Trace Elements in Human Skeletal Material from the Great Moravian Burial Site at Mikulčice-Kostelisko

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Differences in the inorganic bone content of trace elements between a group of adult males and females as well as between social groups with different grave equipment were discovered at the Slavic burial site of Mikulčice-Kostelisko in the sub-castle. Significant sexual dimorphisms were shown in the case of the elements Ca, Sr, Zn, Pb, Mg, Cu and Ba. For example, a higher content of zinc (406.1 ±586 μg Zn/kg of bone) was observed in the bones of males (N13) compared to those of females (N15) (161±53 μg Zn/kg of bone) (p< 0.02). The diet of males contained a higher proportion of proteins, most probably originating from a meat-based diet. When using the Spearman’s rank correlation coefficient to test femurs, it was found that concentration of certain elements decrease with age, significantly so in males – in the case of Yb (p= 0.008), even when correlated to calcium, while in the case of Ni, Y, Dy, Er, Tin, Lu, this association is borderline significant p= 0.053. The significance of multi-elemental analysis for the reconstruction of diet lies in the possibility of comparing elemental relationships that are not obvious in small groups of analysed elements.

Key words: trace elements – human skeletal remains – Great Moravian population – social status – diet – environment

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1. Introduction

The improvement of analytical methods in the past decades has made it possible to employ trace elements found in bone remains for the reconstruction of diet. The human diet is converted into the language of elements: not the separate components of the diet itself, but rather the sources of elements are determined. By determining the element whose movement within the food-chain is known, it is possible to determine whether the dietary regimen several years before death was based on a vegetal diet or on animal proteins.

Measurement of strontium concentrations in human bones became the basis for the method that led to the evaluation of the contribution of plant components and animal proteins to diet (BROWN 1973, 1974; SCHOENINGER 1979; SILLEN 1981; PRICE/KAVANAGH 1982; BLAKELY 1989; BURTON/PRICE 1999).

The metabolic similarities between strontium and calcium (incorporation into the bone, occupation of the same area within the apatite matrix, as well as the small fluctuations of calcium concentrations among living individuals) inspired some authors to use a strontium-calcium index as a gauge for the amount of strontium ingested in food (COMAR/SCOTT-RUSSELL/WASSERMAN 1957; PRICE/KAVANAGH 1982; SILLEN/KAVANAGH 1982). Another group assumed that the fluctuation of bone calcium

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is negligible and used strontium for the reconstruction separately (Toots/Voohries 1965; Brown 1974; Schoeninger 1979; Lambert/Szpunar/Buikstra 1979).

Like calcium, Sr$^{90}$ also moves within the food-chain and is easily absorbed by plants. It is accessible to grazing animals on the surface of leaves. When it is absorbed into the leaves, it mixes in various proportions with plant calcium. In man, it is deposited and retained in bones and transported into milk and the developing foetus. Nuclear explosions endanger the biosphere with the presence of Sr$^{90}$. It is a paradox that this originally purely military research, aimed at the destruction of the human population, also provided information that gave us the impulse to understand life in the past.

Comparison of Sr and barium concentrations in carnivores and herbivores showed that Sr is much less sensitive to dietary variations than is Ba. Barium is concentrated on the bone surface like manganese (Lambert et al. 1984). Parker/Toots (1980) documented a certain degree of contamination. Barium is thus excellent for distinguishing the type of diet. However, archaeological results are probably endangered by diagenetic effects.

Zn seems to be exempt from diagenetic processes, at least in the case of burial grounds in non-acid soils, several thousands years old. Whilst Sr decreases in the succession from herbivores 400-500 ppm, omnivores 150-400 ppm, to carnivores 100-300 ppm, Zn concentrations increase in the same series from 90-150, 120-220, 175-250 ppm (Lambert et al. 1984; Reinhold et al. 1973; Reinhold 1975). Relatively high values of zinc are found in meat, and high concentrations of Zn can also be found in nuts and molluscs (Gilbert 1977).

Lead analysis provides pathological information that remains useful even in an anthropological context (Wittmers/Alich/Aufderheide 1981; Aufderheide et al. 1981, 1985; Aufderheide 1989).

Our objective was to reconstruct diet based on the analysis of elements contained in human skeletal remains. We did not determine individual components of the diet, but rather the sources of elements, which predominated in the given population.

The only elements that can be used for diet reconstruction are those whose concentration is minimally affected by the influence of the surrounding environment, or whose changes in concentration can be expressed mathematically. For this purpose, we chose zinc and strontium, as their concentrations in a buried skeleton are influenced only minimally by soil composition (Smrčka 2005; Sandford 1992). The chemistry of the enamel reflects that obtained through the diet of early childhood and thus has the potential to inform about changes in childhood diet (e.g. nursing and weaning). Also, because enamel retains elements of the diet during early childhood, it is most valuable for the study of human mobility and provenience. The amounts of strontium, barium and lead can vary geographically and thus may be potentially used in enamel to distinguish places of human origin (Burton 2008).

Multi-elemental chemical analysis was used to study skeletons from the Great Moravian burial site at Mikulčice-Kostelisko. The aim of this work was to determine the content of elements in the skeletons of the adult human population in order to reconstruct the diet and learn about the environment inhabited by the people from Mikulčice and its surroundings.

2. Materials

Samples from the proximal right femurs opposite the small trochanter were taken for the anthropological determination of age and sex from the twenty eight human adult skeletons (aged between 20-50 years) from the Slavic burial site at Mikulčice-Kostelisko in the sub-castle (Velemínský et al. 2005), deposited in the National Museum (Smrčka 2005).

The analysed sample of human skeletons from Mikulčice-Kostelisko included 14 males and 14 females (see Table 1).

Bone fragments were rid of any earth remnants using a PVC brush. They were then washed in deionised water and soaked in formic acid in order
to remove the diagenetically affected sections. Following thorough rinsing with deionised water and subsequent drying, the bone samples were coated in an agate dish for analytical precision.

Samples of soil from the given location were taken concomitantly with the bone samples. The soils were sifted using a sieve with 2 mm large apertures (mesh) and then dried in the laboratory until a constant weight was attained. Once dried, the soils were homogenised in an agate mill to an analytical precision.

### 3. Methods

#### 3.1 Chemical analysis

Exactly about 0.2 g of the bone sample was weighed out and transferred into a 50 ml graduated glass and 5 ml of concentrated HNO₃ was poured over it. The sample was then slowly dissolved by careful warming on a heater plate, at approx. 80° C. Upon cooling, deionised water was added to each graduated glass. A blind test was prepared for every series of ten samples. The
overall mineralisation of the soil was conducted as follows. Exactly around 0.2 g of the soil placed in a platinum dish was annealed in an oven (Linn, FRG) up to a temperature of 450°C. Upon cooling, 10 ml of concentrated HF and 0.5 ml of concentrated HClO₄ were poured over the sample, which was then defumed in a fume chamber into moist residue. Subsequently, the residue in the dish was dissolved in water and with the addition of 2 ml HNO₃ transferred to a 100 ml graduated glass. Merck brand acids and deionised water from MilliQPlus (Millipore, USA) were used to prepare the solutions.

The contents of Ca, Mg, K, Fe, Na in the mineralised samples was determined using flame atomic absorption spectrometry (Spectra AA 200 HT, Varian Australia) under the conditions recommended by the manufacturer.

The contents of V, Cr, Co, Ni, As, Y, Cd, Ba, Pb, U, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu in the mineralised samples was determined using mass spectrometry with induction-bound plasma (PQ 3, VG Elemental, Great Britain) under the following conditions: energy output ICP 1350 W, measuring mode “peak jump”, duration of measurement 3 x 50 s, \(^{115}\)In optimised parameters of ionic optics, gas flow volume 13.5 l/min cooling, 0.7 l/min auxiliary, 0.65 l/min nebuliser, internal standards In, Re, Sc.

The Asrasol brand solution (Analytika, Czech Republic) was used to calibrate both measurements.

The method was tested in certified reference bone material, NIST SRM 1486.

3.2 Statistical analysis

Twenty-eight samples from human adult skeletons were used for statistical analysis.

Trace elements following the analysis of samples from 28 human skeletons were statistically tested from the aspect of sexual dimorphisms in groups of males (N=14) and females (N=14) and in all groups together.

Furthermore, testing was conducted in groups of garnitures according to grave equipment, in accordance with Stloukal’s classification (1-rich grave equipment, 2-poor grave equipment) (Stloukal 1970). Division according to V. Hrubý (1955) was not conducted in view of the small number of elements within the groups.

The Statistika programs, version 7.0 from StatSoft were used in the evaluation.

4. Results

4.1 Sexual dimorphisms in the content of trace elements in human skeletons

An overview of the content of elements in the skeletons from the burial site at Mikulčice-Kostelisko for which statistically significant differences were recorded is detailed in Table 2.

Differences between the groups of adult males and females with both rich (“Stloukal 1”) and poor (“Stloukal 2”) grave equipment were found in the bone contents of the elements Ca, Sr, Zn, Pb, Mg, Cu (see Table 2a). An analysis for every statistically significant element was conducted on the basis of testing of the elements content in the skeletons using the variance analysis according to sex (see Table 2b, 2c).

Ca contents in the bones of females (N=15) (294 ± 313.3 ± 31 643 μg Ca/g of bone) were higher than in those of males (N=13) (278 661 ± 312 33 μg Ca/g of bone). Even in the bones of eleven females with poor grave equipment (304255 ± 23611 μg), the Ca contents were higher than in the case of the nine males (287694 ± 26811 μg) (p<0,044) with poor grave equipment. The soil from the burial site contained 6825 μg Ca/g of soil.

Sr contents in the bones of males (N=13) (390 ± 112 μg Sr/g of bone) were higher than in those of females (N=15) (348 ± 83 μg Sr/g of bone). Even in the bones of eleven females with poor grave equipment (304255 ± 23611 μg), the Sr contents were higher than in the case of the nine males (287694 ± 26811 μg) (p<0,044) with poor grave equipment. The soil from the burial site contained 6825 μg Ca/g of soil.

Sr contents in the bones of males (N=13) (390 ± 112 μg Sr/g of bone) were higher than in those of females (N=15) (348 ± 83 μg Sr/g of bone). Even in the bones of nine males with poor grave equipment (418 ± 107 μg/g), the Sr contents were higher than in the case of the eleven females (364 ± 72 μg/g) also with poor equipment.

The Sr contents in the bones of four males with rich grave equipment (329 ± 111 μg/g) were higher than in the case of the four females also with rich equipment (302 ± 106 μg/g).
The soil from the burial site contained 94 μg Sr/g soil.

Zn contents in the bones of males (N=13) were higher (406.1 ± 586 μg Zn/g of bone) than in those of females (N=15) (161 ± 53 μg Zn/g of bone) (p<0.02). Even in the bones of eight males with poor grave equipment (494 ± 674 μg/g), the Zn contents were higher than in the case of eleven females (154 ± 44μg) (p=0.048) also with poor equipment.

The soil from the burial site contained 29 μg Zn/g of soil.

Pb contents in the bones of males (N=13) (2.22 ± 5.31 μg Pb/g of bone) were higher than in those of females (N=15) (0.25 ± 0.23 μg Pb/g of bone). In nine males with poor grave equipment, the bone content of lead (2.91 ± 6.36 μg/g) was higher than in the case of the eleven females (0.27 ± 0.27 μg/g) (p<0.001) also with poor equipment.

The Pb content in the bones of four males with rich grave equipment (0.65 ± 0.5 μg/g) was also higher than in the case of four females with rich equipment (0.18 ± 0.06 μg/g) (p<0.09).
soil from the burial site contained 10.8 μg Pb/g of soil.

*Mg contents* in the bones of females (N=15) (1029.7 ± 165 μg Mg/g of bone) were higher than in those of males (N=13) (933.8 ± 160.64 μg Mg/g of bone). Even in the bones of eleven females with poor grave equipment (1046 ± 109 μg/g), the magnesium contents were higher than in the case of nine males (967 ± 149 μg/g) (p=0.09) with poor equipment. The soil from the burial site contained 1614 μg Mg/g of soil.

*Cu contents* in the bones of males (N=13) (5.4 ± 2.42 μg Cu/g of bone) were higher than in those of females (N=15) (4.2 ± 2.02 μg Cu/g of bone). Even in four males with rich grave equipment (5.23 ± 2.12 μg Cu/g of bone), the copper contents were higher than in the case of four females (3.25 ± 2.01 μg/g) (p=0.083) with rich equipment. The soil from the burial site contained 14.30 μg Cu/g of soil.

*Ba contents* in the bones of males (N=13) (57.1 ± 27.57 μg Ba/g of bone) were higher than in those of females (N=15) (4.2 ± 2.02 μg Ba/g of bone). Even in nine males with poor grave equipment (67.17 ± 26.57 μg/g), the Ba contents were higher than in the case of eleven females (43.4 ± 17.59) (p=0.028) also with poor equipment. The soil from the burial site contained 370 μg Ba/g of soil.

### 4.2 Bone contents of trace elements in 28 individuals from the burial site at Mikulčice and association with age

Significant differences (p<0.008 up to 0.053) in the bone content were discovered in the case of the following elements: Ni, Y, Dy, Er, Tm,Yb, Lu (Table 3). When testing femurs using the Spearman’s rank correlation coefficient, we found a decrease in the content of elements in association with age. This decrement was significant in males – in the case of Yb (p=0.008), even when correlated to calcium. In the case of Ni, Y, Dy, Er, Tm, Lu, this association was borderline significant p=0.053.

### 4.3 Social relations deduced from the different bone contents of elements in the case of graves with rich and poor equipment at the burial site

We took the grave equipment as the basis for our evaluation of social relations. We took into account the classification according to Stloukal (1970) (1 = rich graves, 2 = poor graves), as well as that of Hrubý (1955) (1, 2 = rich graves, 3 to 5 = poor graves).

Five (of eight) males with poor grave equipment (grave 1794, grave 1907, grave 1860, grave 1861, grave 1945) had a higher zinc content than did the four wealthy males (207 ± 29 μg/g) (grave 1809, grave 908, grave 1912, grave 1975). A content of over 300 μg Zn/g of bone was found in graves 1907 and 1794. Males had the highest content of zinc (Graph 1a).

Rich males had more zinc than rich females. Only one female, rich /1/ according to Stloukal and poor according to Hrubý (grade 3) (1777A), had more zinc than rich males (Graph 2a, 2b).

Tables 4a, 4b summarise the statistical results of the double sorting analysis, which tested concurrently the hypothesis regarding the influence of sex and grave paraphernalia. Significant results were acquired for zinc, lead and barium.

### 5. Discussion

The allocation of trace elements into distribution groups of the human population (males and females) at the Slavic burial site allowed us to study the content of individual elements in the skeletal remains of these groups, as well as the relationships between these two groups.

*In the human population of Slavs*, the bone content of the elements Zn, Pb, Mn, Fe, Cu, Mg, Cr, Co, Ni, As, Y, La, Ce, Pr, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu and U follows the age growth curve. In this case, these elements were analysed in the adult population of males and females. They included metal elements predominating in the proximal and distal sections of bones, of which many play a role during growth in the form of metalloenzymes (Smrčka 2005).
In the femurs of Slavs from the Mikulčice burial site at Kostelisko, testing using Spearman’s rank correlation coefficient uncovered a decrease of certain elements with age, namely in males. We recorded a significant decrease in males of Yb (p=0.008) in correlation with calcium. In the case of Ni, Y, Dy, Er, Tm, Lu, this correlation was borderline significant p=0.053.

The decrease of the essential element, nickel whose daily-recommended dose is 25-35 μg (NIELSEN 2000) was quite notable. Increased losses may occur in persons with increased intake of fats, sugars and diary products (UNDERWOOD 1977).

The diet of adult individuals from the Mikulčice-Kostelisko burial site differed, depending on sex and wealth.

Higher contents of zinc (406.1 ± 586 μg Zn/g of bone) were discovered in the bones of males (N=13) compared to those of females (N=15) (161 ± 53 μg Zn/g of bone) (p ≤ 0.02). The diet of males consisted of a higher proportion of proteins, probably from a meat-based diet. The relatively high values of bone zinc originate from a meat-based diet, nuts and molluscs (GILBERT 1977, 1985).

The diet of females consisted predominantly of a vegetal component. All females had more strontium than zinc. None of the females had strontium levels over 500 μg and none of the females had zinc levels over 300 μg. One woman (grave 1648) had a zinc content of less than 100 μg, and such a value was not recorded in any man (Graph 1b).
The female from grave 1973 (over 50 years) with rich grave equipment and the male from grave 1809 (35-50 years) both had an increased bone content of sodium, which could confirm an increased proportion of a millet-based diet (Boardman 1975).

According to historical reports, the Slavs took a liking to millet. It was an expensive cereal, though. Millet mash was often a festive or prestigious meal (Beranová 2005).

6. Conclusion

The bone content of adult individuals from the Great Moravian burial site at Mikulčice-Kostelisko differed, depending on sex and wealth. Significant sexual dimorphisms were shown in the case of the elements Ca, Sr, Zn, Pb, Mg, Cu and Ba. For example, higher contents of zinc (406.1 ± 586 μg Zn/g of bone) were found in the bones of males (N=13) compared to those of females (p<0.02). The diet of males consisted of a higher proportion of proteins, probably from a meat-based diet.

Testing of femurs using the Spearman’s rank correlation coefficient showed a decrease in element of content with age, significantly so in males – in the case of Yb (p=0.008), even when correlated to calcium. In the case of Ni, Y, Dy, Er, Tm, Lu, this association was borderline significant p=0.053.

The importance of multi-elemental analysis in the reconstruction of diet lies in the possibility of comparing elemental relations that are not obvious in the case of small groups of analysed elements.

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Table 2. Contents of stable elements in the skeletons from the Mikulčice-Kostelisko burial site.

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Table 2a. Contents of stable elements in the skeletons from the Mikulčice-Kostelisko burial site in relation to sex and grave goods.

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<td>160.64</td>
<td>984.00</td>
<td>1029.65</td>
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<td>38.73</td>
<td>145.80</td>
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<td>80.63</td>
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Table 2b. Analysis of the variance of element content in the skeleton from Mikulčice-Kostelisko in relation to sex.

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<tr>
<th>Element</th>
<th>Group rich (Stloukal 1970) (Males=4, Females=4)</th>
<th>Group poor (Stloukal 1970) (Males = 9(8), Females = 11)</th>
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</thead>
<tbody>
<tr>
<td>Ca</td>
<td>0.113 1 6 0.748 0.485 0.386</td>
<td>Ca 0.112 1 6 0.745 0.533 0.444</td>
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<tr>
<td>Sr</td>
<td>0.114 1 6 0.747 0.908 0.564</td>
<td>Sr 0.113 1 6 0.201 0.176 0.210</td>
</tr>
<tr>
<td>Zn</td>
<td>0.405 1 6 0.548 0.300 0.564</td>
<td>Zn 5.746 1 17 0.028 0.029 0.048 5% 5%</td>
</tr>
<tr>
<td>Pb</td>
<td>4.073 1 6 0.090 0.007 0.149</td>
<td>Pb 16.309 1 17 0.001 0.776 0.002 5% 1%</td>
</tr>
<tr>
<td>Mn</td>
<td>0.191 1 6 0.677 0.525 0.386</td>
<td>Mn 1.066 1 18 0.316 0.141 0.184</td>
</tr>
<tr>
<td>Fe</td>
<td>0.010 1 6 0.922 0.782 0.564</td>
<td>Fe 0.156 1 18 0.697 0.302 0.621</td>
</tr>
<tr>
<td>Mg</td>
<td>0.531 1 6 0.494 0.304 0.386</td>
<td>Mg 1.888 1 18 0.186 0.452 0.087 10%</td>
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<td>K</td>
<td>2.704 1 6 0.151 0.084 0.248</td>
<td>K 0.040 1 18 0.844 0.371 0.790</td>
</tr>
<tr>
<td>Na</td>
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<td>Na 0.032 1 18 0.860 0.889 0.676</td>
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<tr>
<td>Cu</td>
<td>1.846 1 6 0.223 0.696 0.083</td>
<td>Cu 0.923 1 18 0.349 0.356 0.470</td>
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<tr>
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<tr>
<td>Cr</td>
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<tr>
<td>As</td>
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<td>As 0.163 1 18 0.691 0.347 0.342</td>
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Table 2c. Analysis of the variance of element content in the skeleton from Mikulci-Kostelisko in relation to sex.

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<th>Group rich (Stloukal 1970) (Males=4, Females=4)</th>
<th>Group poor (Stloukal 1970) (Males = 9(8), Females =11)</th>
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<tr>
<td>Ba</td>
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<td>Ho</td>
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<tr>
<td>Er</td>
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<tr>
<td>Tm</td>
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<tr>
<td>Yb</td>
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<tr>
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<th>p</th>
<th>Levene p</th>
<th>Mann-Whitney p</th>
<th>Signif. AOV</th>
<th>Signif. MWh</th>
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<tr>
<td>Ratio</td>
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<td>SV error</td>
<td>p</td>
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<td>Signif. AOV</td>
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Table 3. Association with age of the trace element content within the burial site Mikulčice-Kostelisko using Spearman's rank correlation coefficient.

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Tables 4a, 4b. Analysis of the variance of the double sorting of the influence of sex and grave equipment on the content of trace elements in the skeletons from the Mikulčice-Kostelisko burial site.

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