

ANALYSIS OF TRACE ELEMENTS IN THE TEETH OF INDIVIDUALS FROM THE FORMER CRYPT IN ST. CATHERINE MONASTERY IN DECHTICE (DISTRICT TRNAVA, SLOVAKIA)

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ABSTRACT

The present article provides results of analyses of trace elements from dental tissues and their relations with social status, dietary habits, and pathological changes in vertebrae in the skeletal remains from the former family crypt in the St. Catherine Church ruins. Three aristocratic families (the Labsánszky from the Korlátko castle, the Erdődys and the Apponyis) were buried under St. Catherine church in the 18th century. Skeletal material from one of the three crypts was investigated. The family allegiance of these skeletal remains is still not known; our considerations based on the historical sources indicated the Labsánszky family. The concentrations of Ca, Sr and Zn in 8 permanent teeth obtained from 8 individuals were analysed. The number of analysed teeth was limited by the number of buried individuals and the preservation state of the skulls. Concentrations of the trace elements and their ratios – a relatively low content of strontium and a higher concentration of zinc – indicated a rich protein diet. Despite the small number of teeth analysed, the results are relatively homogenous and show that probably all of the buried persons had belonged to a higher society. The results were also confirmed indirectly by the palaeopathological findings in the bones of the postcranial skeleton. The Forestier disease (DISH) was diagnosed in three individuals at minimum, which can also indicate that they suffered from obesity or type 2 diabetes. However, DISH is a hereditary disease; therefore we must also consider the familiar appearance.

INTRODUCTION

Analyses of trace elements and stable isotopes have become a very useful research tool in physical anthropology for the

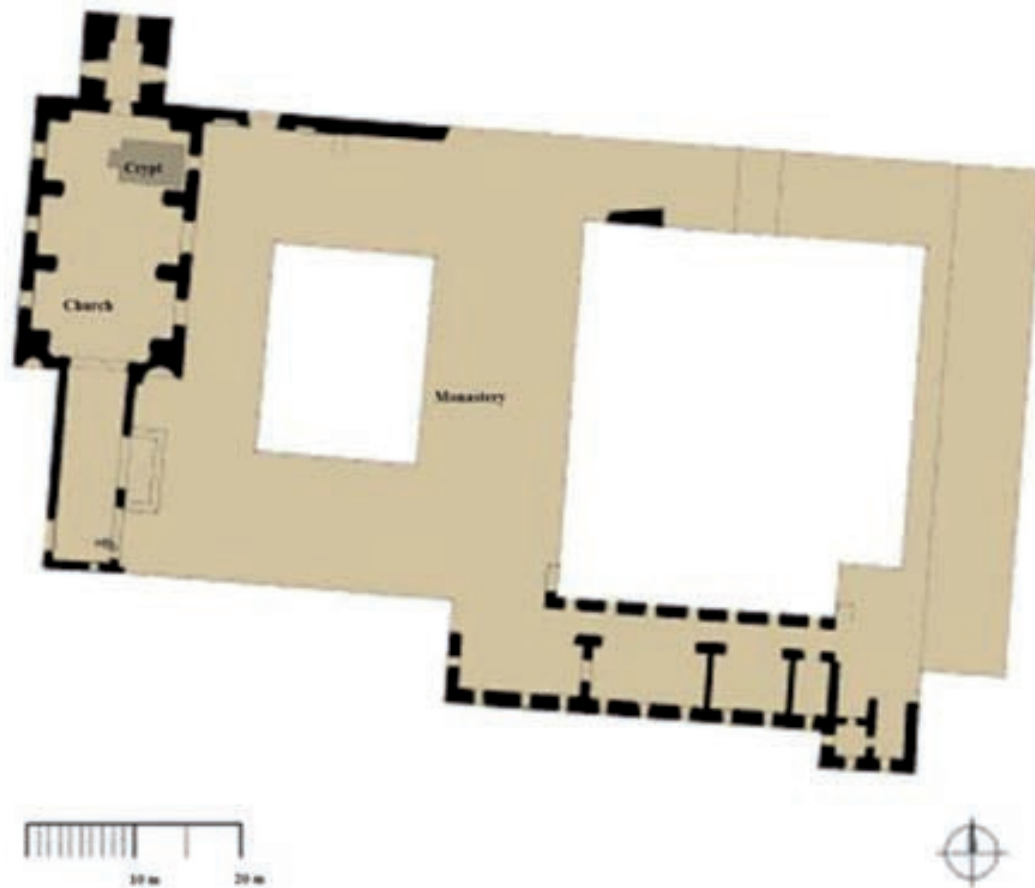


Figure 1
The ground plan of the crypt

last three decades. They offer the possibility of studying the biological condition of human groups [1, 2, 3, 4], dietary habits of past populations [5, 6, 7, 8], processes of diagenesis [9, 10], as well as the aetiology of some diseases [11]. The trace element analyses are also used for studying the type of nutrition in connection with the socioeconomic position of individuals or whole population groups [12].

In palaeoanthropological research focused on diet reconstruction a wide spectrum of macro- and microelements has usually been analysed. Strontium, zinc, calcium, barium, copper, and iron provide the best information about the biological status of the studied populations [6, 12].

The reliability of zinc concentration for diet reconstruction has been widely discussed [13]. Some authors [8, 13] proposed that the relation between zinc concentration and the type of nutrition is disputable. On the other hand, the

existence of a positive correlation between the amount of accumulated zinc and protein diet has been confirmed [14, 15]. However, strontium absorption and accumulation is in inverse proportion to an organism's position in the trophic pyramid. Plants absorb and accumulate strontium directly from the environment, while mammals accumulate it indirectly, by consumption of plants and other animals [7, 8, 11, 16, 17]. Some authors indicate that strontium and Sr/Ca ratio do not provide direct information on diet components and cannot be used as a straight indicator of the trophic position of organisms [8, 18].

However, in our study we tried to reconstruct the quality of alimentation based on the mentioned trace elements and to find relations to Forestier disease (DISH) and the aristocratic origin of individuals from the former family crypt of the St. Catherine church.



Figure 2
The 9th -12th thoracic vertebrae with suspected DISH

HISTORICAL AND ARCHAEOLOGICAL CONTEXT

The monastic complex of St. Catherine is situated in Western Slovakia, around 20 kilometres north of Trnava, close to the villages of Dechtice and Naháč.

The beginning of the monastery dates back to the end of 16th century, when St. Catherine appeared reputedly several times in this place. The cloister was built in 1618 by Count Christopher Erdődy for 12 Franciscan monks. Soon after the foundation the monastery was demolished and a larger one was built on its place. After rebuilding, which Count Erdődy's son Gabriel with his wife had finished, the church occupied rather a large area. There were eight altars at the time of dedication. Three familial vaults were also situated in the church, in which members of three aristocratic families (Erdődy, Labsánszky, and Apponyi from Korlátko) were buried.

In the 17th century, the monastery was on several occasions close to ruination. The monastery and church were stripped and burned by the armies of Juraj Rákóczi during the first rebellion. Later Turks hijacked the monastery and finally soldiers of Imre Thököly sacked the complex.

The monastery was closed on 22nd July 1786 by the order of Joseph II. After evacuation of the monks, the building was occupied by the Trnava's soldiers with army disabilities. After the closure of the monastery the vaults were robbed on many occasions. According to written sources, the first forced entry into aristocratic vaults was carried out in 1793. Count Joseph Erdődy, out of respect to the remains of his forefathers, purchased the desolate objects in 1797. However, he was unable to prevent the monastery from falling into ruins.

Further raids were made in the 19th century, when the inhabitants of the surrounding villages, who disassembled the buildings for use as building material, systematically looted the church and monastery. The temple floor and the crypt arch were also dismantled within this period. Some bones were bundled out of the coffins and later repeatedly placed in the crypt together with building waste.

In 1995, the volunteers and devotees began with conservation of the ruins. Archeological examination of this locality started in 1997 and its goals are to verify the written history of the monastery [19].

In 2000 and 2001, a part of the circumferential stall of the monastic building and of the crypt was revealed. Archeological examination in the church proceeded with geophysical investigation, specialised in searching for crypts and other cavities. As a result of this investigation, 3–4 places indicating the presence of cavities were located, while a marked anomaly was registered to the left side near the entrance [20, 21]. According to the canonical visitation of 1782, one of the three aristocratic crypts should be present here. A probe in this area uncovered a partially destroyed and disarranged crypt.

The crypt was built of brick; its inside had a rectangular shape with the dimensions of 350 cm x 190 cm and the end of the first third it was divided by a partition (*Figure 1*) [22]. The rolling vault finishing narrowly under the temple floor initially vaulted the crypt. All filling layers yielded skeletal remains mixed with building waste and stones. Only one incomplete skeleton in anatomical position was found in the crypt bottom. From the coffins, only a few fragments of wood ornamented with hemispherical rivets were preserved. Copper-plate, initially gold-plated, rivets were organised into letters, but the bad condition of disintegrated wood did not allow reconstruction of the text.

Although the remains were found in non-anatomical positions, we have supposed for the last 8 years that they had belonged exclusively to members of the Labsánszky family. Furthermore, the families of Erdődy and Apponyi had their own crypt, and there are records regarding removal of the remains from the church. If our assumptions are right will probably show the opening of the two remaining crypts in the next years.

MATERIAL AND METHODS

Anthropological and palaeopathological analysis

As the bones were mixed with the filling, craniums and postcranial bones were individually examined. A departure from this was one individual torso (pelvis and lower limb bones) lying in an anatomical position in the crypt bottom [23].

After counting the long bones, namely the number of humeri, it was determined that there were remains of at least 26 individuals present. The number of skulls was lower; however, it was possible to differentiate 24 skulls in different stages of preservation. It was not possible to allocate some of the fragments to any of the skull, thus it is probable that the number of craniums in the crypt was higher. The distribution of the skulls based on age and gender is shown in *Table 1*, the number of postcranial bones with regard to the laterality is in *Table 2*.

It is also very difficult to estimate the number of individuals actually buried in the crypt. According to the archaeologist, the crypt is rather small for storing more than 20 individuals. It is possible that some of the skeletal remains came secondarily from another crypt in the times of raids.

The skeletal material was examined using standard morphoscopic and morphometric methods [24, 25]. Gender and age were determined only in the craniological material using the methods of Acsádi and Nemeskéri [26] and Lovejoy [27].

We also observed developmental defects, pathological changes, and traumatic lesions in all the examined remains.

Table 1

Distribution of the skulls based on age (in years) and gender

Age	Juv	Ad	Ad II-Mat I	Mat	Mat II-Sen	Sen	Indeterminate	Total
Sex	(16–20)	(20–40)	(30–50)	(40–60)	(50–60)	(60+)		
Male		2	3	1	1			7
Probably male	1			2	1			4
Female	2	1			1			4
Probably female					2			2
Indeterminate		3				1	3	7
Total	3	6	3	3	5	1	3	24

Table 2

The number of postcranial bones with regard to laterality

	Dexter	Sinister	Indeterminate	Total
Clavicle	17	10	1	28
Scapula	17	17		34
Humerus	26	26		52
Ulna	24	16		40
Radius	17	19		36
Sacrum	19	x	x	19
Os coxae	20	19		39
Femur	23	23		46
Tibia	23	21	3	47
Fibula	12	11	2	25

Laboratory methods

The concentrations of trace elements in 8 permanent teeth obtained from 8 individuals were analysed (Table 3). The number of analysed teeth was limited by the preservation state of the odontological material as well as by the relatively low number of skulls. In order to minimise the effects of diagenesis, which is frequent in bones, the analysis was restricted to teeth [7]. All analysed teeth were well-preserved, intact, without dental caries and abrasion.

Before the analysis, each tooth was washed with distilled water and dried at room temperature. Then the teeth were crushed in an agate mortar and homogenised. Altogether, 0.5g of each sample was subjected to wet mineralisation in a mixture of 10ml HF, 1 ml HNO₃, and 1 ml HClO₄. On the next day, the samples were evaporated in a water bath to

approximately 1 ml volume. Then, the evaporation on a sand bath with a gradual addition of HF, HNO₃ and HClO₄ continued until the escape of dense smoke. Finally, 5 ml of saturated solution of H₃BO₃, 1 ml HNO₃ and HClO₄ was added and 1 ml of the samples was evaporated on a sand bath until dry. The dry residues were diluted with redistilled water, warmed in a water bath and, after the addition of 5 ml HNO₃, heated to a temperature of about 150 °C on a sand bath for 5 minutes. After cooling, the samples were transferred into 50 ml measuring flasks and diluted with spectrally pure water. Blind tests were prepared parallel to the sample preparation to determine the analytical background. Analyses were made by optical emission spectrometry with inductively coupled plasma using the spectrometer ICP OES Jobin Yvon 70 Plus (France).

The contents of Ca, Zn, and Sr were examined. Two soil samples obtained during excavation of the grave were also subjected to analysis, the pH value and the same elemental concentrations were determined.

Statistical analysis

Data analysis was performed in R [28]. We used the Kolmogorov – Smirnov Goodness-of-Fit test for testing data normality (for all of the variables we did not reject null hypothesis about normality – all p-values were greater than 0.8). One sample Student t-test was used for testing if the content of trace elements in the teeth could be affected by diagenetic processes in the soil. T-test for zero linear correlation (measured by the Pearson product-moment correlation coefficient) was used to test association between the variables (Ca-Zn, Zn-Sr, and Sr-Ca). For all statistical tests, the significance level α was equal to 0.05.

RESULTS AND DISCUSSION

The pH values of the soil from the crypt were 8.66, or 8.91. The concentrations of the examined elements determined in the soil are listed in *Table 4*; descriptive statistics of the concentrations of elements in human teeth are shown in *Table 5*. Linear association between the variables Ca-Zn, Zn-Sr, and Sr-Ca was not statistically significant (all p-values were greater than 0.2).

Due to the small number of soil samples, we tested the equality of the mean value of each parameter in teeth (Ca, Sr, and Zn) with the representative soil samples for determination of possible diagenetic processes (*Table 6*).

According to the concentration gradient theory, ions from higher concentration areas tend to move to sites of lower concentration [8]. We did not find any differences between the concentrations of Sr in teeth and soil samples. On the other hand, significant differences were found in the Ca and Zn concentrations between teeth and soil. However, in the case of strontium diagenetic processes cannot be excluded, concentrations of calcium and zinc were not affected by diagenesis and indicate that ionic movement from soil to teeth would not be expected.

Since the buried individuals probably belonged to members of an aristocratic family, we supposed that their diet had been abundant in animal proteins. The results of the trace element analysis as well as the ratios of examined elements confirmed our assumptions; a relatively low content of strontium and a higher concentration of zinc expressly indicated a rich protein diet (*Table 5*). In spite of a small sample of teeth, the results are relatively homogenous and indicate that all of the buried persons could belong to a higher society. Similar

Table 3

List of analysed skulls and teeth

Skull	Gender	Age (in years)	Analysed tooth (FDI)
K01	M	30–40	17
K03	M	30–50	35
K04	M	30–50	45
K05	NA	20–40	28
K07	M	20–30	18
K09	M	45+	15
K12	M	30–50	48
K13	pM	16–20	14

FDI – World Dental Federation notation

M – Male

pM – Probably male

NA – Indeterminate gender

results were found by Prokeš and Hegrová [29]. Chemical analysis of the skeletal remains of Dietrichstein family members showed that they had consumed food rich in meat and pastry, and poor in milk, fruit, and vegetables.

We tried to confirm these facts also indirectly by the palaeopathological findings in bones of the postcranial skeleton. All pathological lesions were examined in isolated bones because, as mentioned above, all remains were mixed up in the crypt.

In the sample examined, a suspicion of DISH was discovered in 7 thoracic vertebrae from the total number of 130 (5.4 %), which belong to a minimum of one, and to a maximum of three separate individuals. In the first case, two incompletely connected thoracic vertebrae had monumental bridging osteophytes in the right frontal surface of the body (*Figure 2*). The next case was a thoracic vertebra with a monumental osteophyte in the right frontal side of the corpus bottom. The last finding consisted of four vertebrae (9th–12th thoracic vertebrae from one individual). The first vertebra is not fused; the last three ones have already formed a block (*Figure 3*). In the last case the diagnosis of DISH is more likely; on the other hand, in the first two cases it is uncertain because of the small number of vertebrae.

Hyperostotic changes were also found in the further three pelvic bones (the frequency of this pathological change reached 7.7 %); this could be related to the DISH illness, too.

Table 4

Concentrations of trace elements in the soil from the crypt

Sample	pH	Ca (%)	Sr (mg/kg)	Zn (mg/kg)	Sr/Zn	Zn/Ca	Sr/Ca
K 1	8.66	13.75	74.9	34.9	2.14	2.54	5.44
K 2	8.91	20.03	73.9	28.2	2.62	1.41	3.69

Table 5

Concentration of trace elements in human teeth – descriptive statistics

	N	Median	Mean	SD
Ca	8	30.10	30.00	0.80
Sr	8	70.55	72.78	12.35
Zn	8	226.00	233.88	56.23
Sr/Zn	8	0.32	0.34	0.13
Zn/Ca	8	7.51	7.81	1.91
Sr/Ca	8	2.34	2.43	0.44

SD – standard deviation

Table 6

Results of one sample Student t-test used for testing the possible diagenetic processes

	Soil sample	t-statistics	p-value
Ca	K1	57.78	<0.0001*
	K2	35.46	<0.0001*
Sr	K1	-0.49	0.6413
	K2	-0.26	0.8040
Zn	K1	10.01	<0.0001*
	K2	10.35	<0.0001*

*statistically significant results (p-value <α=0.05)

In the sample of skeletal remains from St. Catherine's Monastery, one case of DISH was already described, namely in the torso of an individual lying on the crypt floor [23]. The skeletal remains belonged to a male, who died at the age of 44–52 years. The marked hyperostotic changes in the last three thoracic vertebrae with a tendency of connection to the block were observed in this individual. Hyperostotic changes were also present at the upper perimeter of pubic bones, within the *tuberositas iliaca*, *tuber ischiadicum*, and *ramus ossis ischii*, and in both trochanters of the femoral bones. The individual also had ossified rib cartilages that remained separated from the sternum. Trace elements in this individual could not be analysed due to the absence of the skull. However, it cannot be excluded that one of the exhumed skulls had belonged to this individual.

The DISH, known as Forestier disease, is characterised by changes in the spine, which are the result of ossification of the anterior longitudinal ligament and other spinal ligaments. Although, they may occur in all areas of the spine, they are usually most prominent in the thoracic region [30]. In the course of time, the ossification leads to ankylosis of variable numbers of vertebrae, but the intervertebral disc spaces and the facet joints are normal in the absence of other pathology. One of the features of the spinal manifestations of DISH is that changes are only found on the right-hand side of the thoracic region [31]. The aetiology of DISH is by no means certain, but one of the earliest suggestions was that it was related to obesity and insulin-independent diabetes mellitus. Later studies have, however, failed to confirm the relationship between DISH and diabetes, but have noted a number



Figure 3
The monumental bridging osteophytes in two thoracic vertebrae

of other metabolic abnormalities, including variations in lipid metabolism and hyperuricaemia and elevated levels of growth hormones [32]. DISH occurs frequently in human skeletal remains, particularly in those recovered from monastic cemeteries. It is hypothesised that “the monastic way of life” can predispose to DISH, and obesity as well as diabetes mellitus are considered as the potential co-factors for its development [31].

The familial appearance of DISH was considered to be rare, because the illness usually manifests itself in old age and genetic connections are difficult to evaluate retroactively. On the strength of the familial studies, a highly probable autosomal dominant heredity of DISH was found, and it also explains the connection with some metabolic diseases [33, 34].

CONCLUSION

The results of our analysis indicate that the skeletal remains were most likely members of higher society; they could belong to an aristocratic family. The results of the trace element analysis indicate that the examined population enjoyed a diet rich in proteins. Indirectly, it can also confirm the high prevalence of DISH. However, the high prevalence of Forestier disease can indicate the family relationships between the buried individuals. We know that the teeth sample examined is relatively small, but we try to use all possibilities that would help us to identify a family allegiance of the buried skeletal remains.

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