Original paper

Searching for a relationship between the elemental composition of archaeological bones and the occurrence of caries

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Abstract

Background. Although the macroscopic assessment of dental caries and the assessment of bone elemental composition are quite different, efforts can be made to identify commonalities in the assessment of health and nutritional quality. Both indicators are correlated with dietary habits and are dependent on taphonomic processes occurring in the postmortem substrate. However, teeth exhibit structural resilience of their hard tissues to adverse environmental factors.

Objectives. The aim of the study was to establish a correlation between the elemental composition of bones and the presence of carious lesions.

Material and methods. The study material consisted of the following skeletal parts: 161 permanent teeth from 36 individuals and bridge fragments of 36 ribs. The presence of caries was assessed visually using a modified International Caries Detection & Assessment System (ICDAS II) scale. The rib samples were subjected to elemental analysis (zinc (Zn), iron (Fe), magnesium (Mg), calcium (Ca), phosphorus (P), strontium (Sr), barium (Ba)) using spectroscopic methods.

Results. The odontological and chemical analyses did not reveal any statistically significant relationships between the Ca/P diagenesis index and dental features. Postmortem tooth loss showed a weak correlation with the diagenesis index.

Conclusions. Discoloration, cracks and flaking of the dental crown surfaces may be associated with the intensity of Ca/P diagenesis. However, no significant correlation was found between these phenomena. Among other elements, only Zn levels exhibited a correlation with the caries index.

Keywords: diagenesis, bioarchaeology, bone elemental concentration

Background

One of the goals of dental studies of ancient populations is to accurately assess carious lesions on the surfaces of dental crowns of deciduous and permanent teeth. The loss of hard tooth tissue is significantly influenced by chemical and physical factors. The effective detection and quantification of carious lesions enables the estimation of the incidence and severity of caries within the studied population.[1]

The modern theories of the formation of carious lesions unanimously emphasize that caries is a dynamic process that varies in time and involves many microbiological factors as well as chemical and physical phenomena, resulting in the destruction of hard tooth tissues.[2] Among these, exogenous nutritional factors play an important role. Exogenous factors include processed foods with high viscosity and high levels of simple carbohydrates. The consumption of these products may result in an increased prevalence of advanced caries.[3]

Prior to the formation of medium and deep enamel defects as well as the destruction of dentin, characteristic discoloration is observed on the surfaces of dental crowns. This discoloration typically manifests as dull, chalky white spots associated with the loss of dihydroxyapatite. In the subsequent phase, black and brown spots can be observed within enamel micropores, which are caused by food-derived dyes.[4]

The biggest challenge in evaluating caries indicators, such as the severity of caries in ancient human populations, is accurately identifying the earliest carious lesions. This can be particularly challenging due to taphonomic changes, which can both mask the presence of early carious lesions or mimic them.[5] Another obstacle is postmortem tooth loss, which precludes the assessment of caries on absent teeth.

Incorrect or imprecise estimation of caries indicators in bioarchaeological studies affects the assessment of the masticatory organ condition and, indirectly, the hygienic and nutritional status of the examined population.[6] These studies implicitly provide indirect information on nutritional status, hygiene, eating habits, and socio-economic stratification in the population. Inaccurate assessment of the state of the masticatory organ indirectly results in an erroneous evaluation of the aforementioned aspects of the studied population.[1,7]

Chemical analysis of preserved hard tissues can be employed to obtain similar information about a given population. However, the relationship between diet and the elemental composition of bones obtained at archaeological sites is far from the simple "you are what you eat" model, and the method itself is prone to errors and overinterpretations. Nevertheless, with proper procedures, some metals can be successfully used in bioarchaeological research.[8] However, it is worth emphasizing that there is still a significant challenge (especially in the context of archaeological research) of determining the extent to which the original chemical composition of human bones has changed as a result of processes that occur after the death of an individual.[9,10] The knowledge about diagenetic changes (related to postmortem bone degradation) regarding the addition or removal of specific elements from human bones is incomplete.[10]

The most useful elements in the chemical analysis of bone excavation materials are calcium (Ca) and phosphorus (P). While not indicative of an individual's dietary habits, the calcium-to-phosphorus (Ca/P) ratio can be used to estimate the extent of diagenetic changes in decomposing bone, which are influenced by the chemical composition of the surrounding substrate. In the absence of substrate chemical influence, the Ca/P ratio in bone is expected to remain stable at approx. 2.1. However, due to diagenesis, it can increase significantly.[11–13]

The studies of other elements, such as barium (Ba), iron (Fe), magnesium (Mg), strontium (Sr), and zinc (Zn) remain more controversial. Theoretically, these elements could be used as potential biomarkers of diet assessment quality and its components, as well as indirectly to assess socio-environmental and economic differences that existed between the studied human groups.[14] However, in practice, the usefulness of elemental bone analysis is significantly impaired by postmortem diagenetic changes.[15] A bone deposited in soil is susceptible to element substitution, which results in composition changes over time.[16] For this reason, methods of diet reconstruction based on the elemental composition of extracted bone samples, quite commonly used in the 1970s and 1980s, have been the subject of increased criticism.[8,16,17] However, with careful analysis of the results and proper procedures, they can still be used successfully.[8,11,17]

Although the macroscopic assessment of dental caries and the assessment of elemental composition of bones are different markers, it is still possible to identify common points in the assessment of health and nutritional quality. Both indicators are correlated with diet and dependent on the taphonomic processes occurring in the postmortem substrate, despite the observed resilience of teeth's hard tissues to adverse environmental factors.[5] On the one hand, taphonomic processes occurring at the place of deposition of bone remains may have a secondary impact on the state of dentition (including the number of teeth preserved), the state of alveolar processes, enamel discoloration, etc., and thus the detection of caries. On the other hand, taphonomic processes can also impact the reliability of chemical analysis of elements.

The concentrations of Ba, Sr, Fe, Mg, and Zn in the rib samples were found to be outside the ranges of concentrations of these elements reported in bones of modern humans (without the influence of diagenesis), which were used as reference data. Therefore, we assumed that the diagenesis process may have influenced the concentration in the examined bones. Conversely, if the concentration of a given element in the examined bone did not differ significantly from the so-called reference data, it was assumed that the diagenesis process had no impact on its concentration. Instead, its origin was attributed to the diet (i.e., the food and water consumed and drank by the individual to whom this bone belonged) and/or the biological condition of the individual's body. This interpretation is consistent with that used in other studies concerning the impact of diagenesis on the concentration of elements in human bones.[8] The reference data was obtained from the literature and is presented in Table 1.

However, this issue is highly complex.[27] Based on the aforementioned methodology, it is not possible to determine the standard limit of the concentration of a given element in human bone, above or below which (in the case of hypothetical loss of elements from bone) the processes of diagenesis occur. This is attributable to inter-individual variations in the concentration of these elements in the bones of living individuals. Therefore, only a significant deviation from the so-called reference data may be useful in determining whether the concentration of a specific element in bone is of diagenetic origin.[8,10]

Objectives

The question of whether there are significant links between these indicators remains open at present, given the indicated common dependencies. The answer to this question may be an attempt to assess the potential stochastic relationships between the state of dentition and the elemental composition of bones.

Material and methods

Population characteristics

The research material consisted of the following skeletal parts: 161 permanent teeth from 36 individuals and bridge fragments of 36 ribs from the collection of the Department of Anatomy, Wroclaw Medical University, Poland. Bone remains were obtained during the exploration of the archaeological site in the former church cemetery of St. Barbara in Wrocław, Poland (Fig. 1).

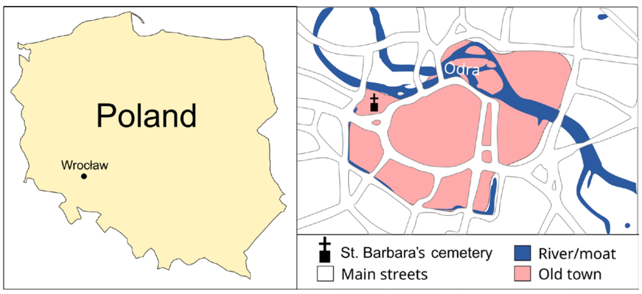

Fig. 1. Location of the cemetery from which the material was extracted

Table 1. Review of the concentrations of the elements analyzed in the bones of modern human populations

Element	Study	Rib bone samples (*n*)	Mean value (range) [μg/g]
Ba	Samudralwar and Robertson[19]	F&M (80)	2.54 (0.57–7.70)
	Zaichick et al.[18,20]	F&M (168)	0.94
	Brätter et al.[21]	F&M (12) cortical	36
		F&M (12) cancellous	19
	Yoshinaga et al.[22]	F (38)	2.86 (1.13–7.70)
		M (42)	2.24 (0.57–5.22)
Sr	Samudralwar and Robertson[19]	F&M (80)	29 (36–1,163)
	Takata et al.[23]	F&M (69)	400
	Yoshinaga et al.[22]	F (38)	334 (36–1,163)
		M (42)	252 (58–701)
	Yoshinaga et al.[24]	F&M (18)	176 (87–345)
	Brätter et al.[21]	F&M (12) cortical	62
		F&M (12) cancellous	58
Fe	Samudralwar and Robertson[19]	F&M (80)	140 (23.4–448)
	Yoshinaga et al.[24]	F&M (18)	31.2
	Crawford and Crawford[25]	F&M (13)	842 (224–917)
	Koch et al.[26]	F&M (45)	71
	Brätter et al.[21]	F&M (12) cortical	23
		F&M (12) cancellous	77
Mg	Samudralwar and Robertson[19]	F&M (80)	2,139 (1,118–2,876)
	Simpson et al.[27]	F&M (8)	620
	Crawford and Crawford[25]	F&M (18)	4,184 (2,886–5,321)
	Koch et al.[26]	F&M (45)	2,850
		F (28)	2,920
		M (17)	2,730
	Brätter et al.[21]	F&M (12) cortical	2,600
		F&M (12) cancellous	2,700
Zn	Samudralwar and Robertson[19]	F&M (80)	92.8 (53.6–128.0)
	Burak and Okólska[28]	F&M (2)	26 (18–34)
	Crawford and Crawford[25]	F&M (13)	220 (154–278)
	Brätter et al.[21]	F&M (12) cortical	180
		F&M (12) cancellous	144

Ba – barium; Sr – strontium; Fe – iron; Mg – magnesium; Zn – zinc; M – male; F – female.

The dating of the archaeological site at the church of St. Barbara was based on relative methodology, including historical sources, fragments of preserved epitaphs displayed in the church, stratigraphic research, and radioisotope methods.[28] The content of ^{14}C carbon isotope in the bone samples from 18 individuals across all stratigraphic levels indicates that the cemetery was in use between 1695 and 1919 (±25 years). Based on the historical verification of the data, it can be inferred that the individuals whose bone remains were examined had been buried between the end of the late 17th and late 18th centuries.[11]

The cemetery was located in close proximity to the Gothic cemetery temple dedicated to Saint Barbara, a patron of hospitalization and poverty (Fig. 1). Historical sources, tombstone epitaphs and urban sociotopography from the 16th to 19th centuries indicate several distinct social groups interred in the cemetery, from which the archaeological material used in the research was derived. The groups include the poorer craftsmen, patients of numerous hospitals and shelters surrounding the cemetery, and a few wealthy townsmen from Protestant families.[28]

All skeletal remains used in the current study were excavated from the soil. As a result, they were subjected to diagenesis and a variety of taphonomic processes. The degree of diagenesis for each individual was estimated by calculating the Ca/P ratio.

Sex and age at death

Sex was determined by examining skull morphology and, when possible, the characteristics of the pelvic bone.[29,30] The age of individuals was estimated using both cranial and post-cranial skeletal features, including changes in the auricular surfaces of the ilia and pubic symphyses,[29,30] as well as the closure of cranial sutures and tooth crown wear.[30–32] If the post-cranial skeleton was not available, age estimation relied solely on the cranial skeleton.

Due to the poor state of preservation of the bone remains, it was not possible to determine the sex and biological age at the time of death in all cases (morphological gender was determined in 20 cases) (Table 2). The bone material and permanent dentition were subjected to further analysis in 28 adults and 8 juveniles (Table 2).

Table 2. Distribution of sex and age among the examined individuals

Variable		Individuals, *n*
Sex	male	16
	female	4
	unknown	16
Age	juveniles (<18 years)	8
	adults (≥18 years)	28

Evaluation of caries

The intensity of caries was visually assessed using a modified International Caries Detection & Assessment System (ICDAS II) scale, which is suitable for both daily clinical practice and epidemiological studies.[33] This scale has been modified for the study of archaeological materials.[6] The modified scoring system is presented in Table 3.

Table 3. Modified International Caries Detection & Assessment System (ICDAS II) scale criteria

Code	Criterion
1	No caries detected.
2	Distinct visual change in enamel.
3	Localized enamel breakdown (without clinical visual signs of dentinal involvement).
4	Underlying dark shadow from dentin.
5	Distinct cavity with visible dentin.
6	Extensive distinct cavity with visible dentin (>50% of the surface).

The objective of the individual assessment of caries intensity was to determine the stage of carious lesions with the highest degree among all lesions described in the upper and lower alveolar arches using the ICDAS II scale. In addition to evaluating the presence of caries on individual teeth, indicators were employed to estimate the intensity of caries in the examined individuals. The commonly used indicator, the caries index, was calculated by dividing the number of teeth with caries by the number of preserved teeth.[34]

Mineralization of research material

The samples were subjected to wet mineralization using a closed MARS 6 microwave system (CEM Corporation, Matthews, USA).

A homogeneous sample weighing between 0.1 g and 0.5 g was placed in preparation vessels, to which 5 cm^3 of concentrated nitric acid (V) (Chempur, Piekary Śląskie, Poland) was added. The samples were subjected to mineralization in the microwave sample preparation system. The minerals were quantitatively transferred to 10-cm^3 measuring vessels using redistilled water. Mineralization was conducted in accordance with the Polish standard PN-EN 13805:2003. Foodstuffs – Determination of trace elements – Pressure digestion.[35]

Determination of elements in the research material

The atomic absorption or emission spectrometry with an acetylene/air flame was employed to determine the Fe, Zn, Mg, and Ca contents in the materials. The analysis was conducted using a SpectraAA atomic absorption spectrometer (Spectralab Scientific Inc., Markham, Canada) and a Varian AA240FS flame attachment (Spectralab Scientific Inc.). The Sr and Ba levels were determined using inductively coupled plasma optical emission spectrometry (ICP-OES). The phosphorus content was quantified with a spectrophotometer (Thermo Spectronic Unicam UV-300; Thermo Fisher Scientific, Waltham, USA).

The accuracy of the method was validated using the National Institute of Standards and Technology (NIST) 1486 Bone Meal (Sigma-Aldrich, St. Louis, USA). The estimated measurement uncertainty was 5%.

The elements were determined in accordance with the following standards:

- Zn, Fe: PN-EN 12143:2000. Fruit and vegetable juices. Determination of soluble matter content by refractometric method[36];
- Mg, Ca: PN-EN ISO 6869:2000. Feedstuffs. Determination of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc by atomic absorption spectrometry.[37]

In order to assess the occurrence and intensity of diagenesis in examined rib bone samples, the mass ratio of Ca to P was calculated. A ratio above 2.16 is indicative of structural degradation of the bone (higher values indicate stronger bone degradation).[12,13]

Statistical analysis

The normality of the distribution for each variable was evaluated using the Shapiro–Wilk test. Pearson's analyses were performed using Statistica 13.1 software (TIBCO Software, Palo Alto, USA) to determine the correlation between dental condition traits, the concentration of the analyzed elements in the examined bones, and the diagenesis index. The assumptions of Pearson's correlation test were satisfied because at least 1 variable pair was normally distributed in each comparison.[38] The results were considered statistically significant for a p-value <0.05.

Results

Analysis of the prevalence of caries

The dental condition characteristics of the early modern population of Wrocław were based on an examination of carious changes in crowns and dental roots. This examination was conducted through a visual assessment of 161 permanent teeth obtained from 36 individuals. The total number of teeth in the alveolar arches was determined, including both carious and non-carious teeth, as well as those that had been lost antemortem. Information related to taphonomic processes included the number of teeth lost postmortem, as evidenced by the number of empty alveolar sockets. The characteristics of the severity of carious cavities were also presented, with a particular emphasis on the specific "predominance state", which represents the highest degree in the ICDAS II classification. Therefore, individuals were classified according to the most advanced form of dental caries, with the weaker forms of the disease on dental crowns being disregarded. In addition to the individual assessment of caries severity, caries intensity indexes were also calculated. The list of parameters for assessing the state of dentition in the form of descriptive statistics is presented in Table 4.

Table 4. Statistical data regarding the masticatory organs of the studied population

Variable	*Me*	Minimum	Maximum
Missing teeth, *n*	13	5	26
Teeth antemortem, *n*	0	0	24
Teeth postmortem, *n*	11	2	24
Teeth in the upper jaw, *n*	0	0	10
Teeth in the lower jaw, *n*	3	0	11
Teeth with caries, *n*	2	0	12
Teeth with caries in the crown, *n*	3	1	12
Teeth with caries in the root, *n*	0	0	2
Teeth with the highest caries grade, *n*	3	1	6

Me – median.

A considerable number of missing teeth were observed in the examined material. On average, approx. 13 teeth were outside the possibilities of dental assessment, with nearly 11 teeth lost postmortem. Undoubtedly, the decrease in the number of teeth available for examination may have influenced the further assessment of the distribution of caries. On average, 80% of the preserved teeth in the examined individuals were affected by carious lesions, with the maximum number of teeth affected by caries in a single case not exceeding 12. Among the carious lesions observed in 36 individuals, the most prevalent defect was classified as code 3 on the ICDAS II scale. An important finding about the nature of the disease in the study group is the relatively low average number of teeth lost antemortem, which may be linked to cariogenic processes (Table 4).

Diagenesis and the content of selected micronutrients

The range of values obtained for the diagenesis index (Ca/P ratio) for the examined human rib samples (n = 35; minimum Ca/P ratio = 2.099, maximum Ca/P ratio = 5.048) and the mean value of this trait (3.296) is indicative of the influence of diagenesis processes on these bones (with the exception of 1 sample, where the index value did not exceed 2.16) (Table 5). These results were expected due to 2 significant factors: the bones were derived from a cemetery dating to the 17^{th}–18^{th} centuries AD; and ribs are more susceptible to diagenesis processes compared to other bones, such as femur or skull bones.[39]

The mean concentration of Ba obtained in this study was 44.9 µg/g (range: 3.8–194.7 µg/g) (Table 5). Although this value is higher than the reference mean for this element in most specimens (approx. 65%) of the examined sample, the Ba concentration is within the widest reference range established for modern human ribs based on the available literature data (0.94–36.00 µg/g) (Table 1). Thus, the results indicate that diagenesis had a significant impact on the Ba concentration in approx. 35% of the examined bones.

Table 5. Summary statistics of the concentration of the analyzed elements in the rib bone sample

Element	Samples analyzed, *n*	*M* [μg/g]	*SD* [μg/g]	Range (min–max) [μg/g]
Ba	35	44.9	38.72	3.8–194.7
Sr	35	145.5	43.79	55.3–280.2
Fe	36	4,818.9	4,380.24	350.7–16,600.7
Mg	36	3,285.0	1,957.51	288.2–9,558.3
Zn	36	569.0	222.94	166.8–1,157.0
Ca	36	315,685.3	48,175.34	259,801.8–445,511.9
P	35	11,942.4	23,699.98	3,523.9–96,709.1
Ca/P ratio	35	3.3	0.62	2.1–5.0

Ca – calcium; P – phosphorus; *M* – mean; *SD* – standard deviation.

The mean Sr concentration in the examined rib samples (145.5 μg/g) (Table 5) was lower than the reference value (e.g., means: 252 μg/g or 334 μg/g) (Table 1). Furthermore, the obtained Sr concentrations (range: 55.3–280.2 μg/g) fall within the range of variability of this trait established for ribs of modern adult humans (36–1,163 μg/g) (Table 1). This suggests that diagenesis had no impact on the Sr concentration in the examined bones.

The mean Fe concentration was found to be 4,818.9 μg/g (range: 350.7–16,600.7 μg/g) (Table 5). This value is higher than the mean reference value (Table 1), and for most specimens, it is above the upper limit of the widest referential range (23.4–917.0 μg/g) (Table 1). Considering the prevailing view that elevated Fe levels in human bones from archaeological studies are likely caused by diagenesis,[8,40,41] a diagenetic origin is also suggested in this study.

The mean Mg concentration was 3,285 μg/g (range: 288.2–9,558.3 μg/g) (Table 5). According to the reference data, the widest range of variability for this trait (mean range: 620–4,184 μg/g) includes the Mg concentration values obtained for the majority of the specimens analyzed in this study. Therefore, a biogenic origin of Mg can be suggested for these specimens. A smaller subset of specimens included in the sample indicates higher Mg values (>4,184 μg/g). However, these values do not significantly deviate from the upper limit of the abovementioned reference range. In summary, the results indicate that the majority of the sample can be attributed to a biogenic origin of Mg.

The mean concentration of Zn (569 μg/g) significantly exceeds the reference averages established for the ribs of modern humans (e.g., 26 μg/g or 220 μg/g) (Table 1). Almost all Zn concentrations determined for the tested specimens (except for 1 individual) are higher than the upper limit of the widest reference range for this feature (18–278 μg/g) (Table 1). According to the method employed in this study, a large deviation from the upper limit of the reference range suggests a diagenetic origin of the Zn in the majority of the examined samples.

Correlation matrix: dental condition vs. elements and Ca/P ratio

The results of Pearson's analyses, conducted to determine the statistical relationship between element concentration and dental condition, indicated that only 2 correlations were statistically significant or close to the threshold of the confidence interval: first, a positive and weak correlation between the Ca/P ratio and the number of missing teeth; second, a negative and weak correlation between Zn concentration and the caries index (Table 6). No statistically significant correlation was found between any of the dental condition indicators and the Ca/P ratio.

Discussion

The objective of this study was to explore potential relationships between the elemental composition of bones and the state of dentition, as assessed through the analysis

Table 6. Results of the correlation analysis between the dental condition indicators and the content of selected micronutrients in the bone material

Variable	Zn		Fe		Mg		Ca		P		Sr		Ba		Ca/P ratio	
	r	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value
Missing teeth	0.049	0.782	–0.119	0.501	–0.027	0.879	0.012	0.948	0.084	0.641	–0.225	0.208	–0.211	0.238	0.326	0.064
Teeth antemortem	–0.006	0.976	–0.255	0.146	0.185	0.296	–0.031	0.861	0.013	0.942	–0.143	0.427	–0.104	0.564	0.037	0.839
Teeth postmortem	0.059	0.742	0.151	0.393	–0.231	0.188	0.047	0.793	0.076	0.676	–0.084	0.644	–0.112	0.536	0.308	0.081
Teeth in the upper jaw	0.336	0.052	0.144	0.416	0.216	0.220	0.026	0.884	–0.131	0.467	–0.042	0.815	–0.156	0.386	0.019	0.917
Teeth in the lower jaw	0.109	0.540	0.039	0.826	–0.009	0.959	–0.088	0.622	–0.184	0.306	0.024	0.895	–0.027	0.882	–0.166	0.356
Teeth with caries	0.247	0.146	0.203	0.235	0.162	0.345	–0.196	0.251	–0.238	0.169	0.087	0.618	0.035	0.844	–0.205	0.238
Teeth with caries in the crown	0.263	0.133	0.179	0.311	0.190	0.282	–0.083	0.641	–0.189	0.293	0.095	0.601	0.018	0.920	–0.164	0.361
Teeth with caries in the root	0.173	0.327	0.220	0.212	–0.257	0.143	–0.064	0.720	–0.105	0.560	–0.052	0.774	–0.125	0.488	0.093	0.605
Teeth with the highest caries grade	–0.099	0.576	0.072	0.684	–0.146	0.411	–0.087	0.627	0.007	0.969	–0.077	0.671	–0.229	0.200	0.074	0.684
Caries index	–0.336*	0.045*	0.177	0.303	–0.004	0.981	–0.204	0.234	0.254	0.142	0.028	0.871	0.285	0.097	–0.048	0.782

* statistically significant ($p < 0.05$, Pearson's correlation).

of carious lesions. The impact of secondary diagenesis processes was also taken into account.

We aimed to detect a relationship between the concentration of specific elements (Ba, Sr, Mg, Fe, and Zn) in bones, the index of the diagenesis intensity (Ca/P ratio) of these bones, and the broader dental condition traits of the examined individuals, while taking into account the potential impact of diagenesis on the concentration of the abovementioned elements. With regard to the diagenesis index, the study aimed to assess the relationship between the intensity of diagenesis in human bones and 2 types of features characterizing the condition of the teeth: the number of teeth (the number of teeth lost postmortem may be positively related to the intensity of diagenesis influencing the bones); and the intensity of caries development (a positive correlation between the index of caries intensity and the intensity of diagenesis suggests the influence of diagenesis on the occurrence of changes that mimic caries, particularly in early caries).

Macroscopic observations of carious cavities indicate that the disease course was more intense among the studied population from early modern Wrocław. However, the reduction in the number of observations resulting from postmortem tooth loss could have affected the assessment of caries intensity. The individual nature of carious defects, according to modern standards, indicates moderate caries localized to specific areas. On average, carious lesions were recorded at code 3 on the ICDAS II scale, indicating limited local penetration into dental crowns without affecting the dentin.

The results of the odontological and chemical analyses did not reveal statistically significant relationships between the Ca/P diagenesis index and any of the macroscopic variables (Table 6). For the number of teeth lost postmortem ($r = 0.308$, $p = 0.081$), the result is on the verge of statistical significance, suggesting a possible but weak dependence.

Changes observed on the surface of dental crowns and exposed parts of dental roots, such as macroscopically visible discoloration, flaky surfaces, cracks, and chips, were hypothesized to be most related to the Ca/P diagenesis index. This association was considered plausible given that secondary diagenesis processes often correlate indirectly with the poor macroscopic preservation of remains.[42] This, in turn, could result in false positive assessments of lesions conducted macroscopically, which may appear similar to cariogenic defects but are actually specific pseudopathologies. However, the analysis demonstrated no correlation between the caries index and the Ca/P ratio ($r = -0.048$, $p = 0.782$). This suggests that dental assessment of the teeth condition should be considered an independent phenomenon in relation to the process of secondary diagenesis, at least in the context of the tested archaeological material. Numerous studies indicate that dentition is minimally affected by postmortem environmental factors and remains largely unaltered.[43]

Bone tissue undergoes antemortem modifications due to metabolic activity (e.g., as a consequence of disease, inflammatory processes and hyperplasia) and mechanical injuries. In contrast, its secondary transformations occur postmortem due to taphonomic factors, such as substrate pH, water flow and the chemical composition of the burial site. The passage of time significantly affects the preservation of bone material.[44] Thus, the mineral composition of the deposited remains may vary depending on the type and intensity of diagenetic factors affecting the deposited bones, such as those in burial pits. Changes in the chemical composition of bone tissue may be the basis for further transformations and damages of a macroscopic nature, both in terms of its volume and structure.[45]

In the case of searching for potential correlations between the concentration of the analyzed element in the bones and traits of the dental condition, the results suggest a biogenic origin for Sr and Mg, and partially for Ba (significant diagenesis is suggested in approx. 35% of the sampled bones). The majority of the examined bones exhibited predominantly diagenetic origins for Fe and Zn.

The prevailing view is that the concentration of Ba and Sr in human bones is related to diet.[8,46–48] The majority of studies suggest that diagenesis has no effect on the levels of these elements in bones. However, contrary results have been reported, indicating that diagenetic influences may be present.[49–52] Thus, the comparison of the results obtained with the so-called reference data was helpful in determining whether, in the case of analyzed bone samples, the level of these elements is related to the influence of diagenesis. Notably, the relationship between the geographical area inhabited by human populations (especially archaeological) and the specific concentration of Ba and Sr in bones is a consequence of the composition of available water and the type of vegetation occurring in a given area. It is commonly believed that modifying one's diet to include a greater proportion of plant-based foods will result in the elevated levels of Sr and Ba in bones.[53,54] However, the relationship between the Sr and Ba content in food and Sr and Ba levels in the bones of an individual consuming that food remains unclear.[55] In light of our interpretation that the influence of diagenesis on the Ba concentration concerned about 35% of bones in the sample, the presence of Ba of diagenetic origin could potentially influence the results of the statistical analysis, as evidenced by the absence of a significant correlation between the level of this element and dental condition traits.

Studies on the impact of a diet without Sr in rats and guinea pigs indicated reduced calcification of bones and teeth and an increase in the occurrence of dental caries.[56] The results of these studies may, therefore, suggest a relationship between the concentration of this element in human bones and dental condition. Thus, it is probable that a low concentration of Sr in bones may be associated with a higher risk of the occurrence of caries. The statistical analysis of the sample revealed no statistically significant relationships between the Sr level and the analyzed dental condition features, including those describing the occurrence of caries. The relatively narrow range of Sr concentrations obtained in our sample, when compared to the available data (Table 1), suggests that the diets of the examined individuals were likely similar, as was the composition of the water they consumed. The limited variability in Sr concentration may have reduced the likelihood of detecting a relationship between this element and the occurrence of dental caries. Therefore, further research on this matter is necessary, including a sample of individuals with more diverse diets. Considering the high metabolic turnover rate of rib bones,[39] future studies should also include other types of bones, such as the femur.

Previous studies on the impact of diagenesis on Mg concentration in human bones have yielded disparate results, with some indicating the influence of diagenesis on Mg levels and others suggesting no such influence.[52,57,58] Diagenesis processes may cause a decrease or an increase in Mg concentration in bones.[59] Magnesium is important for overall body functioning, including bone mineralization and its development.[60–62] Approximately 60% of Mg present in the human body is stored in bones.[63] Its deficiency affects bone density,[62] and animal studies have demonstrated that a diet with inadequate Mg contributes to the development of osteoporosis.[62] Elevated levels of Mg in bones may influence bone metabolism and cause defective mineralization.[64] In this study, the statistical analysis results showed no relationship between the dental condition traits and Mg concentration in bones. Further studies with larger bone samples (free from the effects of diagenesis) are required to determine whether a positive relationship exists between Mg concentration and good dental condition.

If our interpretation that Fe and Zn in the examined bones predominantly originate from diagenesis is correct, the possibility of detecting potential relationships between the concentration of these elements of biogenic origin and the traits of the dental condition is strongly obscured by the phenomenon of diagenesis. Thus, the significant correlation found in this study between Zn concentration and 2 traits of the dental condition is difficult to explain. The negative and weak correlation between Zn concentration and the caries intensity index ($r = -0.336$, $p = 0.045$) may be attributed to the influence of diagenesis on Zn concentration.

This result is close to the threshold of statistical significance, which suggests that caution should be exercised against dismissing it as a false positive result. Nevertheless, in the absence of a correlation between caries indicators and other chemical elements, several premises indicate that this correlation may not be merely coincidental.

Regarding Zn compounds, the variations in Zn ion concentration in bone material attributable to the type of sediment and soil acidity result from the substitution of Ca in hydroxyapatite crystals. This implies that the proportion of Zn ions in postmortem bones may be greater than in new bone tissue. The degree of tissue Zn saturation is more intense in wet and acidic deposits, similar to the concentration changes observed for other metals.[44] However, these changes are not significant and should not deviate from the typical range observed in new bone tissue. In addition, the elevated concentration of Zn ions in bone tissue may be the result of an individual's diet consumed during their lifetime.[39]

Zinc compounds participate in the prevention of caries at multiple levels, through direct and indirect interactions with tooth tissue. In both cases, the endogenous distribution of Zn ions is dependent on the presence of suitable nutrients.

The indirect effect focuses on the presence of Zn compounds in saliva, the concentration of which varies depending on the salivary gland responsible for their production. The parotid glands are the source of the lowest concentration of Zn compounds, while the sublingual and submandibular salivary glands are the primary sources of these compounds. Zinc plays an important role in the protective function of saliva. It is hypothesized that Zn compounds present in saliva are a constant source of enamel remineralization. During this process, the lost Ca ions in the hydroxyapatite are replaced with Zn ions.[65] As with fluorine compounds, which play a similar role in layer remodeling, a decline in Zn concentrations in enamel is observed during the second and third decades of life. Zinc ions in saliva compete with Ca ions for a place in the enamel. The loss of Ca ions is a direct consequence of the development of caries.

It is not possible to exclude with certainty the potential importance of biogenic Zn contained in bones in shaping the negative correlation between Zn concentration and the caries intensity index. The corresponding differences in bone Zn levels may also be attributed to dietary differences, accompanied by an increase in Zn level due to diagenesis. Such variations may have influenced the results. Maintaining an adequately high Zn level in a living organism (within the natural range needed to maintain internal homeostasis) is associated with overall biological health.[27] The second significant correlation obtained in this study is the positive correlation between the Zn concentration and the number of examined teeth (i.e., individuals with higher Zn concentration tended to have more preserved teeth). A greater number of preserved teeth indicates a lower rate of postmortem loss, as well as a lower rate of antemortem loss. This suggests that individuals with a greater number of preserved teeth may exhibit healthier biological conditions. Unless influenced by diagenesis, this correlation could suggest the importance of Zn as a marker of overall bodily health.

Conclusions

The evaluation of bone mineral composition as a method for assessing the quality of life in historical populations has been a subject of discussion for many years. Linking the elemental content in bone tissue provides a potentially valuable tool for the reconstruction of lifestyle factors related to socioeconomic aspects such as diet, masticatory organ health and general health awareness.

However, proper evaluation in chemical studies of hard tissues of the human body is associated with certain limitations, which must be considered when drawing conclusions based on the elemental composition of dental or bone material. The current research on early modern urban populations highlights 2 main limitations. First, the degree of diagenesis has a significant impact on the obtained results. Second, the chemical composition of bones exhibits variability and instability during ontogenesis, which limits the applicability of results to a narrow period, spanning a few weeks to a few months before death.

Therefore, the assessment of the presence and severity of long-term diseases such as caries provides insights into the mineral composition of bone tissue and may potentially correlate it with persistent nutritional deficiencies. Despite the influence of diagenetic factors on the preservation of the studied elements in bone minerals, elevated concentrations of Zn ions in bone material may be attributable to specific dietary habits influenced by socioeconomic factors. Although the results only approached statistical significance, in light of the limitations associated with diagenesis, it is important to mention the potential association between elevated Zn levels and reduced caries intensity among former inhabitants of Wrocław.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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References

1. Temple DH. Caries: The Ancient Scourge. In: Irish JD, Scott GR, eds. *A Companion to Dental Anthropology*. 1st ed. Hoboken, NJ: John Wiley & Sons, Inc.; 2015:433–449. doi:10.1002/9781118845486.ch26
2. Pitts NB, Zero DT, Marsh PD, et al. Dental caries. *Nat Rev Dis Primers*. 2017;3:17030. doi:10.1038/nrdp.2017.30
3. van Meijeren-van Lunteren AW, Voortman T, Wolvius EB, Kragt L. Adherence to dietary guidelines and dental caries among children: A longitudinal cohort study. *Eur J Public Health*. 2023;33(4):653–658. doi:10.1093/eurpub/ckad097
4. Bilge NH, Yeşiltepe S, Törenek Ağırman K, Çağlayan F, Bilge OM. Investigation of prevalence of dental anomalies by using digital panoramic radiographs. *Folia Morphol (Warsz)*. 2018;77(2):323–328. doi:10.5603/FM.a2017.0087
5. Schmidt CW, Quataert R, Zalzala F, D'Anastasio R. Taphonomy of Teeth. In: Schotsmans EMJ, Márquez-Grant N, Forbes SL, eds. *Taphonomy of Human Remains: Forensic Analysis of the Dead and the Depositional Environment*. 1st ed. Hoboken, NJ: John Wiley & Sons, Inc.; 2017:92–100. doi:10.1002/9781118953358.ch7
6. Dąbrowski P, Grzelak J, Kulus M, Staniowski T. Diagnodent and VistaCam may be unsuitable for the evaluation of dental caries in archeological teeth. *Am J Phys Anthropol*. 2019;168(4):797–808. doi:10.1002/ajpa.23785
7. Cox M, Mays S, eds. *Human Osteology in Archaeology and Forensic Science*. Cambridge, UK: Cambridge University Press; 2006:228–236.
8. Rasmussen KL, Milner G, Skytte L, Lynnerup N, Thomsen JL, Boldsen JL. Mapping diagenesis in archaeological human bones. *Herit Sci*. 2019;7(1):41. doi:10.1186/s40494-019-0285-7
9. Price TD, Blitz J, Burton J, Ezzo JA. Diagenesis in prehistoric bone: Problems and solutions. *J Archaeol Sci*. 1992;19(5):513–529. doi:10.1016/0305-4403(92)90026-Y
10. Rasmussen KL, Milner GR, Delbey T, et al. Trace element distribution in human cortical bone microstructure: The potential for unravelling diet and social status in archaeological bones. *Herit Sci*. 2020;8(1):111. doi:10.1186/s40494-020-00457-1
11. Dąbrowski P, Kulus MJ, Grzelak J, Olchowy C, Staniowski T, Paulsen F. Nutritional reconstruction in an early modern population: Searching for a relationship between dental microwear and bone element composition. *Ann Anat*. 2022;240:151884. doi:10.1016/j.aanat.2021.151884
12. Demirci S, Kayaturk N. Chemical analysis of fossil bones. *PACT (Rixensart)*. 1995;(45):111–118. https://open.metu.edu.tr/handle/11511/7199. Accessed June 26, 2024.
13. Hedges REM. Bone diagenesis: An overview of processes. *Archaeometry*. 2002;44(3):319–328. doi:10.1111/1475-4754.00064
14. Lambert JB, Weydert-Homeyer JM. The fundamental relationship between ancient diet and the inorganic constituents of bone as derived from feeding experiments. *Archaeometry*. 1993;35(2):279–294. doi:10.1111/j.1475-4754.1993.tb01043.x
15. Szostek K, Stepańczak B, Szczepanek A, et al. Diagenetic signals from ancient human remains – Bioarchaeological applications. *Mineralogia*. 2011;42(2–3):93–112. doi:10.2478/v10002-011-0009-4
16. Burton JH, Price TD. The Use and Abuse of Trace Elements for Paleodietary Research. In: Ambrose SH, Katzenberg MA, eds. *Advances in Archaeological and Museum Science. Volume 5. Biogeochemical Approaches to Paleodietary Analysis*. Dordrecht, the Netherlands: Kluwer Academic Publishers; 2002:159–171. doi:10.1007/0-306-47194-9_8
17. Wathen CA, Isaksson S, Lidén K. On the road again – A review of pretreatment methods for the decontamination of skeletal materials for strontium isotopic and concentration analysis. *Archaeol Anthropol Sci*. 2022;14(3):45. doi:10.1007/s12520-022-01517-2
18. Zaichick V, Zaichick S, Karandashev V, Nosenko S. The effect of age and gender on Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Sr, V, and Zn contents in rib bone of healthy humans. *Biol Trace Elem Res*. 2009;129(1–3):107–115. doi:10.1007/s12011-008-8302-9
19. Samudralwar DL, Robertson JD. Determination of major and trace elements in bones by simultaneous PIXE/PIGE analysis. *J Radioanal Nucl Chem*. 1993;169(1):259–267. doi:10.1007/BF02046801
20. Zaichick S, Zaichick V, Karandashev V, Nosenko S. Accumulation of rare earth elements in human bone within the lifespan. *Metallomics*. 2011;3(2):186–194. doi:10.1039/c0mt00069h
21. Brätter P, Gawlik D, Lausch J, Rösick U. On the distribution of trace elements in human skeletons. *J Radioanal Chem*. 1977;37(1):393–403. doi:10.1007/BF02520545
22. Yoshinaga J, Suzuki T, Morita M. Sex- and age-related variation in elemental concentrations of contemporary Japanese ribs. *Sci Total Environ*. 1989;79(3):209–221. doi:10.1016/0048-9697(89)90337-9
23. Takata MK, Saiki M, Sumita NM, Saldiva PHN, Pasqualucci CA. Activation analysis methods and applications. *J Radioanal Nucl Chem*. 2005;264(1):5–8. doi:10.1007/s10967-005-0666-0
24. Yoshinaga J, Suzuki T, Morita M, Hayakawa M. Trace elements in ribs of elderly people and elemental variation in the presence of chronic diseases. *Sci Total Environ*. 1995;162(2–3):239–252. doi:10.1016/0048-9697(95)04470-I

25. Crawford MD, Crawford T. Lead content of bones in a soft and a hard water area. *Lancet*. 1969;1(7597):699–701. doi:10.1016/s0140-6736(69)92649-x
26. Koch HJ, Smith ER, McNeely J. Analysis of trace elements in human tissues. II. The lymphomatous diseases. *Cancer*. 1957;10(1):151–160. doi:10.1002/1097-0142(195701/02)10:1<151::aid-cncr2820100122>3.0.co;2-p
27. Simpson R, Cooper DML, Swanston T, Coulthard I, Varney TL. Historical overview and new directions in bioarchaeological trace element analysis: A review. *Archaeol Anthropol Sci*. 2021;13(1):24. doi:10.1007/s12520-020-01262-4
28. Burak M, Okólska H. *Cmentarze dawnego Wrocławia*. Wrocław, Poland: Muzeum Architektury we Wrocławiu; 2007:100–128.
29. White TD, Black MT, Folkens PA. *Human Osteology*. 3rd ed. Cambridge, MA: Elsevier Academic Press; 2012:51–99.
30. Buikstra JE, Ubelaker DH, eds. *Standards for Data Collection from Human Skeletal Remains*. Arkansas Archeological Survey; 1994:17–38.
31. Lovejoy CO. Dental wear in the Libben population: Its functional pattern and role in the determination of adult skeletal age at death. *Am J Phys Anthropol*. 1985;68(1):47–56. doi:10.1002/ajpa.1330680105
32. Meindl RS, Lovejoy CO. Ectocranial suture closure: A revised method for the determination of skeletal age at death based on the lateral-anterior sutures. *Am J Phys Anthropol*. 1985;68(1):57–66. doi:10.1002/ajpa.1330680106
33. Braga MM, Oliveira LB, Bonini GAVC, Bönecker M, Mendes FM. Feasibility of the International Caries Detection and Assessment System (ICDAS-II) in epidemiological surveys and comparability with standard World Health Organization Criteria. *Caries Res*. 2009;43(4):245–249. doi:10.1159/000217855
34. Erdal YS, Duyar İ. A new correction procedure for calibrating dental caries frequency. *Am J Biol Anthropol*. 1999;108(2):237–240. doi:10.1002/(SICI)1096-8644(199902)108:2<237::AID-AJPA8>3.0.CO;2-Z
35. gov.pl. Zakres działalności laboratorium badania żywności. https://www.gov.pl/attachment/75016d78-d3db-4192-a077-f09ad186f030. Accessed July 19, 2024.
36. Institute of Agricultural and Food Biotechnology – State Research Institute. Propozycje dotyczące metod oznaczania jakości, metod próbobrania, niezbędnego wyposażenia wykorzystywanego do oceny jakości, znakowania, opakowań i warunków przechowywania najbardziej perspektywicznych produktów rolno-spożywczych oferowanych na Platformie Żywnościowej. https://platformazywnosciowa.com.pl/wp-content/uploads/2019/12/RAPORT-NR-4-IBPRS.pdf. Accessed July 19, 2024.
37. ISO 6869:2000. Animal feeding stuffs – Determination of the contents of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc – Method using atomic absorption spectrometry. https://www.iso.org/standard/33707.html. Accessed July 19, 2024.
38. Sedgwick P. Pearson's correlation coefficient. *BMJ*. 2012;345:e4483. doi:10.1136/bmj.e4483
39. López-Costas O, Lantes-Suárez Ó, Martínez Cortizas A. Chemical compositional changes in archaeological human bones due to diagenesis: Type of bone vs soil environment. *J Archaeol Sci*. 2016;67:43–51. doi:10.1016/j.jas.2016.02.001
40. Buikstra JE. Multiple Elements: Multiple Expectations. In: Price TD, ed. *The Chemistry of Prehistoric Human Bone*. Cambridge, UK: Cambridge University Press; 1989:155–210.
41. Williams AMM, Siegele R. Iron deposition in modern and archaeological teeth. *Nucl Instrum Methods Phys Res*. 2014;335:19–23. doi:10.1016/j.nimb.2014.06.003
42. Kendall C, Eriksen AMH, Kontopoulos I, Collins MJ, Turner-Walker G. Diagenesis of archaeological bone and tooth. *Palaeogeogr Palaeoclimatol Palaeoecol*. 2018;491:21–37. doi:10.1016/j.palaeo.2017.11.041
43. Humphrey LT. Chemical and Isotopic Analyses of Dental Tissues. In: Irish JD, Scott GR, eds. *A Companion to Dental Anthropology*. 1st ed. Hoboken, NJ: Wiley-Blackwell; 2015:499–513. doi:10.1002/9781118845486.ch30
44. Krajcarz MT. Alteration of the metal content in animal bones after 2.5-year experimental exposure to sediments. *Archaeol Anthropol Sci*. 2019;11(1):361–372. doi:10.1007/s12520-017-0533-2
45. Sorg MH. Differentiating trauma from taphonomic alterations. *Forensic Sci Int*. 2019;302:109893. doi:10.1016/j.forsciint.2019.109893
46. Rasmussen KL, Skytte L, Pilekær C, et al. The distribution of mercury and other trace elements in the bones of two human individuals from medieval Denmark – The chemical life history hypothesis. *Herit Sci*. 2013;1(1):10. doi:10.1186/2050-7445-1-10
47. Schutkowski H, Herrmann B, Wiedemann F, Bocherens H, Grupe G. Diet, status and decomposition at Weingarten: Trace element and isotope analyses on early mediaeval skeletal material. *J Archaeol Sci*. 1999;26(6):675–685. doi:10.1006/jasc.1998.0384
48. Lösch S, Moghaddam N, Grossschmidt K, Risser DU, Kanz F. Stable isotope and trace element studies on gladiators and contemporary Romans from Ephesus (Turkey, 2nd and 3rd Ct. AD) – Implications for differences in diet. *PLoS One*. 2014;9(10):e110489. doi:10.1371/journal.pone.0110489
49. Trueman CN, Tuross N. Trace elements in recent and fossil bone apatite. *Rev Mineral Geochem*. 2002;48(1):489–521. doi:10.2138/rmg.2002.48.13
50. Fabig A, Herrmann B. Trace elements in buried human bones: Intra-population variability of Sr/Ca and Ba/Ca ratios – Diet or diagenesis? *Naturwissenschaften*. 2002;89(3):115–119. doi:10.1007/s00114-001-0294-7
51. Hancock RGV, Grynpas MD, Alpert B. Are archaeological bones similar to modern bones? An INAA assessment. *J Radioanal Nucl Chem*. 1987;110(1):283–291. doi:10.1007/BF02055031
52. Lambert JB, Vlasak S, Simpson, Szpunar CB, Buikstra JE. Bone diagenesis and dietary analysis. *J Hum Evol*. 1985;14(5):477–482. doi:10.1016/S0047-2484(85)80026-9
53. González-Weller D, Rubio C, Gutiérrez ÁJ, et al. Dietary intake of barium, bismuth, chromium, lithium, and strontium in a Spanish population (Canary Islands, Spain). *Food Chem Toxicol*. 2013;62:856–868. PMID:24416776.
54. Burton JH, Price TD, Cahue L, Wright LE. The use of barium and strontium abundances in human skeletal tissues to determine their geographic origins. *Int J Osteoarchaeol*. 2003;13(1–2):88–95. doi:10.1002/oa.661
55. Burton JH, Wright LE. Nonlinearity in the relationship between bone Sr/Ca and diet: Paleodietary implications. *Am J Phys Anthropol*. 1995;96(3):273–282. doi:10.1002/ajpa.1330960305
56. Underwood EJ. *Trace Elements in Human and Animal Nutrition*. 4th ed. Cambridge, MA: Elsevier Academic Press; 2012:183.
57. Lambert JB, Xue L, Buikstra JE. Physical removal of contaminative inorganic material from buried human bone. *J Archaeol Sci*. 1989;16(4):427–436. doi:10.1016/0305-4403(89)90017-4
58. Byrne KB, Parris DC. Reconstruction of the diet of the Middle Woodland Amerindian population at Abbott Farm by bone trace-element analysis. *Am J Biol Anthropol*. 1987;74(3):373–384. doi:10.1002/ajpa.1330740309
59. Parker RB, Toots H. Trace Elements in Bones as Paleobiological Indicators. In: Behrensmeyer AK, Hill AP, eds. *Fossils in the Making: Vertebrate Taphonomy and Paleoecology*. Chicago, IL: University of Chicago Press; 1988:197.
60. Maguire ME, Cowan JA. Magnesium chemistry and biochemistry. *Biometals*. 2002;15(3):203–210. doi:10.1023/A:1016058229972
61. Leidi M, Dellera F, Mariotti M, Maier JAM. High magnesium inhibits human osteoblast differentiation in vitro. *Magnes Res*. 2011;24(1):1–6. doi:10.1684/mrh.2011.0271
62. Castiglioni S, Cazzaniga A, Albisetti W, Maier JAM. Magnesium and osteoporosis: Current state of knowledge and future research directions. *Nutrients*. 2013;5(8):3022–3033. doi:10.3390/nu5083022
63. Saris NE, Mervaala E, Karppanen H, Khawaja JA, Lewenstam A. Magnesium: An update on physiological, clinical and analytical aspects. *Clin Chim Acta*. 2000;294(1–2):1–26. doi:10.1016/s0009-8981(99)00258-2
64. Navarro-González JF, Mora-Fernández C, García-Pérez J. Clinical implications of disordered magnesium homeostasis in chronic renal failure and dialysis. *Semin Dial*. 2009;22(1):37–44. doi:10.1111/j.1525-139X.2008.00530.x
65. Sejdini M, Begzati A, Salihu S, Krasniqi S, Berisha N, Aliu N. The role and impact of salivary Zn levels on dental caries. *Int J Dent*. 2018;2018:8137915. doi:10.1155/2018/8137915