Journal of Trace Elements in Medicine and Biology 51 (2019) 115–122

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

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

Epidemiology

The influence of the place of residence, smoking and alcohol consumption on bone mineral content in the facial skeleton

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ARTICLE INFO

Keywords:
Environmental factors
Facial skeleton
Trace elements
Tobacco smoking
Alcohol consumption

ABSTRACT

Background: Environmental factors exert their influence on the living organism throughout ontogeny. More and more often, researchers find correlations between specific environmental factors and the so-called diseases of affluence. Deficits and excess of essential elements also leave their mark on the skeleton.

Aim: To investigate the influence of alcohol consumption, tobacco smoking and place of residence, according to sex and calendar age, on the concentrations of micro-, macro- and toxic elements in human facial bones.

Material & methods: Patients undergoing surgical treatment were examined for the mineral content in the collected bone material. The bone contents of the following elements were determined: Ca, K, Mg, Na, P, Fe, Zn, Mo, Ba, Mn, Li, Be, Co, B, Sr, Cr, Pb, Cd, Ni, and Al, depending on the type of facial bone, sex, calendar age, alcohol consumption, tobacco smoking and place of residence.

Results: Sex and alcohol consumption showed the highest degree of correlation with the content of the minerals included in the study. Alcohol drinking was found to exert the strongest influence on women's bodies, the highest number of statistically significant correlations was demonstrated between the content of minerals in the examined bones and alcohol drinking in women. Other factors included in the analysis had a different impact on men and women, the concentrations of elements included in the study differed depending on age, tobacco smoking and place of residence.

Conclusions: The observed differences in the element mineral composition of the human facial skeleton may be explained by developmental specifics and functional adaptation. However, general biological characteristics (sex, age), environmental factors (place of residence), as well as smoking and alcohol use may exert significant influence on the concentrations of micro-, macro- and toxic elements in particular regions of the human skeleton. The impact of environmental factors is a very complex phenomenon, which may be stronger or more subtle, leaving its mark on the bone structure. The environmental factors included in the analysis had a different influence on men than women.

1. Introduction

Epidemiological data and animal studies indicate that environmental factors, particularly those associated with exposure to heavy metals, contribute to the risk of developing many diseases [1]. Exposure to environmental factors, especially in industrial areas, may be linked to the increased prevalence of numerous diseases of affluence and autoimmune disorders [2], Parkinson's disease [3,4], diabetes [5,6], cardiovascular disease [7,8], hypertension [6], asthma [9], and even obesity [10]. Simultaneously, the vast multitude of different factors influencing the accumulation of minerals in the human body make it impossible to examine the influence of individual environmental factor and describe it in detail.

The osseous tissue (in the maxillofacial area also) is actively and functionally adapted to static and dynamic loads acting upon it. These adaptations are manifested as changes in bone density, primarily in the content of minerals, and in the concentration and composition of elements taking part in the mineral segment construction. At the same

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https://doi.org/10.1016/j.jtemb.2018.10.012
Received 1 August 2018; Received in revised form 7 October 2018; Accepted 9 October 2018

time, bone mineral content is associated with environmental conditions, diet, geographical range, occupational exposure and health status of populations [11].

Environmental exposures are also to some extent conditioned by culture. Some behaviours are perceived differently depending on sex. For instance, excessive consumption of alcohol by women is frowned upon due to their maternal role. The same applies to smoking, although regrettably, today smoking is socially acceptable in both sexes. Furthermore, environmental factors change over time in response to cultural trends, e.g. tattoos, exercise or eco-friendly lifestyles. Nevertheless, all environmental factors exert their influence with varying strength and direction, depending on the surrounding circumstances, throughout the person's lifetime, leading to physiological and biochemical changes in the body.

Few quantitative studies on the elemental composition of particular functionally and anatomically defined sections of the temporal bone have been published to date due to the difficulty in obtaining material from the facial skeleton of patients undergoing surgical procedures [12]. Therefore, the aim of this study was to determine the levels of selected macroelements (Ca, K, Mg, Na, P), microelements (Fe, Zn, Mo, Ba, Mn, Li, Be, Co, B, Sr, Cr), and toxic elements (Pb, Cd, Ni, Al) in the bones of the human facial skeleton collected as by-products in the course of surgical treatment. Material was collected from: the frontal bone *(os frontale)*, zygomatic bone *(os zygomaticum)*, maxilla (*os maxillare)* and mandible *(mandibula)*. Samples of the frontal bone (which is part of the neurocranium) were collected in very small quantities and the majority of the analyses are based on samples of facial bones only.

In this paper, we explore the influence of general biological characteristics (sex, age), environmental factors (place of residence), as well as the influence of smoking and alcohol use on the concentrations of selected elements.

2. Material and methods

The study group included men (n = 81) aged 16–79 years (37.4 ± 14.9) and women (n = 53) aged 16–82 years (38.4 ± 17.2). In the study, we used material collected from the frontal bone (2.2%, n = 3), zygomatic bone (16.4%, n = 22), maxilla (33.9%, n = 45) and mandible (47.8%, n = 64) of patients (n = 134) hospitalised at the Department of Maxillofacial Surgery of the Hospital of the Ministry of Interior in Kielce (Poland) in 2017. Bone material was obtained from patients presenting with disorders classified into the following groups: (I) cysts – affecting the jaws (maxilla or mandible) which required surgical intervention (excision). Due to the intraosseous location of these lesions, during the surgical access it was necessary to remove a fragment of the bone tissue surrounding the cyst. These bone fragments, which had to be removed to access and remove the cyst, and which would normally be discarded (disposed of), were collected for the purposes of biochemical research. (II) tumours – this group included odontogenic tumours (odontomas and ameloblastomas). Bone material was collected both during exploratory surgery (extraction of samples for histopathological examination), where it was necessary to remove a bone fragment to access the tumour, and in tumour resection procedures in which bone fragments were excised to enable access to the tumour and its removal. These fragments were then used in biochemical analysis. (III) impacted teeth – for the most part, these were upper and lower wisdom teeth covered by bone, which due to their positioning (blockage from other teeth), orthodontic indications or recurrent inflammation related to eruption problems presented indications for extraction. To access the impacted tooth, it was necessary to remove a fragment of the adjacent bone. Such fragments, which in standard medical practice would be disposed of, were instead collected for the purposes of biochemical research. (IV) trauma – in the case of fractures involving the facial skeleton (maxilla, mandible, zygomatic bone) which required treatment by open surgery (reduction and stable osteosynthesis), after accessing the site of the fracture and finding (particularly with comminuted fractures) small, free-floating splinters not covered by periosteum, which did not qualify for fixation, these were curetted and removed from the surgical incision wound. Such fragments, if not useful for depositing, were used for research purposes. (V) sinus disorders – in the case of maxillary sinus disorders requiring treatment by open surgery (in the majority of cases, the technique used was the Caldwell-Luc procedure), it was necessary to make an osteotomy opening in the canine fossa region in order to enter the maxillary sinus. The fragments which would normally be disposed of were collected for research purposes. (VI) gangrenous teeth – in patients who, due to comorbidities, needed to be hospitalised to have teeth removed. Following multiple tooth extractions, osteoplasty was performed on the alveolar process of the maxilla or mandible, to remove sharp, protruding bone fragments of the interdental and inter-root septa. The removed bone fragments, which would normally be disposed of, were sent for biochemical tests. (VII) necrosis – in cases of MRONJ (medication-related osteonecrosis of the jaw) – necrosis of the jawbone in response to medication – primarily bisphosphonates. In these patients, surgery involved the removal of sequestra and inflammatory granulation tissue surrounding the sequestra. Most of the removed bone, with the granulation tissue, was sent for histopathological examination, and small fragments of dead bone were used in biochemical tests. (VIII) inflammation – in cases of osteomyelitis, where bone decompression (decortication) was performed, involving the removal of compact bone fragments affected by the bone inflammation process, the removed fragments were sent in part for histopathological examination, and small fragments were used in biochemical tests.

Once the patient was admitted to the hospital, a standard blood panel was ordered. On the basis of the patient's history, physical examination and analysis of diagnostic imaging results, it was decided whether the patient qualified for surgery. Following anaesthesia consultation, procedures were performed under local or general anaesthesia. All patients underwent surgery using standard surgical procedure – corresponding to disease classification, test results (laboratory, microbiological, histopathological, imaging) and the patient's general status. There was not a single case where bone tissue would be collected without indications applicable to the patient's condition. The scope of bone removal corresponded to the scope of the surgical procedure – the bone fragment collected was never larger than necessary to conduct the procedure, and therefore the patients were never exposed to any risk arising from this study. The bone collected for biochemical tests was redundant waste (cysts, tumours, impacted teeth, trauma, sinus disorders, dental gangrene), which would otherwise have been subject to disposal. In cases of necrosis or inflammation, the removed bone tissue for the most part was sent for histopathological examination, and only small fragments were used in biochemical tests – again without any threat for the patients undergoing treatment.

2.1. Bone element content analysis

Laboratory tests for the purposes of biochemical analyses were performed at the Department of Biochemistry and Medical Chemistry, Pomeranian Medical University in Szczecin, Poland.

After the removal of remaining ligaments and muscles, the samples were stored frozen at −20 °C until analysis. Compact bone was used in the analysis. The samples were thawed at room temperature and dried overnight at 70 °C to constant weight after cleaning of all adherent tissue. The bones were ground into powder in a porcelain mortar. The weight of each bone sample donated for research was no less than 0.1 g. The samples were transferred to clean polypropylene tubes. 1 mL of 65% HNO_3 (Suprapur, Merck) was added to each vial and each sample was allowed 30 min' pre-reaction time in the clean hood. After the pre-reaction time, 1 mL of non-stabilized 30% H_2O_2 solution (Suprapur, Merck) was added to each vial. Once the addition of all reagents was complete, the samples were placed in special Teflon vessels and mineralized using the microwave digestion system MARS 5, CEM (USA)

for 35 min at 180 °C (15 min ramp time to 180 °C and 20 min hold time at 180 °C). At the end of digestion, all samples were removed from the microwave and allowed to cool to room temperature. In the clean hood, samples were transferred to acid-washed 15 mL polypropylene sample tubes.

A further 100-fold dilution was performed prior to ICP-OES measurement. Samples were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES, ICAP 7400 Duo, Thermo Scientific) equipped with a concentric nebulizer and a cyclonic spray chamber to determine levels of selected macroelements (Ca, Mg, Na, K, P), microelements (Fe, Zn, Co, Li, Be, Cr, Mn, Sr, Mo, Ba, B) and toxic elements (Pb, Cd, Ni, Al). The volume of 100 µL was taken from each digest. The samples were spiked with an internal standard to provide a final concentration of 0.5 mg/L Ytrium, 1 ml of 1% Triton (Triton X-100, Sigma) and diluted to the final volume of 10 mL with 0.075% nitric acid (Suprapur, Merck). Samples were stored in a monitored refrigerator at a nominal temperature of 8 °C until analysis.

Blank samples were prepared by adding concentrated nitric acid (50 µL) to tubes without sample and subsequently diluted in the same manner as described above.

Multi-element calibration standards (ICP multi-element standard solution IV, Merck for Ca, K, Mg, Na, Zn, Co, Li, Cr, Mn, Ni, Ba, B, Sr, Fe, Al, Cd, Pb; ICP multi-element standard solution XVI, Merck for Be, Mo and ICAP 6000 Multi-Element Test Solution, Thermo Scientific for P) were prepared with different concentrations of inorganic elements in the same manner as in blanks and samples. Deionized water (Direct Q UV, Millipore, approximately 18.0 MΩ) was used in the preparation of all solutions.

2.2. Validation of analytical proceedings

The analytical procedure was validated by the analysis of standard reference material with known concentrations: NIST SRM 1486 Bone Meal. Concentrations of minerals in the reference material provided by the manufacturer and our own determinations are shown in Table 1.

Table 1
The analysis of NIST-SRM 1486 (Bone Meal) and Seronorm™ Trace Elements Serum L-1 LOT 0903106 by ICP-OES.

Chemical elements The wavelengths (nm)	Bone Meal SRM NIST 1486 [mg/kg d m.]		Recovery (%)
	Certified	Measured	
Ca [315.887]	265.8 ± 2.4	261.7 ± 22.4	100.49
K [766.490]	0.412 ± 0.004	0.410 ± 8.6	100.48
Mg [279.553]	4.66 ± 0.17	4.50 ± 0.3	95.74
Na [589.592]	5.0 ± 0.00	5.00 ± 0.00	100.00
P [178.766]	123.0 ± 1.9	125.0 ± 0.3	101.626
Fe [259.940]	0.099 ± 0.008	0.1100 ± 0.00	111.00
Zn [206.200]	0.147 ± 0.016	0.150 ± 0.001	102.72
Mn [670.784]	0.001 ± 0.00	0.001 ± 0.000	100.00
Sr [205.56]	0.264 ± 0.007	0.260 ± 0.005	100.30
Pb[228.802]	0.001335 ± 0.00014	0.0013 ± 0.000	97.37
Cd [221.647]	0.00003 ± 0.00	0.000029 ± 0.00	96.66
Al [396.152]	< 0.001	0.00098	98.00
Seronorm™ Trace Elements Serum L-1 LOT 0903106 [µg/L].			
Mo [233.527]	0.700	0.720-	102.86
Ba [257.610]	125.00	125.00-	100.00
Li [313.042]	5535	5655	102.168
Be [238.892]	1.80 [ng/L]	1.84 [ng/L]	102.22
Co [208.959]	1.20	1.25	104.16
B[421.552]	145.00	140.00	103.57
Cr [220.353]	1.50	1.45	96.60
Ni [396.152]	5.80	5.75	99.14

2.3. Statistical analysis

The normality of mineral concentrations in bone samples (consistency with normal distribution) was tested by the Shapiro-Wilk test. Data distribution differed from normal distribution and that is why the non-parametric Kruskal-Wallis ANOVA test by ranks was employed in further analysis. The analysis of the relationships between the concentrations of elements included in the study and categorised data was based on the Spearman rank correlation test. Student's *t*-test was used to compare element concentrations by sex.

To examine the strength of influence of general biological data, environmental factors, smoking and alcohol use on the concentrations of analysed elements, backward stepwise multiple regression analysis was performed. The regression model was built separately for each element as the variable dependent on: calendar age, place of residence, tobacco smoking and alcohol consumption. The backward stepwise multiple regression analysis demonstrates causal relationships among variables in an equation. Such a statistical model starts the equation with all the variables included in the analysis. In the subsequent stages (steps), the variables which are insignificantly related are eliminated out of the equation, and each time the statistical significance of the remaining variables is tested. The final result (adjusted R^2) is not always higher from those in earlier stages. However, it reveals the mutual dependencies of the variables included in the equation. The application of this technique allows for a comprehensive assessment of the intensity of the impact of the explanatory variables included in the model upon the concentrations of analysed elements in bone samples.

The statistical significance level was set at $p \leq 0.05$. Statistical analysis of the results was conducted using the Statistica 12.0 PL software.

3. Results

The concentrations of trace elements in the examined bones obtained from patients undergoing surgical treatment, both men and women, are presented in Tables 2 and 3. The comparison of total elements concentrations in examined bone material in men and women is presented in Table 4.

The analysis of elements concentrations in facial bone specimens collected from men revealed that Cr content differs depending on the origin of the sample $H(3, n = 81) = 9.23$, $p = 0.03$ (Table 2). Cr content in the maxilla was significantly higher ($p = 0.02$) than that in the zygomatic bone. Also in the case of Al statistically significant differences were found depending on the collection site of the material $H(3, n = 81) = 16.61$, $p = 0.00$ (Table 2). Significantly higher concentrations of this element were observed in the maxilla compared to the zygomatic bone and the mandible ($p = 0.0035$), and in the maxilla to the zygomatic bone ($p = 0.004$) as well as the maxilla to the mandible ($p = 0.004$).

In bones collected from women, depending on the collection site, statistically significant differences were found for Pb, Cr, Ni and B (respectively for H(2), n = 53: 8.55, p = 0.01; 10.01, p = 0.01; 8.62, p = 0.01 and 7.68, p = 0.02). The content of Pb in the mandible was observed to be more than twice as high as that in the maxilla and the zygomatic bone ($p = 0.01$). Statistically significant differences were also found in the concentrations of Cr, which in the mandible amounted to more than double the level found in the zygomatic bones ($p = 0.01$). The difference between Ni concentration in the mandible samples and that in the maxilla samples was statistically significant, too ($p = 0.01$). In turn, the content of B maintained at comparable level in the maxilla and the mandible, but differed in a statistically significant manner from that in the zygomatic bone $H(2, n = 53) = 7.68$, $p = 0.02$ (Table 3). The B content in the zygomatic bone was also significantly different from that in the maxilla ($p = 0.02$).

Analysing bone elements concentrations according to sex, statistically significant differences were found only in the concentrations of

Table 2
The concentrations of trace elements (in mg/kg dry weight) and differences between them in the examined bone material collected from the men included in the study (x, arithmetic mean; SD, standard deviation; frontal bone *(os frontale)*; zygomatic bone *(os zygomaticum)*; maxilla *(maxilla)*; mandible *(mandibula)*; Kruskal-Wallis ANOVA test by ranks; p – level of significance).

	frontal bone (n = 3)		zygomatic bone (n = 19)		maxilla (n = 23)		mandible (n = 36)		Kruskal-Wallis test H (3, n = 81)	p
	x ± SD	min-max	x ± SD	min-max	x ± SD	min-max	x ± SD	min-max		
Ca	2952.86 ± 2664.54	1263.71–6024.51	5579.38 ± 6033.23	527.00–24322.10	6058.10 ± 5675.87	492.17–25423.32	5516.15 ± 5404.55	87.21–21515.28	1.63	0.65
K	26.09 ± 2.89	23.22–29.01	58.08 ± 22.73	19.08–96.77	43.34 ± 26.66	13.32–90.82	48.72 ± 21.70	17.50–100.08	7.72	0.05
Mg	47.83 ± 41.34	21.93–.95.50	83.17 ± 85.04	6.79–343.66	79.59 ± 69.17	7.39–317.59	74.95 ± 66.89	1.75–276.49	0.91	0.82
Na	80.94 ± 72.05	26.76–162.71	158.01 ± 156.87	37.71–659.81	181.38 ± 122.32	25.56–434.35	156.61 ± 122.12	22.21–482.21	3.30	0.35
P	1528.41 ± 1417.63	617.42–3161.72	2656.69 ± 2913.54	226.47–11448.08	2988.78 ± 2888.89	207.72–12524.77	2665.10 ± 2631.92	40.05–10999.72	1.54	0.67
Fe	0.47 ± 0.18	0.31–0.66	0.59 ± 0.56	0.21–2.72	0.67 ± 1.05	0.22–5.41	0.81 ± 1.50	0.17–8.27	0.06	0.99
Zn	1.825 ± 1.12	1.11–3.11	3.35 ± 3.54	0.41–12.73	2.95 ± 2.27	0.41–10.74	2.66 ± 2.39	0.11–11.47	1.00	0.80
Mo	2.08 ± 0.28	1.75–2.27	1.80 ± 0.32	1.21–2.31	1.97 ± 0.18	1.61–2.31	0.78 ± 0.31	1.16–2.18	6.79	0.08
Ba	1.04 ± 0.24	0.87–1.32	0.80 ± 0.26	0.46–1.45	0.98 ± 0.45	0.59–2.71	0.82 ± 0.29	0.48–1.93	5.93	0.12
Mn	0.01 ± 0.0.004	0.005–0.012	0.01 ± 0.002	0.004–0.02	0.01 ± 0.01	0.001–0.03	0.01 ± 0.01	0.001–0.05	4.81	0.19
Li	1.63 ± 0.26	1.40–1.99	1.91 ± 0.40	1.24–2.39	1.74 ± 0.44	1.07–2.59	1.63 ± 0.60	0.01–2.24	3.48	0.32
Be	0.08 ± 0.004	0.08–0.08	0.10 ± 0.03	0.05–0.14	0.08 ± 0.02	0.04–0.12	0.08 ± 0.03	0.02–0.14	6.88	0.08
Co	0.96 ± 0.14	0.87–1.12	0.90 ± 0.22	0.33–1.16	0.98 ± 0.28	0.64–2.00	0.92 ± 0.16	0.67–1.23	0.63	0.89
B	0.57 ± 0.25	0.35–0.85	0.32 ± 0.19	0.00–0.78	0.39 ± 0.11	0.08–0.53	0.35 ± 0.17	0.01–0.74	5.02	0.17
Sr	1.17 ± 0.75	0.33–1.77	2.23 ± 2.09	0.42–7.39	1.88 ± 1.30	0.40–6.40	1.75 ± 1.32	0.31–6.22	1.27	0.74
Cr	0.36 ± 0.02	0.34–0.38	**0.17 ± 0.17**	0.00–0.51	**0.35 ± 0.11**	0.02–0.51	0.25 ± 0.19	0.004–0.58	**9.23**	**0.03**
Pb	0.49 ± 0.30	0.18–0.77	0.67 ± 0.43	0.03–1.88	1.34 ± 1.8	0.0004–5.13	0.89 ± 1.14	0.005–4.54	0.93	0.82
Cd	0.10 ± 0.01	0.10–0.11	0.09 ± 0.02	0.06–0.14	0.10 ± 0.02	0.08–0.15	0.09 ± 0.02	0.06–0.14	6.21	0.10
Ni	0.63 ± 0.05	0.60–0.68	0.50 ± 0.16	0.31–0.91	0.62 ± 0.18	0.34–0.92	0.54 ± 0.19	0.30–0.91	5.81	0.12
Al	0.39 ± 0.11	0.32–0.51	**0.27 ± 0.13**	0.13–0.64	**0.39 ± 0.11**	0.14–0.60	**0.28 ± 0.12**	0.12–0.73	**16.61**	**0.00**

essential elements like Na (p = 0.00), K (p = 0.01), Mn (p = 0.00) and toxic elements like Cr (p = 0.01) and Cd (p = 0.02). Concentrations of these elements in women's bones were higher than in men's bones (Table 4).

3.1. Correlation analysis

Correlation analysis was employed in order to determine the influence of factors including: place of residence, sex, calendar age, tobacco smoking and alcohol use on the concentrations of elements in the bone tissue of participating patients.

3.2. Place of residence vs. bone element content

Out of n = 81 examined men, more than 43% reported that they live in a rural area; 34.6% in a large city, and 22.2% in a smaller town. Correlation analysis demonstrated a statistically significant but rather weak relationship between the concentrations of Li, K and Al in the bone material tested and the place of residence (Rs = 0.3, p = 0.003; Rs = 0.2, p = 0.04; and Rs = -0.3, p = 0.02, respectively). Such correlation was also confirmed by regression analysis. In regression analysis, the place of residence of the participating men was found to be significant in explaining the variation in Li, K and Al. For the remaining elements, their bone concentrations were independent of the place of living (Tables 5 and 6). Out of n = 53 examined women, 32.1% reported that they live in a rural area, 30.2% in a large city, and 37.7% in a smaller town. Correlation analysis showed that the concentrations of: Cd, Pb, P, Li, Be, Na, Mg, Ca, Sr, Zn and Mo in the tested bone material were dependent on the place where the women lived (Rs = −0.4, p = 0.001; Rs = 0.3, p = 0.05; Rs = −0.4 p = 0.01; Rs = −0.4, p = 0.004; Rs = −0.3, p = 0.04; Rs = −0.4, p = 0.004; Rs = −0.4, p = 0.001; Rs = −0.4, p = 0.01; Rs = −0.3, p = 0.02; Rs = −0.3, p = 0.02; Rs = −0.3, p = 0.01; and Rs = −0.3, p = 0.03, respectively). In regression analysis, the factor of "residence" was statistically significant in explaining the variability of Cd, P, Li, Be, Na, Mg, Ca, Zn and Mo levels (Tables 5 and 6).

3.3. Participants' calendar age vs. bone element content

Correlation analysis demonstrated a weak relationship between the concentrations of Cd, Cr, Al and Mo in the analysed bone material and the women's calendar age (Rs = −0.3, p = 0.043; Rs = −0.3, p = 0.047; Rs = −0.3, p = 0.012; and Rs = −0.3, p = 0.012, respectively). In regression analysis "participant's age" was statistically significant in explaining the variability of Al and Mo levels (Table 6). Among the men included in the study, correlation analysis did not reveal any associations between age and the concentrations of studied elements.

3.4. Alcohol consumption vs. bone element content

As many as 79% of studied men admitted that they consumed alcohol (either often or at a frequency that may be regarded as social/regular drinking), and 21% described themselves as non-drinkers. Correlation analysis showed that alcohol consumption among men correlated weakly with concentrations of Pb, Li and Al in the examined bone material (Rs = −0.4, p = 0.001; Rs = 0.4, p = 0.001; and Rs = −0.3, p = 0.012, respectively). Regression analysis confirmed that the factor "alcohol consumption" was statistically significant in explaining the variability of the bone content of Li. For the remaining elements, their bone concentrations were independent of the factor. The frequency of alcohol consumption among the women included in the study was almost the reverse of that of men. Nearly 70% of the studied women declared they did not drink alcohol at all. Only 30.2% declared that they drank socially or very rarely. Among the women, levels of Co, Ba, Li, K, Sr, Mn, Ni, B, Mo in the collected bone material correlated

Table 3
The concentrations of trace elements (in mg/kg dry weight) and differences between them in the examined bone material collected from the women included in the study (x, arithmetic mean; SD, standard deviation; zygomatic bone *(os zygomaticum);* maxilla (*maxilla);* mandible *(mandibula)*; Kruskal-Wallis ANOVA test by ranks; p – level of significance).

	zygomatic bone (n = 3)		maxilla (n = 22)		mandible (n = 28)		Kruskal-Wallis test H (2, n = 53)	p
	x ± SD	min-max	x ± SD	min-max	x ± SD	min-max		
Ca	11455.64 ± 6225.05	4575.60-16698.45	5737.73 ± 5052.87	947.67-17357.62	6786.39 ± 6587.35	560.35-23103.64	2.54	0.28
K	74.29 ± 14.94	57.04-83.46	65.96 ± 28.57	3.11-96.39	55.44 ± 33.30	12.35-107.76	1.47	0.48
Mg	155.83 ± 64.01	87.36-214.16	78.99 ± 66.02	13.21-229.08	87.56 ± 78.83	7.20-284.12	3.04	0.22
Na	333.61 ± 179.42	137.32-489.15	237.76 ± 104.90	48.74-445.62	225.21 ± 149.26	27.98-566.81	1.48	0.48
P	5473.87 ± 3153.43	1977.23-8102.10	2757.35 ± 2473.97	432.57-8479.71	3150.24 ± 3003.08	268.93-10661.42	2.41	0.30
Fe	0.74 ± 0.34	0.45-1.11	0.56 ± 0.27	0.24-1.17	0.61 ± 0.55	0.26-2.76	2.38	0.30
Zn	5.46 ± 2.95	2.08-7.51	2.99 ± 2.33	0.53-7.88	3.28 ± 2.71	0.34-10.74	2.00	0.37
Mo	1.77 ± 0.37	1.35-2.01	1.83 ± 0.26	1.26-2.31	1.89 ± 0.27	1.25-2.28	1.91	0.38
Ba	0.74 ± 0.21	0.54-0.96	0.77 ± 0.26	0.48-1.71	1.54 ± 3.75	0.54-20-64	2.19	0.33
Mn	0.01 ± 0.003	0.01-0.01	0.02 ± 0.01	0.004-0.06	0.02 ± 0.03	0.002-0.14	3.46	0.18
Li	1.86 ± 0.66	1.10-2.35	1.68 ± 0.40	1.07-2.27	1.74 ± 0.53	0.66-2.67	0.53	0.77
Be	0.11 ± 0.01	0.10-0.12	0.09 ± 0.02	0.04-0.13	0.08 ± 0.04	0.02-0.16	2.14	0.34
Co	0.97 ± 0.12	0.85-1.08	1.02 ± 0.18	0.68-1.27	0.96 ± 0.23	0.48-1.31	1.16	0.56
B	**0.04 ± 0.04**	0.01-0.08	**0.40 ± 0.20**	0.09-0.89	**0.37 ± 0.17**	0.02-0.76	**7.68**	**0.02**
Sr	4.07 ± 1.80	2.16-5.74	1.70 ± 1.21	0.34-5.01	2.22 ± 2.14	0.20-7.62	3.76	0.15
Cr	**0.15 ± 0.08**	0.05-0.22	0.32 ± 0.09	0.02-0.46	**0.36 ± 0.07**	0.22-0.52	**10.01**	**0.01**
Pb	0.50 ± 0.21	0.35-0.74	**0.67 ± 0.96**	0.02-4.54	**1.40 ± 1.22**	0.06-4.48	**8.55**	**0.01**
Cd	0.10 ± 0.04	0.07-0.14	0.10 ± 0.02	0.07-0.13	0.11 ± 0.04	0.05-0.24	0.43	0.81
Ni	0.40 ± 0.07	0.33-0.46	**0.47 ± 0.17**	0.31-1.11	**0.59 ± 0.19**	0.35-1.00	**8.62**	**0.01**
Al	0.27 ± 0.05	0.22-0.33	0.33 ± 0.13	0.18-0.60	0.35 ± 0.12	0.14-0.67	1.41	0.49

Table 4
The comparison of total mineral concentrations (in mg/kg dry mass) in examined bone material for men and women (x, arithmetic mean; SD, standard deviation; Student *t*-test; p – level of significance.

	Men (n = 81)		Women (n = 53)		p
	x ± SD	min-max	x ± SD	min-max	
Ca	5589.93 ± 5507.45	87.21-25423.32	6615.39 ± 6002.13	560.35-23103.64	0.31
K	48.55 ± 23.79	13.32-100.08	60.88 ± 30.84	3.12-107.76	**0.01***
Mg	77.19 ± 70.63	1.75-343.66	87.86 ± 73.79	7.20-31.48	0.40
Na	161.17 ± 129.14	22.21-659.81	236.56 ± 133.65	27.98-566.81	**0.00***
P	2712.94 ± 2695.88	40.05-12524.77	3118.68 ± 2812.95	268.93-10661.42	0.40
Fe	0.71 ± 1.17	0.17-8.27	0.59 ± 0.44	0.24-2.76	0.51
Zn	2.87 ± 2.62	0.11-12.73	3.28 ± 2.58	0.34-10.74	0.38
Mo	1.85 ± 0.27	1.16-2.31	1.86 ± 0.27	1.25-2.31	0.88
Ba	0.87 ± 0.34	0.46-2.71	1.18 ± 2.74	0.48-20.64	0.32
Mn	0.01 ± 0.01	0.001-0.05	0.02 ± 0.03	0.002-0.14	**0.00***
Li	1.73 ± 0.51	0.009-2.59	1.72 ± 0.48	0.66-2.67	0.94
Be	0.09 ± 0.03	0.02-0.14	0.08 ± 0.03	0.02-0.16	0.94
Co	0.93 ± 0.21	0.33-2.00	0.98 ± 0.21	0.49-1.31	0.15
B	0.36 ± 0.17	0.00-0.85	0.37 ± 0.20	0.01-0.23	0.91
Sr	1.88 ± 1.51	0.31-7.39	2.11 ± 1.84	0.20-7.62	0.43
Cr	0.26 ± 0.17	0.00-0.58	0.33 ± 0.09	0.02-0.52	**0.01***
Pb	0.95 ± 1.13	0.001-5.13	1.05 ± 1.14	0.02-4.54	0.64
Cd	0.09 ± 0.02	0.06-0.15	0.11 ± 0.03	0.05-0.24	**0.02***
Ni	0.56 ± 0.18	0.30-0.92	0.53 ± 0.19	0.31-1.11	0.37
Al	0.31 ± 0.13	0.12-0.73	0.33 ± 0.12	0.14-0.67	0.40

weakly or moderately with alcohol consumption (Rs = −0.4, p = 0.010; Rs = 0.4, p = 0.003; Rs = 0.3, p = 0.030; Rs = −0.5, p = 0.000; Rs = 0.3, p = 0.045; Rs = −0.3, p = 0.012; Rs = 0.4, p = 0.007; Rs = −0.3, p = 0.027; and Rs = 0.3, p = 0.015, respectively). Regression analysis demonstrated that "alcohol consumption" as an explanatory variable had statistical significance in explaining

Table 5
Environmental factors and self-reported tobacco smoking and alcohol consumption.

	Men (n = 81)	Women (n = 53)
Place of residence		
small town	18 (22.2%)	20 (37.7%)
city	28 (34.6%)	16 (30.2%)
rural	35 (43.2%)	17 (32.1%)
alcohol consumption	17 (21.0%)	37 (69.8%)
no	64 (79.0%)	16 (30.2%)
yes (socially)		
tobacco smoking	41 (50.6%)	48 (90.6%)
no	40 (49.4%)	5 (9.4%)
yes		

Table 6
Summary of findings from the analysis of the influence of tobacco smoking, alcohol consumption, calendar age and place of residence on the levels of analyzed minerals (stepwise regression analysis). Statistically significant results marked * for men, and # for women, p ≤ 0.05.

	Tobacco smoking	Alcohol consumption	Age	Place of residence
Ca				#
K	*	#		*#
Mg				#
Na				#
P				#
Zn				#
Mo	*	#	#	#
Ba		#		
Mn	*	#		
Li		*		*#
Be	*#	#		#
Co	*	#		#
B	#			
Cr	*#		*	
Pb	*			
Cd				#
Ni	*	#		
Al	*		#	*

No statistically significant conclusions came from the Fe and Sr analysis.

variations in the levels of Co, Ba, K, Mn, Ni and Mo in the women's bones (Table 6).

3.5. Smoking vs. bone element content

Close to a half of the studied men admitted to *habitual* tobacco smoking (49.4%). The remainder were non-smokers (50.6%). Correlation analysis demonstrated that tobacco smoking among the men correlated weakly or moderately with the levels of Co, Ba, Be, K, Cr, Ni, Al and Mo ($Rs = 0.2$ $p = 0.04$; $Rs = -0.3$ $p = 0.01$; $Rs = 0.5$, $p = 0.00$; $Rs = 0.4$, $p = 0.001$; $Rs = -0.5$, $p = 0.000$; $Rs = -0.3$, $p = 0.002$; $Rs = 0.3$, $p = 0.003$; and $Rs = -0.6$, $p = 0.000$, respectively).

In case of women, correlation analysis showed that the concentrations of Cr and B correlated weakly or moderately with tobacco smoking ($Rs = -0.4$ $p = 0.005$ and $Rs = -0.3$ $p = 0.23$, respectively). The influence of smoking on the levels of analysed elements was also confirmed by regression analysis. Among both the men and women studied, elements which demonstrated statistically significant correlation with tobacco smoking were also found to have a statistically significant influence on explaining their variation in regression analysis (Table 6).

4. Discussion

According to Lalonde's report, the health of populations is influenced by the following four groups of factors (the so-called "health fields"): human biology, lifestyle, environment and healthcare organization [13]. In turn, the authors of Poland's National Health Program (1996–2005) expressed the opinion that 50–60% of the health of the population is determined by lifestyle, approx. 20% – by environmental factors, approx. 20% – by human biology, with healthcare organization as the least important factor, accounting for 10–15% of the influence [14].

The influence of sex on element concentrations in human bone was analyzed in a number of studies [15–19]. Significant sex differences were noted in the concentrations of Pb [15,17], Cr and Mn [18], as well as Ca, Mg, P, Sr and Fe [16]. One study demonstrated no gender-related differences in the contents of all analyzed elements [19].

In our study, the content of Cr and Al in the men's maxilla was higher than in the zygomatic bone. In bone samples collected from women, Pb content was found to be statistically significantly more than twice as high in the mandible than in the maxilla, and moreover the content of Cr and Ni was statistically significantly more than twice as high in the mandible than in the zygomatic bone, however the B content was relatively similar in the zygomatic bone, maxilla, and mandible. In our research, by analysing elemental concentrations in bones according to the patients' sex, we have demonstrated significant differences in the concentrations of essential elements: Na, K, Mn and toxic elements: Cr and Cd. Concentrations of these elements in women's bones were higher than in men's bones.

The place of residence may be a variable that affects human bone metabolism and the concentration of elements in bone. In our study this factor turned out to have a weak but significant impact on the concentrations of Li, K and Al in the studied bone material. In regression analysis, the place of living of the participating men was found to be significant in explaining the variation in Li, K and Al. For the remaining elements, their concentrations in the men's bones were independent of the place of residence. Statistical analyses demonstrated that the levels of Cd, Pb, P, Li, Be, Na, Mg, Ca, Sr, Zn and Mo were dependent on the place of residence of the studied women. Moreover, in regression analysis, the factor of the "place of residence" featured significantly in explaining the variation in 9 out of all the elements included in the analysis. According to other studies, the place of living reflects the exposure of the human body to environmental pollution, and therefore mineral concentrations in the bone [17,18,20]. Malara et al. detected significantly lower concentrations of Pb, Cd, Cr, Cu, Mn and Zn in the bones of inhabitants of less polluted areas compared to the material collected from inhabitants of polluted areas [18]. Kubaszewski et al. found a significant correlation between the bone concentration of Ni and place of living [20]. Lanocha-Arendarczyk et al. demonstrated that among many analyzed elements, only Se concentration in bone depended on geographical location [17].

Age is a variable with a major impact on disease prevalence. There have been studies investigating the influence of age on the content of elements in human bones [16,17,20,21,19]. They demonstrated age-related alterations in the contents of Ca, Mg and P [16,19], Pb [20], F^- [17], and other trace elements [19]. Our study, too, confirms the influence of calendar age on the concentrations of Cd, Cr, Al and Mo in the analysed bone material collected from women.

Alcohol consumption and tobacco smoking, belonging to the group of lifestyle factors, have significant impact on human health worldwide. Approximately 4% of the global burden of disease is attributable to alcohol, which is responsible for as much death and disability globally as tobacco and hypertension [22]. The European WHO region has the highest levels of alcohol consumption, resulting in the highest prevalence of alcohol dependence and alcohol-use disorders of all the WHO regions [23]. Alcohol is causally associated with more than 60 different medical conditions, including malignant neoplasms (mouth and oropharynx cancers, oesophageal cancer, liver cancer, breast cancer), neuropsychiatric disorders (unipolar depressive disorders, epilepsy, alcohol dependence), diabetes mellitus, cardiovascular disorders (ischaemic heart disease, haemorrhagic stroke, ischaemic stroke) and gastrointestinal diseases (cirrhosis of the liver) [22]. This seems to be important due to identifying a relationship between alcohol consumption and the accumulation of elements, notably Pb, Ni and Al which are well-known environmental factors in the development of neurodegenerative, autoimmune, and cardiovascular disorders [1]. Several studies review the effects of alcohol on bone [24–26]. By altering the organization of cell membranes, ethanol may non-selectively disrupt a multitude of regulatory pathways in bone cells [26]. However, when trying to assess the effects of alcohol on bone, the following variables also need to be taken into consideration: age, skeletal site evaluated, duration, and pattern of drinking [26].

Cigarette smoking is the single most important cause of preventable disease and premature mortality in high income countries [27]. In 2015, smoking was responsible for 1 in 10 deaths worldwide, killing more than 6 million people [28]. WHO estimates that in 2015 about 27% of Poland's population (aged 15 years and over) smoked tobacco (32.6% men and 21.9% women) [29]. Cigarette smoke contains more than 7000 chemicals, including numerous toxic and carcinogenic compounds to which the smoker is exposed [27]. Cigarette smoking causes a broad spectrum of immediate, intermediate and long-term adverse health effects [27]. Among the long-term health effects of smoking are diseases that constitute the major causes of death in middle and high-income countries: coronary heart disease, cancer, and chronic obstructive pulmonary disease (COPD). Tobacco smoking also negatively affects bone health, resulting in increased risk of hip fractures and low bone density in postmenopausal women [27]. In our study, nearly half of the male participants admitted to smoking (49.4%). Tobacco smoking was found to be positively correlated with the levels of: Co, Ba, Be, K, Cr, Ni, Al and Mo. The influence of smoking on the levels of analysed elements was also confirmed by regression analysis. Moreover, the smoking habit among the studied men in regression analysis was statistically significant in explaining the variation of elemental concentrations, namely Co, Be, K, Cr, Mn, Ni, Al, Mo and Pb. The influence of tobacco smoking on the content of elements in human bone was investigated in different studies [15,30,31,17]. The findings have been ambiguous to date, with some studies demonstrating a significantly higher concentration of Pb in smokers' bones [30,31,17], a markedly higher concentration of Cd in smokers' bones [17], and others showing insignificant differences in the concentration of Cd between

smokers' and non-smokers' bones [30,31], or no effect of smoking on the content of Pb, Sr, P, Na and Mg [15]. In our study the factor of tobacco smoking turned out to have a weak but significant impact on the concentrations of Pb, Li and Al in the bones collected from the male participants. For the remaining elements, their bone concentrations were independent of the factor. In turn, in the studied women the concentrations of Co, Ba, Li, K, Sr, Mn, Ni, B, Mo in the bone material revealed correlations with alcohol consumption.

It should be noted that our study has some limitations, which mainly concern the lack of information about systemic diseases of the patients from whom face bone samples were taken for multi-element analysis. In patients referred to the department of maxillofacial surgery, no detailed medical examinations are performed, except those that are necessary to perform the surgical procedure, i.e. those that allow to assess the patient's condition for surgery. No additional information was collected from patients for the purpose of our study. It is known, however, that none of the patients had osteoporosis which could significantly affect the bone mineral metabolism. In addition, it can be concluded that patients in this department were in good general health, because otherwise they would not be eligible for surgery. It should be noted, however, that gathering homogeneous groups of patients, for example, those qualified for abscess incision procedure and suffering from hypertension, in sufficient numbers for statistical analysis, would be very difficult.

Another limitation of our study is the lack of detailed information on the amount and frequency of alcohol and/or cigarettes consumed, which does not allow for a more detailed analysis and demonstration of the impact of alcohol drinking/cigarette smoking intensity on the concentration of elements in bones.

5. Conclusions

The observed differences in the element composition found in this particular region of the human skeleton may be explained by developmental specifics and functional adaptation. At the same time, general biological characteristics (sex, age), environmental factors (place of residence), as well as smoking and alcohol use exert a significant influence on the levels of some elements in the human facial skeleton. The impact of the environmental factors included in the analysis was different in men and women.

Ethical considerations

All participants signed informed consent agreements to take part in biochemical research which was conducted concurrently with necessary surgical procedures. None of the patients underwent additional invasive procedures, other than the surgical treatment. The collected biological material which was used in the bone element analysis would otherwise have been disposed of by the hospital.

Conflict of interest statement

The authors declare that they have no conflict of interest regarding the publication of this paper.

Acknowledgments

Our sincere thanks go out to the Patients who were involved in this study. Despite their pain and suffering, they gave their consent so that biological material collected in the course of their treatment at the Maxillofacial Surgery Ward of the Hospital of the Ministry of Interior in Kielce, Poland, could be used anonymously for research purposes. This study was supported by the statutory budget of the Department of Biochemistry and Medical Chemistry, Pomeranian Medical University.

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