016/j.jtemb.2016.04.008>.
- Milnerowicz, H., Ścisłalska, M., Dul, M., 2015. Pro-inflammatory effects of metals in persons and animals exposed to tobacco smoke. *J Trace Elem Med Biol* 29, 1–10. <https://doi.org/10.1016/j.jtemb.2014.04.008>.
- Mortada, W.I., Sobh, M.A., El-Defrawy, M.M., 2004. The exposure to cadmium, lead and mercury from smoking and its impact on renal integrity. *Med Sci Monit* 10 (3), 112–116.
- Pappas, R.S., 2011. Toxic elements in tobacco and in cigarette smoke: inflammation and sensitization. *Metallomics* 3 (11), 1181–1198. <https://doi.org/10.1039/c1mt00066g>.
- Pateva, I.B., Kerling, E.H., Reddy, M., Chen, D., Carlson, S.E., Tancabelic, J., 2015. Effect of maternal cigarette smoking on newborn iron stores. *Clin Res Trials* 1 (1), 4–7.
- Pinto, E., Cruz, M., Ramos, P., Santos, A., Almeida, A., 2017. Metals transfer from tobacco to cigarette smoke: Evidences in smokers' lung tissue. *J Hazard Mater* 325, 31–35. <https://doi.org/10.1016/j.jhazmat.2016.11.069>.
- Practice Committee of the American Society for Reproductive Medicine, 2018. Smoking and infertility: a committee opinion. *Fertil. Steril.* 110 (4), 611–618. <https://doi.org/10.1016/j.fertnstert.2018.06.016>.
- Qin, Y., Xu, C., Li, W., Jian, B., Wu, B., Chen, M., Sun, H., Hong, H., 2021. Metal/metalloid levels in hair of Shenzhen residents and the associated influencing factors. *Ecotoxicol Environ Saf* 220, 112375. <https://doi.org/10.1016/j.ecoenv.2021.112375>.
- Rivera-Mancía, S., Colín-Ramírez, E., Montes, S., Cartas-Rosado, R., Vargas-Barrón, J., Vallejo, M., 2017. Dyslipidemia and tobacco smoking synergistically increase serum manganese. *Invest Clin* 58 (3), 238–249.
- Schneider, G., Krivna, V., 1993. Multi-element analysis of tobacco and smoke condensate by instrumental neutron activation analysis and atomic absorption spectrometry. *Int J Environ Anal Chem* 53, 87–100. <https://doi.org/10.1080/03067319308044438>.
- Shakeri, M.T., Nezami, H., Nakhaee, S., Aaseth, J., Mehrpour, O., 2021. Assessing heavy metal burden among cigarette smokers and non-smoking individuals in Iran: cluster Analysis and principal component analysis. *Biol Trace Elem Res* 199 (11), 4036–4044. <https://doi.org/10.1007/s12011-020-02537-6>.
- Skalis, G., Archontakis, S., Thomopoulos, C., Andrianopoulou, I., Papazachou, O., Vamvakou, G., Aznaouridis, K., Katsi, V., Makris, T., 2021. A single-center, prospective, observational study on maternal smoking during pregnancy in Greece: the HELENA study. *Tob Prev Cessation* 7, 16. <https://doi.org/10.18332/tpc/131824>.
- Skalny, A.V., Skalnaya, M.G., Tinkov, A.A., Serebryansky, E.P., Demidov, V.A., Lobanova, Y.N., Grabeklis, A.R., Berezkina, E.S., Gryazeva, I.V., Skalny, A.A., Skalnaya, O.A., Zhivaev, N.G., Nikonov, A.A., 2015. Hair concentration of essential trace elements in adult non-exposed Russian population. *Environ Monit Assess* 187, 677. <https://doi.org/10.1007/s10661-015-4903-x>.
- Slezakova, K., Pereira, M.C., Alvim-Ferraz, M.C., 2009. Influence of tobacco smoke on the elemental composition of indoor particles of different sizes. *Atmos. Environ.* 43, 486–493.
- Song, Y., Elias, V., Loban, A., Scrimgeour, A.G., Ho, E., 2010. Marginal zinc deficiency increases oxidative DNA damage in the prostate after chronic exercise. *Free Radic Biol Med* 48, 82–88.
- Stavriles, J.C., 2006. Lung carcinogenesis: pivotal role of metals in tobacco smoke. *Free Radic Biol Med* 41 (7), 1017–1030. <https://doi.org/10.1016/j.freeradbiomed.2006.06.024>.
- Stefanowicz, F.A., Talwar, D., O'Reilly, D.S., Dickinson, N., Atkinson, J., Hursthouse, A. S., Rankin, J., Duncan, A., 2013. Erythrocyte selenium concentration as a marker of selenium status. *Clin Nutr* 32, 837–842. <https://doi.org/10.1016/j.clnu.2013.01.005>.
- Suadicaní, P., Hein, H.O., Gynzelberg, F., 2012. Serum selenium level and risk of lung cancer mortality: a 16-year follow-up of the Copenhagen Male Study. *Eur Respir J* 39 (6), 1443–1448. <https://doi.org/10.1183/09031936.00102711>.
- Suarez-Varela, M.M., Llopis-González, A., González Albert, V., López-Izquierdo, R., González-Manzano, I., Chaves, J., Biosca, V.H., Martín-Escudero, J.C., 2015. Zinc and smoking habits in the setting of hypertension in a Spanish population. *Hypertens Res* 38 (2), 149–154. <https://doi.org/10.1038/hr.2014.150>.
- Takser, L., Lafond, J., Bouchard, M., St-Amour, G., Mergler, D., 2004. Manganese levels during pregnancy and at birth: relation to environmental factors and smoking in a Southwest Quebec population. *Environ Res* 95 (2), 119–125. <https://doi.org/10.1016/j.envres.2003.11.002>.
- Talhout, R., Schulz, T., Florek, E., van Benthem, J., Wester, P., Opperhuizen, A., 2011. Hazardous compounds in tobacco smoke. *Int J Environ Res Public Health* 8 (2), 613–628. <https://doi.org/10.3390/ijerph8020613>.
- Tungtrongchitr, R., Pongpaew, P., Phonrat, B., Supawan, V., Chanjanakitskul, S., Vudhivai, N., Schelp, F.P., 2002. The effect of cigarette smoking on ceruloplasmin and C3 complement: risk of cardiovascular disease (atherosclerosis). *Asian Pac J Allergy Immunol* 20 (1), 23–28.
- Vinceti, S.R., Filippini, T., 2021. Public health and public law issues for the toxicological risk assessment of chemical mixtures. *Pub Health Tox* 1 (2), 6. <https://doi.org/10.18332/pht/144201>.
- Vinceti, M., Grill, P., Malagoli, C., Filippini, T., Storani, S., Malavolti, M., Michalke, B., 2015. Selenium speciation in human serum and its implications for epidemiologic research: a cross-sectional study. *J Trace Elem Med Biol* 31, 1–10. <https://doi.org/10.1016/j.jtemb.2015.02.001>.
- Wang, F., Zou, Y., Shen, Y., Zhong, Y., Lv, Y., Huang, D., Chen, K., Li, Q., Qing, L., Xia, B., Su, C., Ma, S., Yang, X., 2015. Synergistic impaired effect between smoking and manganese dust exposure on pulmonary ventilation function in Guangxi manganese-exposed workers healthy cohort (GXMEWHC). *PLoS one* 10 (2), e0116558. <https://doi.org/10.1371/journal.pone.0116558>.
- World Health Organization, 2018. WHO Global Report on Trends in Prevalence of Tobacco Smoking 2000–2025, second ed. World Health Organization <https://apps.who.int/iris/bitstream/handle/10665/272694/9789241514170-eng.pdf>.
- World Medical Association, 2013. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 310 (20), 2191–2194. <https://doi.org/10.1001/jama.2013.281053>.