

Hair concentration of essential trace elements in adult non-exposed Russian population

Anatoly V. Skalny · Margarita G. Skalnaya · Alexey A. Tinkov ·
Eugeny P. Serebryansky · Vasily A. Demidov · Yulia N. Lobanova ·
Andrei R. Grabeklis · Elena S. Berezkina · Irina V. Gryazeva · Andrey A. Skalny ·
Oksana A. Skalnaya · Nikolay G. Zhivaev · Alexandr A. Nikonorov

Received: 4 June 2015 / Accepted: 30 September 2015 / Published online: 7 October 2015
© Springer International Publishing Switzerland 2015

Abstract Appropriate reference values of hair trace element content are required for correct interpretation of biomonitoring data. The primary objective of the current study was to estimate the reference values of selected essential trace elements in hair of adult Russian population. Involved in current investigation were 7256 occupationally non-exposed adults aged from 20 to 60 years and living in the European part of Russia. Occipital hair essential metal and metalloid (Co, Cr, Cu, Fe, Mn, Se, V, Zn) content was estimated using inductively coupled plasma mass spectrometry. The reference ranges were calculated in accordance with the International Union of Pure and Applied Chemistry (IUPAC) recommendations. Women were characterized

by 55, 18, 58, and 7 % higher values of hair Co, Cu, Mn, and Zn content as compared to the values observed in men. At the same time, hair Cr, Fe, Se, and V concentration in men significantly exceeded the respective female values by 65, 13, 20, and 56 %. Consequently, the reference ranges of essential hair trace elements content should be separately calculated for both men and women. The obtained reference ranges for hair Co, Cr, Cu, Fe, Mn, Se, V, and Zn in men were 0.11–0.67, 0.007–0.045, 10.4–22.6, 11.1–40.5, 0.24–1.05, 0.089–0.480, 0.014–0.083, and 125.7–262.8 $\mu\text{g/g}$, respectively. The respective values estimated for women were 0.06–0.40, 0.011–0.085, 12.1–44.5, 8.9–25.6, 0.32–2.05, 0.094–0.504, 0.010–0.056, and 140.0–315.1 $\mu\text{g/g}$

A. V. Skalny · A. A. Tinkov (✉) · A. R. Grabeklis ·
E. S. Berezkina
Laboratory of biotechnology and Applied Bioelementology,
Yaroslavl State University, Sovetskaya st., 14, Yaroslavl 150000,
Russia
e-mail: tinkov.a.a@gmail.com

A. V. Skalny
All-Russian Research Institute of Medicinal and Aromatic Plants
(VILAR), Grina St., 7, Moscow 117216, Russia

A. V. Skalny · M. G. Skalnaya · A. A. Tinkov ·
E. P. Serebryansky · V. A. Demidov · Y. N. Lobanova ·
A. R. Grabeklis · E. S. Berezkina · I. V. Gryazeva ·
A. A. Skalny · O. A. Skalnaya
Russian Society of Trace Elements in Medicine, ANO “Centre for
Biotic Medicine”, Zemlyanoy Val St. 46, Moscow 105064, Russia

A. A. Tinkov · A. A. Nikonorov
Department of Biochemistry, Orenburg State Medical University,
Sovetskaya St., 6, Orenburg 460000, Russia

A. A. Skalny
Federal State Scientific Institution “Institute of Toxicology”,
Federal Medico-Biological Agency, Bekhtereva str. 1, St.
Petersburg 192019, Russia

N. G. Zhivaev
Department of Pedagogy and Educational Psychology, Yaroslavl
State University, Sovetskaya st., 14, Yaroslavl 150000, Russia

g. The reference ranges for hair Co (0.07–0.50), Cr (0.009–0.073), Cu (11.8–29.2), Fe (9.6–31.5), Mn (0.29–1.76), Se (0.093–0.482), V (0.011–0.069), and Zn (134.7–301.9) content ($\mu\text{g/g}$) in the general cohort were also calculated.

Keywords Hair · Reference values · Coverage interval · Trace elements · Metals

Introduction

Essential trace elements play a significant role in cellular functioning and are indispensable for life (Fraga 2005). Cobalt, copper, iron, manganese, and zinc are cofactors for multiple enzymatic systems (Underwood 1977). One of the key roles of chromium (Anderson 2000) and vanadium (Mukherjee et al. 2004) in the living organism is modulation of insulin signaling and regulation of carbohydrate homeostasis. Due to involvement in numerous metabolic pathways, clinical signs of trace element deficiency are diverse (Mertz 1981). The use of refined diets has resulted in an increased risk of trace element deficiency. In particular, it has been shown that consumption of refined sugar and white flour results in chromium deficiency (Tuormaa 2000). Despite being essential, a number of trace elements may also exert toxic properties (Goldhaber 2003). In particular, excessive dietary intake or occupational exposure to several essential metals and metalloids may result in metal toxicity through induction of oxidative stress and inflammation (Valko et al. 2005). Consequently, effective health management requires adequate assessment of trace elements status (Goldhaber 2003).

Hair has been widely used for chemical analysis for a long time (Kosanovic and Jokanovic 2011). The main advantages of the use of hair as a sample for trace element analysis are (i) simplicity and non-invasiveness of sampling, (ii) high mineralization of the sample, and (iii) irreversible incorporation of trace elements into hair matrix (Chojnacka et al. 2010). Consequently, hair may be used as a more stable indicator of trace element status in comparison with blood and urine, where the level of metals is rather low and strictly regulated by homeostatic mechanisms (Razagui and Ghribi 2005). Hair analysis may also reflect environmental exposure of human beings to certain metals (Bencko 1995), being a potential tool in biomonitoring and environmental risk assessment (Tamburo et al.

2011). The most common methods used for analysis of inorganic chemicals in human biosamples include atomic absorption spectrometry, neutron activation analysis, and inductively coupled plasma optical emission spectrophotometry and mass spectrometry (Esteban and Castaño 2009). In addition, inductively coupled plasma mass spectrometry (ICP-MS) is supposed to be the best technique for multielement analysis of human hair (Bass et al. 2001).

However, a number of limitations for using hair as a substrate of trace element status analysis exist. In particular, hair metal content may vary due to a number of factors like gender, age, occupation, geographical location, climate, ethnicity, and living and dietary habits (Gordon 1985; Christensen 1995; Aftanas et al. 2011). In order to increase the significance of hair trace element analysis for correct interpretation of biomonitoring data, the appropriate reference values should be used (Hoet et al. 2013). The most recent systematic review has estimated that data on reference values of hair are inconsistent (Mikulewicz et al. 2013). Earlier, using the International Union of Pure and Applied Chemistry (IUPAC) recommendations, we have estimated reference values of hair toxic trace elements (Al, As, Be, Bi, Cd, Hg, Li, Ni, Pb, Sn, and Sr) content in 5908 adult volunteers living in Central Russia (Skalny et al. 2015). However, no study has yet calculated reference values of essential trace elements content in hair using a large sample. Therefore, the primary objective of this research project was to estimate the reference values of selected essential trace elements in the hair of adult Russian population.

Materials and methods

Cohort characterization

A total of 7256 occupationally non-exposed adults aged from 20 to 60 years and living in the European part of Russia were involved in this study. Men represented 29 % of the studied cohort ($n=2095$), and 71 % ($n=5161$) of the examinees were women. The mean age of the men and women in the sample was 37.46 ± 10.49 and 38.36 ± 10.70 , respectively. No significant difference was observed between age values for the men and women in the sample. We obtained informed consent forms from all individual participants included in the study. The analysis was performed in accordance with

the principles of the Declaration of Helsinki for studies involving humans. The protocol of investigation was approved by the Local Ethics Committee.

The following exclusion criteria were used for the participant selection procedure: (i) occupational involvement in heavy industry, (ii) current or former smoking status, (iii) metal implants, (iv) endocrine disorders, (v) surgical and traumatic diseases, (vi) acute inflammatory and infectious diseases, (vii) pregnancy and lactation, (viii) vegetarian diet, (ix) use of synthetic chemical hair dyes, and (ix) frequent alcohol consumption.

Sampling and mineralization

All participants washed hair on the day of the sampling. Occipital scalp hair was collected for analysis using precleaned stainless steel scissors. Only proximal parts of strands were collected in order to diminish possible external contamination of samples. The average weight of hair was 0.05–0.1 g. The preparation of the samples included washing procedures and microwave digestion. Briefly, the hair samples were washed with acetone, rinsed thrice with deionized water (Zhao et al. 2012), and subsequently dried on air at 60 °C. Using acetone as an agent for washing allows removal of dirt and dust from hair samples without removal of exogenously bound trace elements (Morton et al. 2002). Afterwards, 0.05 g of hair samples were introduced into Teflon tubes with concentrated HNO₃. Microwave digestion was performed for 20 min at 170–180 °C using Berghof Speedwave 4 (Berghof Products & Instruments, Germany) system as indicated earlier (Aydin et al. 2010). Afterwards, the obtained solutions were added to a total volume of 15 ml with distilled deionized water. The resulting solution was used for the subsequent analysis.

Analysis, standard solutions, and reference materials

Hair essential trace element content (Co, Cr, Cu, Fe, Mn, Se, V, Zn) was analyzed by means of ICP-MS with NexION 300D (PerkinElmer Inc., Shelton, CT 06484, USA) using Dynamic Reaction Cell (DRC) technology for the removal of major polyatomic interferences (Pick et al. 2010). The apparatus was also equipped with ESI SC-2 DX4 autosampler (Elemental Scientific Inc., Omaha, NE 68122, USA).

The analyzer's preparation was performed using manufacturer's guidelines. Standard solutions with a final concentration of 0.5, 5, 10, and 50 µg/l of metals prepared from Universal Data Acquisition Standards Kit (PerkinElmer Inc., Shelton, CT 06484, USA) by dilution with distilled deionized water and acidification with 1 % HNO₃ were used for calibration. Yttrium-89 isotope was used for internal online standardization. Internal standard containing 10 µg/l yttrium was prepared from Yttrium (Y) Pure Single-Element Standard (PerkinElmer Inc., Shelton, CT 06484, USA) on a matrix containing 8 % 1-butanol (Merck KGaA), 0.8 % Triton X-100 (Sigma-Aldrich, Co.), 0.02 % tetramethylammonium hydroxide (Alfa-Aesar, Ward Hill, MA 01835 USA), and 0.02 % ethylenediaminetetraacetic acid (Sigma-Aldrich, Co).

The certified reference material of human hair GBW09101 (Shanghai Institute of Nuclear Research, Shanghai, China) was used for laboratory quality control. All preparative and analytical procedures were similar to analysis of the obtained hair samples. We identified the highest recovery rates for Fe, Se, V, and Zn (Table 1). The estimated recovery rates for Cr, Co, Cu, and Mg were lower than 95 %. The values for these metals were within the limits estimated as allowable by the manufacturer of the reference materials.

Statistical analysis

Statistical treatment of the data was performed using Statistica 10 software (StatSoft Inc., Tulsa, Oklahoma, USA). The descriptive statistics of hair essential metal content included the estimation of the median, 5 and 95 percentile boundaries, mean values, and respective standard deviations for each variable. Data normality was assessed using Shapiro-Wilk test. The distribution of the data on the hair trace elements content was not Gaussian and required log transformation for further analysis. Group comparisons were performed using one-way ANOVA with Fisher's LSD post hoc test. The difference between the group values was considered significant at $p < 0.05$.

The estimation of reference values of the essential trace element content in hair was based on the calculation of the 0.95 coverage intervals with 0.95 confidence intervals for the upper and lower limits. The analysis was conducted in accordance with the International

Table 1 Comparison of the certified and the measured values of trace elements concentration in the reference material of human hair GBW09101

Element	Certified value, $\mu\text{g/g}$	Lower limit, $\mu\text{g/g}$	Upper limit, $\mu\text{g/g}$	Obtained value, $\mu\text{g/g}$	Recovery rate, %	BEC, ppb	LoD, ppb
Co	0.153	0.138	0.168	0.142 \pm 0.018	93	0.0021	0.0013
Cr	8.74	7.77	9.71	7.91 \pm 0.59	91	0.022	0.023
Cu	33.6	31.3	35.9	32.46 \pm 2.31	96	0.0235	0.0035
Fe	160	144	176	157 \pm 24	98	0.11	0.08
Mn	3.83	3.44	4.22	3.50 \pm 0.35	91	0.03	0.01
Se	0.59	0.55	0.63	0.58 \pm 0.08	98	0.026	0.013
V	0.089	0.089	0.089	0.087 \pm 0.01	98	0.0002	0.0003
Zn	191	175	207	188 \pm 7	98	0.22	0.07

The obtained values are presented as mean \pm standard deviation ($n=72$)

BEC background equivalent concentration, LoD limit of detection, ppb part per billion

Union of Pure and Applied Chemistry (IUPAC) recommendations, using a multistep procedure (Poulsen et al. 1997). First, log transformation and exclusion of the outliers were performed. Second, we estimated the upper and the lower limits of the coverage interval between 2.5 and 97.5 percentiles. Determination of coverage uncertainty (δ) was performed for every cohort. Finally, the obtained data were back-transformed to the initial units of hair metal concentration ($\mu\text{g/g}$ dry weight).

Results and discussion

The data on the hair essential trace elements content indicate that the examined women had 55, 18, 58, and 7 % higher values of hair Co, Cu, Mg, and Zn content as compared to the values observed in the men (Table 2). At the same time, hair Cr, Fe, Se, and V concentration in the men in the sample significantly exceeded the respective values in the women by 65, 13, 20, and 56 %. The obtained data on gender differences in the hair essential trace elements status are partially in agreement with the existing scholarship. In particular, it has been demonstrated that women are characterized by lower values of hair Fe content in comparison to men (Deeming and Weber 1978). Such a difference may be associated with the sex-related difference in blood (serum) iron content (Borel et al. 1991). Our study confirms the previous research pointing out that women have higher hair Zn content (Meng 1998). Among other elements, previous research has found significantly higher values of hair Cu in women in comparison with men (Bales et al. 1990).

Barbieri and the coauthors have also observed higher hair Mg levels in girls in comparison to boys (Barbieri et al. 2011). This finding was contradicted by another study, which failed to detect significant sex-related differences in the hair Mg content in children (Perrone et al. 1996). Our own data contradict the results of a previous study that failed to find sex-related differences in hair Cr levels (Wolfsperger et al. 1994). An earlier investigation involving northern Finns demonstrated higher values of hair Cr content in men (Soininen et al. 2003). Stupar and the coauthors (2007) also observed lower hair Cr levels in healthy women in comparison to the values observed in men. The finding of higher Se levels in men is in agreement with data on different levels of Se intake (Fairweather-Tait et al. 2011). At the same time, a recent study failed to reveal gender differences in hair Se content in healthy population and persons with non-alcoholic steatohepatitis (Pan and Huang 2013). Our research data conform to the results of our previous study indicating higher hair V levels in male students in comparison to females (Zaitseva et al. 2015). The observed gender difference in Cr values is caused by higher intake of the metal in men (Anderson and Kozlovsky 1985). At the same time, higher hair concentration of Mn and Co in women may result from higher intensity of intestinal absorption (Christensen et al. 1993; Finley et al. 1994). Hypothetically, increased V content in men may also have dietary origin. However, the exact mechanism is still to be estimated.

To conclude, trace elements levels in hair significantly differ for men and for women, which necessitates using gender-specific reference ranges. We present the reference limits for hair essential trace elements

Table 2 Descriptive statistics of hair essential trace elements content in adults

Element ($\mu\text{g/g}$)	Males ($n=2095$)					Females ($n=5161$)					Total ($n=7256$)				
	Median	P_5	P_{95}	Mean	SD	Median	P_5	P_{95}	Mean	SD	Median	P_5	P_{95}	Mean	SD
Cr	0.38	0.07	1.27	0.51	0.51	0.23	0.04	0.76	0.29	0.278	0.26	0.04	0.95	0.35	0.374
Co	0.011	0.004	0.066	0.02	0.028	0.017	0.005	0.138	0.037	0.0627	0.015	0.004	0.118	0.032	0.0556
Cu	11.7	8.2	24.4	13.2	5.4	13.9	8.3	40.6	17.5	11.09	13.0	8.3	36.3	16.3	9.99
Fe	13.7	6.4	61.8	20.7	19.4	12.1	6.0	33.8	15.1	9.86	12.5	6.1	41.8	16.7	13.59
Mn	0.38	0.12	1.76	0.59	0.61	0.60	0.16	3.60	1.09	1.426	0.52	0.14	3.15	0.95	1.27
Se	0.335	0.106	0.727	0.38	0.285	0.279	0.050	0.697	0.333	0.2976	0.296	0.061	0.711	0.347	0.295
V	0.039	0.005	0.219	0.065	0.078	0.025	0.005	0.128	0.041	0.0457	0.028	0.005	0.155	0.048	0.058
Zn	177.1	100.5	310.8	187.5	63.9	190.7	114.7	375.9	208.3	81.08	186.4	109.9	356.9	202.3	77.09

P_5 , P_{95} 5 and 95 percentile boundaries, SD standard deviation

Table 3 Coverage intervals of hair essential trace elements content in adult occupationally non-exposed Russian population calculated in accordance with IUPAC recommendations (0.95 level of expectation in coverage interval; 0.95 level of confidence of the coverage uncertainty)

Element ($\mu\text{g/g}$)	Men ($n=2095$)	Women ($n=5161$)	General cohort ($n=7256$)
Cr	0.11–0.67	0.06–0.40	0.07–0.50
Co	0.007–0.045	0.011–0.085	0.009–0.073
Cu	10.4–22.6	12.1–44.5	11.8–29.2
Fe	11.1–40.5	8.9–25.6	9.6–31.5
Mn	0.24–1.05	0.32–2.05	0.29–1.76
Se	0.089–0.480	0.094–0.504	0.093–0.482
V	0.014–0.083	0.010–0.056	0.011–0.069
Zn	125.7–262.8	140.0–315.1	134.7–301.9
δ	0.007	0.004	0.004

δ coverage uncertainty

content in men, women, and general cohort in Table 3. Our data are partially in agreement with the previously reported reference ranges obtained in Sweden (Rodushkin and Axelsson 2000), France (Goullé et al. 2005), and Poland (Chojnacka et al. 2010) (Table 4). At the same time, some disagreement between our and previously published values exists. In particular, our experimental data on hair Cr and Co content were in agreement with the respective values

in the French and Swedish studies, but were significantly lower than those found in the subjects living in Poland. The upper reference limit for the hair Cu concentration was significantly lower in our study than in the previous works. The obtained reference intervals for hair Fe in adults generally conform to earlier indications. However, the values of hair Se content obtained by Rodushkin and Axelsson (2000) and Goullé et al. (2005) were significantly higher than the ones estimated in the present study. At the same time, our reference ranges for hair Zn content were nearly similar to those in Chojnacka et al. (2010), a study of Polish students. The estimated reference values for hair Mg and V are inconsistent with and contradict the previous data. Table 4 represents characteristics of the studied cohorts that may have been responsible for the observed disagreement. In particular, all studies involved persons of various ages. Previous research has demonstrated that age significantly affects the trace element status of an individual (Gordon 1985). Moreover, even though all studies were performed in continental Europe, the geographical location of the studied cohorts was different. In particular, Russia, Poland, Sweden, and France are characterized by different climatic and ecological conditions. Finally, statistical treatment and calculation of the reference ranges also varied and may have changed the resulting reference ranges.

Table 4 Reference ranges of hair trace element content in adult population in different European countries

Reference	Rodushkin and Axelsson 2000	Goullé et al. 2005	Chojnacka et al. 2010
Location	Sweden	France	Poland
Method	ICP-MS	ICP-MS	ICP-MS
Number	112	45	117
Age	1–76	n.d.	21–22
Gender	M/F	M/F	M/F
Statistics	n.d.	5–95 Percentiles	10–90 Percentiles
Cr, $\mu\text{g/g}$	0.046–0.527	0.11–0.52	0.91–1.53
Co, $\mu\text{g/g}$	0.002–0.063	0.004–0.14	0.775–0.985
Cu, $\mu\text{g/g}$	8.5–96	9.0–61.3	8.51–34.97
Fe, $\mu\text{g/g}$	4.9–23	n.d.	16.9–29.6
Mn, $\mu\text{g/g}$	0.080–2.41	0.016–0.57	0.459–1.046
Se, $\mu\text{g/g}$	0.48–1.84	0.37–1.37	n.d.
V, $\mu\text{g/g}$	0.005–0.134	0.001–0.051	0.641–1.182
Zn, $\mu\text{g/g}$	68–198	129–209	140–371

ICP-MS inductively coupled plasma mass spectrometry, M males, F females, n.d. not defined

Conclusions

Generally, our data indicate gender differences in hair trace elements content. Consequently, the use of gender-specific reference ranges is strongly recommended in biomonitoring and assessment studies. The present reference values obtained by ICP-MS using DRC technology based on a large sample of examinees are generally in agreement with the existing scholarship and may be used for biomonitoring.

Acknowledgments The current research is supported by the Russian Ministry of Education and Science within project No. 2014/258-544.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

References

Aftanas, L. I., Berezkina, E. S., Bonitenko, E. Y., Varenik, V. I., Grabeklis, A. R., Demidov, V. A., Kiselev, M. F., Nechiporenko, S. P., Nikolaev, V. A., Skalny, A. V., & Skalnaya, M. G. (2011). Element status of population of Russia. Part II. In A. V. Skalny & M. F. Kiselev (Eds.), *Element status of population of Central Federal Region*. Saint Petersburg: ELBI-SPb.

Anderson, R. A. (2000). Chromium in the prevention and control of diabetes. *Diabetes Metabolism*, 26(1), 22–7.

Anderson, R. A., & Kozlovsky, A. S. (1985). Chromium intake, absorption and excretion of subjects consuming self-selected diets. *The American Journal of Clinical Nutrition*, 41(6), 1177–1183.

Aydin, I., Yuksel, U., Guzel, R., Ziyadanogullari, B., & Aydin, F. (2010). Determination of trace elements in Turkish wines by ICP-OES and HG-ICP-OES. *Atomic Spectroscopy*, 31(2), 67.

Bales, C. W., Freeland-Graves, J. H., Askey, S., Behmardi, F., Pobocik, R. S., Fickel, J. J., & Greenlee, P. (1990). Zinc, magnesium, copper, and protein concentrations in human saliva: age-and sex-related differences. *The American Journal of Clinical Nutrition*, 51(3), 462–469.

Barbieri, F. L., Courmil, A., Sarkis, J. E. S., Bénéfice, E., & Gardon, J. (2011). Hair trace elements concentration to describe polymetallic mining waste exposure in Bolivian

Altiplano. *Biological Trace Element Research*, 139(1), 10–23.

Bass, D. A., Hickok, D., Quig, D., & Urek, K. (2001). Trace element analysis in hair: factors determining accuracy, precision, and reliability. *Alternative Medicine Review*, 6(5), 472–481.

Bencko, V. (1995). Use of human hair as a biomarker in the assessment of exposure to pollutants in occupational and environmental settings. *Toxicology*, 101(1), 29–39.

Borel, M. J., Smith, S. M., Derr, J., & Beard, J. L. (1991). Day-to-day variation in iron-status indices in healthy men and women. *The American Journal of Clinical Nutrition*, 54(4), 729–735.

Chojnacka, K., Zielińska, A., Górecka, H., Dobrzański, Z., & Górecki, H. (2010). Reference values for hair minerals of Polish students. *Environmental Toxicology and Pharmacology*, 29(3), 314–319.

Christensen, J. M. (1995). Human exposure to toxic metals: factors influencing interpretation of biomonitoring results. *Science of the Total Environment*, 166(1), 89–135.

Christensen, J. M., Poulsen, O. M., & Thomsen, M. (1993). A short-term cross-over study on oral administration of soluble and insoluble cobalt compounds: sex differences in biological levels. *International Archives of Occupational and Environmental Health*, 65(4), 233–240.

Deeming, S. B., & Weber, C. W. (1978). Hair analysis of trace minerals in human subjects as influenced by age, sex, and contraceptive drugs. *The American Journal of Clinical Nutrition*, 31(7), 1175–1180.

Esteban, M., & Castaño, A. (2009). Non-invasive matrices in human biomonitoring: a review. *Environment International*, 35(2), 438–449.

Fairweather-Tait, S. J., Bao, Y., Broadley, M. R., Collings, R., Ford, D., Hesketh, J. E., & Hurst, R. (2011). Selenium in human health and disease. *Antioxidants & Redox Signaling*, 14(7), 1337–1383.

Finley, J. W., Johnson, P. E., & Johnson, L. K. (1994). Sex affects manganese absorption and retention by humans from a diet adequate in manganese. *The American Journal of Clinical Nutrition*, 60(6), 949–955.

Fraga, C. G. (2005). Relevance, essentiality and toxicity of trace elements in human health. *Molecular Aspects of Medicine*, 26(4), 235–244.

Goldhaber, S. B. (2003). Trace element risk assessment: essentiality vs. toxicity. *Regulatory Toxicology and Pharmacology*, 38(2), 232–242.

Gordon, G. F. (1985). Sex and age related differences in trace element concentrations in hair. *Science of the Total Environment*, 42(1), 133–147.

Goullé, J. P., Mahieu, L., Castermant, J., Neveu, N., Bonneau, L., Lainé, G., & Lacroix, C. (2005). Metal and metalloid multi-elementary ICP-MS validation in whole blood, plasma, urine and hair: reference values. *Forensic Science International*, 153(1), 39–44.

Hoet, P., Jacquerye, C., Deumer, G., Lison, D., & Haufroid, V. (2013). Reference values and upper reference limits for 26 trace elements in the urine of adults living in Belgium. *Clinical Chemistry and Laboratory Medicine*, 51(4), 839–849.

Kosanovic, M., & Jokanovic, M. (2011). Quantitative analysis of toxic and essential elements in human hair. Clinical validity

- of results. *Environmental monitoring and assessment*, 174(1–4), 635–643.
- Meng, Z. (1998). Age- and sex-related differences in zinc and lead levels in human hair. *Biological Trace Element Research*, 61(1), 79–87.
- Mertz, W. (1981). The essential trace elements. *Science*, 213(4514), 1332–1338.
- Mikulewicz, M., Chojnacka, K., Gedrange, T., & Górecki, H. (2013). Reference values of elements in human hair: a systematic review. *Environmental Toxicology and Pharmacology*, 36(3), 1077–1086.
- Morton, J., Carolan, V. A., & Gardiner, P. H. (2002). Removal of exogenously bound elements from human hair by various washing procedures and determination by inductively coupled plasma mass spectrometry. *Analytica Chimica Acta*, 455(1), 23–34.
- Mukherjee, B., Patra, B., Mahapatra, S., Banerjee, P., Tiwari, A., & Chatterjee, M. (2004). Vanadium—an element of atypical biological significance. *Toxicology Letters*, 150(2), 135–143.
- Pan, D., & Huang, H. (2013). Hair selenium levels in hepatic steatosis patients. *Biological Trace Element Research*, 152(3), 305–309.
- Perrone, L., Moro, R., Caroli, M., Di Toro, R., & Gialanella, G. (1996). Trace elements in hair of healthy children sampled by age and sex. *Biological Trace Element Research*, 51(1), 71–76.
- Pick, D., Leiterer, M., & Einax, J. W. (2010). Reduction of polyatomic interferences in biological material using dynamic reaction cell ICP-MS. *Microchemical Journal*, 95(2), 315–319.
- Poulsen, O. M., Holst, E., & Christensen, J. M. (1997). Calculation and application of coverage intervals for biological reference values (technical report). *Pure and Applied Chemistry*, 69(7), 1601–1612.
- 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., & Nikonov, A. A. (2015). Reference values of hair toxic trace elements content in occupationally non-exposed Russian population. *Environmental Toxicology and Pharmacology*, 40(1), 18–21.
- Razagui, I. B. A., & Ghribi, I. (2005). Maternal and neonatal scalp hair concentrations of zinc, copper, cadmium, and lead. *Biological Trace Element Research*, 106(1), 1–27.
- Rodushkin, I., & Axelsson, M. D. (2000). Application of double focusing sector field ICP-MS for multielemental characterization of human hair and nails Part II. A study of the inhabitants of northern Sweden. *Science of the Total Environment*, 262(1), 21–36.
- Soininen, L., Mussalo-Rauhamaa, H., & Lehto, J. (2003). Hair chromium concentration of northern Finns. *International Journal of Circumpolar Health*, 62(3), 276–283.
- Štupar, J., Vrtovec, M., & Dolinšek, F. (2007). Longitudinal hair chromium profiles of elderly subjects with normal glucose tolerance and type 2 diabetes mellitus. *Metabolism*, 56(1), 94–104.
- Tamburo, E., Dongarrà, G., Varrica, D., & D'Andrea, D. (2011). Trace elements in hair of urban schoolboys: a diagnostic tool in environmental risk assessment. *Geophysical Research Abstracts*, 13, 1157.
- Tuormaa, T. E. (2000). Chromium, selenium and copper and other trace minerals in health and reproduction. *Journal of Orthomolecular Medicine*, 15(3), 145–156.
- Underwood, E. J. (1977). *Trace elements in human and animal nutrition* (4th ed.). New York: Academic.
- Valko, M., Morris, H., & Cronin, M. T. D. (2005). Metals, toxicity and oxidative stress. *Current Medicinal Chemistry*, 12(10), 1161–1208.
- Wolfsperger, M., Hauser, G., Gößler, W., & Schlagenhaufen, C. (1994). Heavy metals in human hair samples from Austria and Italy: influence of sex and smoking habits. *Science of the Total Environment*, 156(3), 235–242.
- Zaitseva, I. P., Skalny, A. A., Tinkov, A. A., Berezkina, E. S., Grabeklis, A. R., & Skalny, A. V. (2015). The influence of physical activity on hair toxic and essential trace element content in male and female students. *Biological Trace Element Research*, 163, 58–66.
- Zhao, L. J., Ren, T., & Zhong, R. G. (2012). Determination of lead in human hair by high resolution continuum source graphite furnace atomic absorption spectrometry with microwave digestion and solid sampling. *Analytical Letters*, 45(16), 2467–2481.