Anthrop. Anz. 70/2 (2013), pp. 229–248
J. Biol. Clinic. Anthrop.
Stuttgart, July 2013

Article

Dietary reconstruction from trace element analysis and dental microwear in an Early Medieval population from Gáň (Galanta district, Slovakia)

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With 6 figures and 8 tables

Summary: The aim of the study was to determine the diet of an historical human population using the trace elements in dental tissues and dental buccal microwear. Although 38 individuals had been buried in the cemetery, preservation of the remains did not allow analysis of all of them. A total of 13 individuals were analysed, of which the samples for trace-element analysis consisted of 12 permanent premolars from 12 individuals. Buccal microwear was studied in a sample of nine teeth from nine individuals. Both trace-element and microwear analyses were performed on eight individuals. All analyzed teeth were intact, with fully developed roots, without dental calculus and macro-abrasion.

Concentrations of Sr, Zn, and Ca, and their ratios, were used to determine the relative proportions of plant and animal protein in the diet. Samples were analyzed using optical emission spectrometry with inductively coupled plasma. The values of the Sr and Zn concentrations indicate that a diet of the investigated population was of a mixed character with approximately the same proportion of plants and meat in their food.

Buccal microwear was studied in molds of buccal surfaces and observed at 100× magnification with a scanning electron microscope (SEM). Length and orientation of striations were determined with the SigmaScan Pro 5.0 image analysis program. The results obtained from microwear analysis correspond with those from trace-element analysis and showed that the population consumed a mixed diet. The density of the scratches indicates that the diet contained a considerable vegetable component. The high number of vertical scratches and their high average length suggest that individuals also consumed a large portion of meat. The results of both analyses showed that there were also individuals whose diet had probably been poor, i.e. richer in animal protein, which probably could be related to their health or social status in the population.

Key words: Paleonutrition, strontium, zinc, buccal microwear, Slavs, Early Middle Age.

DOI: 10.1127/0003-5548/2013/0256 0003-5548/11/0256 $ 5.00

Introduction

There are several approaches to reconstruction of the way of life in historical populations. The concentrations of macro- and microelements in bone and dental tissues, and dental buccal microwear have been used for reconstruction of the paleodiet for several decades (Sillen & Kavanagh 1982, Klepinger 1984, Pérez-Pérez & Lalueza 1992, Wolfsperger 1992, Larsen 1997, Głąb et al. 2001). Analyses of macro- and microelements have offered the possibility of studying the dietary habits, processes of diagenesis (Pérez-Pérez & Lalueza 1992, Wolfsperger 1992, Burton & Wright 1995, Głąb et al. 2001, Bornikowska & Szostek 2001, Szostek et al. 2003[2006]), illnesses and pathological conditions (Gleń-Haduch et al. 1997), as well as the social status of individuals from contemporary and historic populations (Schutkowski 1995, Schutkowski et al. 1999, Szostek & Głąb 2001).

From a wide spectrum of macro- and microelements, the concentrations of calcium, strontium, zinc, phosphorus, barium, copper, and iron provide the most accurate information on the biological status of the examined populations (Schutkowski 1995, Szostek & Głąb 2001). Strontium (Sr) and zinc (Zr) belong to the most frequent trace elements examined in paleodietary reconstruction.

Sr is easily absorbed by plants from the soil, and subsequently in leaves mixed with calcium (Ca) in varying ratios. After the consumption of plant food by humans, Sr, together with Ca, is deposited in the hard tissues, which contain up to 99.1 % of the body's Sr and 98.6 % of its Ca. In the soft tissues, and also in food of animal origin (carnivorous diet), Sr and Ca are deposited in low concentrations (Schroeder et al. 1972). In skeletal material the concentration of Sr is in inverse proportion to an organism's position in the trophic pyramid, hence the higher the individual is in the food chain, the smaller amount of Sr bone and dental tissues contains. The highest concentration of Sr is in plants, because they absorb it directly from the environment, while mammals accumulate Sr indirectly by consumption of plants or other animals. Herbivores have higher Sr concentrations than carnivores, and omnivores (like humans) are intermediate between herbivores and carnivores (Sandford 1992, Larsen 1997, Szostek & Głąb 2001, Szostek et al. 2003, Sponheimer et al. 2005). The highest value of Sr is in herbivores (400–500 ppm), next is omnivores (150–400 ppm), and the lowest is in carnivores (100–300 ppm; Lambert et al. 1984). Low levels of Sr do not necessarily mean that the diet has been enriched with animal protein, because these levels can be masked by consumption of a diet rich in minerals (Szostek et al. 2009, Schutkowski 2000, 2002). It is known that the Sr content in hard tissues changes with the age of individuals. Newborns have low Sr concentrations in bones because of discrimination against Sr by the mother's placenta, and equally low during breastfeeding. Significant fluctuations in the Sr values occur during growth and transition from breastfeeding to solid food; therefore it is recommended to investigate mainly adults (Smrčka 2005).

Zn, in contrast with Sr, is accumulated in soft tissues, so it is presented in higher concentrations in a diet rich in animal protein. The lowest values of Zn are in herbivores (90–150 ppm), next in omnivores (120–220 ppm), and the highest in carnivores (175–250 ppm; Lambert et al. 1984). According to Ezzo (1994), the significance of different bone Zn levels is controversial, because the metabolic pathways of Zn in bone are still not fully understood. Analyses of bone Zn concentrations for paleodiet reconstruction have been widely discussed. Sandford & Weaver (2000) proposed that the relationship between Zn concentration and mode of nutrition was disputable and not wholly objectively documented. On the other hand, however, they indicated a positive correlation between the amount of accumulated zinc and a protein diet. Similar conclusions were propounded by Smrčka (2005), Schutkowski et al. (1999) and Herrmann & Grupe (1988) who reported that Sr, Zn, and Sr/Zn, compared to the value of the Sr/Ca ratio, could be used in studies aimed at determining the dominant dietary components in population assays.

The reconstruction of dietary habits in historical populations is also possible by using microwear changes on occlusal and buccal surfaces of teeth. The microscopic defects on the enamel surface are caused by abrasive particles in the diet, such as phytoliths, particles from the millstones used for grain milling, or sand particles (Gordon 1982). Phytoliths are micro-crystals of silica (SiO_2), calcium oxalate (CaC_2O_4) or carbonates found in the plant tissues. In the process of vegetable food mastication, the phytoliths are liberated and teeth are exposed to their grinding effect. The formation of microwear damage is strongly influenced by the presence of abrasive elements in food, acidity, hardness of diet, force, and also the direction of activity of the maxillo-mandibular apparatus (Teaford 1994).

Grine et al. (2012) recommend assessing the occlusal microwear changes because neither the buccal nor the lingual sides of the molars are employed in food processing and it has never been made clear why they should preserve wear features relating to food items that are processed occlusally. Pérez-Pérez et al. (1994), however, found that the abrasive particles scratch the buccal enamel surface, where pits are rarely formed and scratches are the only relevant features. It has also been argued that, because of its unique formation dynamics, buccal microwear takes longer to form (and turnover) than occlusal microwear and that it therefore overcomes the "Last Supper Effect" (Romero & De Juan 2007). The buccal microwear pattern seems to be conservative within individuals, independent of the analyzed tooth, and the inter-individual variability seems to be significantly larger than the intra-individual variability (Pérez-Pérez et al. 1994, Lalueza et al. 1996, Pérez-Pérez et al. 2003).

The microscopic defects on the enamel surfaces are classified into three categories: scratches, pits, and other surface defects (Gordon 1982). The number, length, and orientation of the microabrasion defects of a buccal dental surface have great informative value for the interpretation of nutritional preferences. It has been proven that, in a population fed by mixed or meat food, there was a tendency to a low density of scratches and a higher proportion of vertical scratches while in agricultural populations, with a higher content of tassel and a higher ratio of abrasive elements (phytoliths) in food, horizontal scratches dominated and their density was higher (Lalueza et al. 1996). It is assumed that the occurrence of scratches presented in historical agricultural populations was also influenced by the grinding of seeds with a stone mill (Del Rincón 1998). Even contemporary populations from arid areas of a desert manifest greater wear because of the presence of abrasive sandy elements in food (Smith 1984).

Research focused on dietary habits in historical populations from Slovakia began only a few years ago. Šefčáková (2003) and Šefčáková & Krištín (2001a, 2001b) analyzed samples of cortex and spongiosis of femurs in individuals from the Early Medieval cemetery in Borovce (Slovakia). Other studies, focused on dietary reconstruction, used only dental tissues for analyses (Bodoriková et al. 2009, Bodoriková et al. 2010, Domonkošová Tibenská et al. 2009, Domonkošová Tibenská & Bodoriková

2010, Domonkošová Tibenská et al. 2010); however in addition to the analysis of trace elements, dental microwear changes were evaluated as well.

The aim of this study was the reconstruction of dietary habits on the basis of Sr and Zn concentrations in dental tissues and dental buccal microwear in a population from the Early Medieval cemetery in Gáň dated to the 9th–10th c. AD. The results of the analysis from Gáň were compared with the previous study (Lalueza et al. 1996) of samples of 153 molar teeth from different modern hunter-gatherer, pastorals, and agriculturalist groups, with different diets (Inuit, "Fuegian" Indians, Bushmen, Australian Aborigines, Andaman Islanders, Indians from Vancouver, Veddahs, Tasmanians, Lapps, and Hindus), preserved in museum collections.

This study continues the research of Domonkošová Tibenská et al. (2010) and Bodoriková et al. (2009) that analyzed cemeteries from the same locality dated to the Migration Period (5th–6th c. AD). Consequently we compared reconstructed dietary habits of two populations from Gáň, which lived in the same area separated by 400–500 years.

Material and methods

The locality of Gáň (Galanta district) is located in western Slovakia about 50 km east of Bratislava (Fig. 1). A cemetery was excavated during rescue archaeological research in 2008. On the basis of burial furnishing, the cemetery was dated back to 9th–10th c. AD. In summer 2007, a cemetery dated to the Migration Period (5th–6th c. AD) was excavated in the same locality. To help distinguish these two burial grounds, the cemetery dated to the 9th–10th c. AD was designated as Gáň C and the cemetery from the Migration period as Gáň A.

Cemetery Gáň C was relatively small, altogether 38 individuals were exhumed. Sex was determined using the methods of Acsádi & Nemeskéri (1970), Brůžek (2002), and Novotný (1979). Age at death was determined by methods of Martin & Saller (1957), Nemeskéri et al. (1960), Acsádi & Nemeskéri (1970), and Ferembach et al. (1980).

Trace-element concentrations were examined in dental tissues. Although 38 individuals were buried in the cemetery, teeth from only 12 individuals were suitable for the chemical analysis (Table 1). We examined 12 permanent teeth; all of them were premolars (ten first premolars and two second premolars). We selected only teeth which were not weakly fixed in

Fig. 1. The map of Slovakia with the cemetery of Gáň.

Table 1. List of examined individuals (N = 13) and analyzed teeth.

Grave number	Gender	Age	Burial furnishing	Analyzed teeth (FDI)	
				Trace elements (n = 12)	Microwear (n = 9)
CH 4	Female	50–60	No	14	–
CH 6	Undeter-mined	20–40	Yes	44	17
CH 7	Female	16–20	Yes	44	47
CH 9	Male	40–50	Yes	24	17
CH 10	Male	20–30	No	14	–
CH 16	Male	20–30	No	34	36
CH 17	Male	16–20	Yes	34	36
CH 19	Male	7–14	No	24	–
CH 23	Undeter-mined	16–20	No	44	–
CH 26	Male	30–40	No	25	36
CH 29	Female	20–30	No	14	16
CH 34	Female	5–6	No	–	46
CH 37	Male	20–30	No	45	45

FDI – World Dental Federation notation of teeth
N – number of individuals
n – number of samples

the alveoli or could be easily removed from the sockets in order to avoid destruction or damage of teeth. The low number of analyzed teeth was dependent on the state of preservation of the odontological material. We analyzed only intact teeth with fully developed roots, without dental caries, calculus, and abrasion.

Before the trace-element analysis, each tooth was washed with distilled water and dried at room temperature. The teeth were then crushed in an agate mortar and homogenized. A half gram of each sample was subjected to wet mineralization in a mixture of 10 ml HF, 1 ml HNO_3, and 1 ml $HClO_4$. The next day, the sample was evaporated on a water bath to approximately 1 ml volume. Evaporation was then continued on a sand bath with a gradual addition of HNO_3, HF, and $HClO_4$ until formation of a dense smoke. Finally, 5 ml of a saturated solution of H_3BO_3, 1 ml HNO_3, and $HClO_4$ were added and 1 ml of the sample was evaporated on a sand bath until dry. The dry residue was diluted with distilled water, warmed in a water bath and, after addition of 5 ml HNO_3, was heated to a temperature of about 150 °C on a sand bath for 5 minutes. After cooling, the sample was transferred to a 50 ml measuring flasks and diluted with spectroscopically pure water. Blind-test samples were prepared to determine the analytical background (Domonkošová Tibenská et al. 2010). The analyses were made by the optical emission spectrometry with inductively coupled plasma using an ICP OES Jobin Yvon 70 Plus (France) spectrometer at Geological Institute (Faculty of Natural Sciences, Comenius University in Bratislava, Slovakia). The amounts of Ca (in %), Zn, and Sr (in mg/kg) were determined.

The pH value and the same elemental concentrations were also determined in the soil samples obtained during grave excavation. We had only four soil samples; nevertheless, we tested them for the possibility of diagenesis.

Microwear changes were observed in nine teeth (eight molars and one premolar) from nine individuals (Table 1). Molds of the buccal surface were obtained for the purposes of dental microwear analysis in order to prevent the destruction of the original teeth. The tooth enamel surfaces were cleaned with 96 % ethanol. Three steps were imposed in the preparation of the replicas. In the first step, negative dental casts were obtained by Polyvinylsiloxane-elite HD light body fast setting and putty soft normal setting easy mix (producer Zhermack, Italy). The positive moulds were made using epoxy resin (Epoxy 372, P 11 – producer Movychem, Slovakia) in the second phase. Finally, the tooth replicas were sputter-coated with a gold layer at the State Geological Institute of Dionýz Štúr in Bratislava. SEM dental pictures were obtained with a JXA-840A-Electron probe micro-analyzer JEOL at the Faculty of Natural Sciences of the Comenius University in Bratislava. SEM pictures were taken on the buccal surface of mesiobuccal caruncula surface of the tooth avoiding both the occlusal and cervical thirds of the tooth. SEM pictures were digitalized as jpg format – 1000 × 800 pixels image. The following parameters were used for digitizing: ampere 3×10^{-9}A to 6×10^{-9}A, accelerating voltage 20 kV, 100× magnifications and working distance between 15 and 39 mm.

The selected area was cropped to include a dental area of 0.56 mm^2 (648 × 648 pixels) by the program ACDSee 7.0. Four categories of microwear scratches were differentiated using 45° orientation with the semi-automatic software SigmaScanPro 5.0: H – horizontal (0° to 22.5° and 157.5° to 180°), MD – mesio-distal (for the lower left and upper right teeth: 112.5 ° to 157.5°; for the lower right and upper left teeth: 22.5° to 67.5°), V – vertical (67.5° to 112.5°), and DM – disto-mesial (for the lower left and upper right teeth: 22.5° to 67.5°; for the lower right and upper left teeth: 112.5° to 157.5°).

Ten variables were analyzed 1) NH – number of H scratches; 2) NMD – number of MD scratches; 3) NV – number of V scratches; 4) NDM – number of DM scratches; 5) NT – total number of striations; 6) XH – average length of the H scratches; 7) XMD – average length of the MD scratches; 8) XV – average length of the V scratches; 9) XDM – average length of the DM scratches; 10) XT – average length of all scratches (Lalueza et al. 1996).

Statistical analyses were performed with R software (R Development Core Team 2012).

All of the hypotheses were tested against two-sided alternatives on significance level $\alpha = 0.05$. For multiple comparisons, Benjamini-Yekutieli (BY) correction of significance level (Benjamini & Yekutieli 2001) was applied. Normality assumption hypothesis was tested by Kolmogorov-Smirnov goodness-of-fit test without BY correction (Venables & Ripley 2002) where the null hypothesis for all of the variables was not rejected.

To test the process of diagenesis of macro- and microelements, we used two-independent sample Student t-tests with Welch approximation of degrees of freedom (Welch 1947) and BY correction comparing mean values of Sr, Zn, and Ca contents with concentrations found in four soil samples. To test sexual dimorphism, to compare non-adult and adult individuals, and individuals with and without burial furnishings by means of mean values of Ca, Sr, Zn, Sr/Zn, Zn/Ca, and Sr/Ca levels we used two-independent sample Student t-tests with Welch approximation of degrees of freedom and BY correction. To compare mean levels of Ca, Sr, Zn, Sr/Zn, Zn/Ca, and Sr/Ca levels with the results of Domonkošová Tibenská et al. (2010) and Bodoriková et al. (2009) we used two-independent sample Student t-tests with Welch approximation of degrees of freedom and BY correction as well.

To test the mean difference of buccal dental microwear density and length by orientation between Gáň cemetery and 11 selected populations (including Gáň A) we used two independent sample Student t-tests with Welch approximation of degrees of freedom and BY correction (for details see Domonkošová Tibenská et al. 2010).

To emphasize the difference between trace-element analysis and microwear analysis, the latter is *meta-analysis* like comparison having only mean, standard deviations and sample sizes from 11 selected populations. In this setting, the comparison was performed based on the effect sizes (mean differences; see Hartung et al. 2008) with Gáň as a control population. Additionally to univariate analyses, multivariate analyses of (1) "density variables" (NH, NMD, NV, NDM, and NT) and (2) "length variables" (XH, XMD, XV, XDM, and XT) were carried out as well. The effect sizes standardized by its standard deviations were used to calculate Euclidean distance matrix between all 12 populations followed by multidimensional scaling to reduce dimensionality of the data. For visualisation purposes, the projection to two dimensions was used. Finally, minimum spanning tree method was applied to connect all the vertices representing 12 populations together where the sum of the distances between connected vertices (sum of the length of all branches) is minimal (Härdle & Simar 2012).

Results and discussion

Trace-element analysis

The descriptive characteristics of location and variability of the examined populations are shown in Table 2. The average concentration of Sr was 130.59 ± 56.99 mg/kg and of Zn was 128.80 ± 20.37 mg/kg. The mean values of Sr and Zn indicate that, in general, the populations consumed a diet with approximately the same proportion of plant and animal food.

At the beginning of the analysis, it is necessary to determine if the chemical composition of dental tissue was not affected by diagenesis, i.e. if there was no diffusion of ions from the soil into the dental tissue or leaching of ions from the teeth to the soil. It is known that the exchange of elements between the osteological material and the soil depends on a number of factors such as the pH of soil, its absorptive properties, occurrence of micro-organisms, chemical properties (concentrations of elements), and water conditions. As we mentioned above, we had only four samples of soil. Samples were taken directly from the graves, and their pH was in the range of 7.82–8.34. Since the concentration of Zn and Ca was significantly higher in the teeth ($p < 0.0001$) and Sr concentration was significantly higher in soil ($p = 0.0064$), we can assume that diagenesis did not occur (Table 3). According to Szostek et al. (2005), both too high and too low acidity may be the cause of changes in the concentrations of trace elements within mineral salt structures in teeth. The diagenetic changes are less likely, because the pH of soil is slightly alkaline (Gordon & Buikstra 1981).

Table 2. Descriptive characteristics of location and variability of concentrations of elements in dental tissues of examined individuals (N = 12).

	Ca	Sr	Zn	Sr/Zn	Sr/Ca	Zn/Ca
n	12	12	12	12	12	12
Mean	31.24	130.59	128.80	1.02	4.18	4.12
SD	1.87	56.99	20.37	0.42	1.55	0.64
Median	31.15	123.00	122.65	0.85	4.01	3.94
Minimum	28.63	71.30	105.10	0.60	2.38	3.33
Maximum	34.38	291.20	166.90	2.06	8.47	5.22

Ca concentration in %, Sr and Zn in mg/kg
N – number of individuals
n – number of samples
SD – standard deviation

Table 3. Results of two-sample testing of mean concentrations of trace-element differences in human teeth and soil from the same site.

	Teeth	Soil	t-statistics	*p*-values
n	12	4		
Ca	31.24	7.04	20.152	*<0.0001*
Sr	135.98	202.73	–3.293	*0.0064*
Zn	128.81	48.14	–10.625	*<0.0001*

Ca concentration in %, Sr and Zn in mg/kg
n – number of samples
Significant *p*-values are written in italics.

The highest value of Sr (291.2 mg/kg) was found in an adult man aged of 20–30 years from grave CH 16 (Fig. 2). This grave disturbed the burial pit of a 10–12 years old child of undetermined sex. The remains of the lower half of the child's body were missing. It is possible that they were thrown from the grave during the digging of the burial pit for the adult male. The adult individual was buried without burial furnishings. In dental tissues the Sr concentration is more than twice that of the mean of the population. This man also had the highest Ca concentration (34.38 %). The Zn content (141.6 mg/kg) was close to the average. Commonly, high Sr concentrations indi-

Fig. 2. The skeletal remains of an adult male from grave CH 16 (the grave disturbed the burial pit of a 10–12 year old child; photo by M. Takács).

cate increased intake of food of vegetable origin (Szostek et al. 2005). It is questionable whether the higher Sr concentration was caused by vegetable food preference or not, because the remains belonged to a young man, and we would expect an increased supply of animal proteins. According to Szostek (2006), higher content of Sr does not directly correlate with the type of diet. High concentration of Sr may be a result of its increased absorption in the intestine (Sillen & Kavanagh 1982). We do not also exclude the possibility that this individual suffered from any disease, although we did not find any pathological changes in the skeletal remains.

The lowest Sr concentration (71.3 mg/kg) was found in a non-adult woman aged 16–20 years buried in grave CH 7 (Fig. 3). Zn and Ca were close to the mean values.

Fig. 3. The skeletal remains of a juvenile female from grave CH 7 (photo by M. Takács).

However, it is interesting that the low Sr value corresponds to the burial furnishing, which was probably the richest in the entire cemetery. In grave CH 7, seven glass beads of yellow, turquoise and dark blue colours, a bronze button, a silver knife and a silver ring were found. The amount of the findings from this grave clearly shows that the young woman had belonged to a higher social class in the given population. The low Sr content, in turn, points out that the woman apparently consumed a diet rich in animal protein. A similar finding was recorded in the cemetery of Gáň A, dated to the Migration Period, where the lowest Sr concentration was found in a woman with the richest burial furnishing (Bodoriková et al. 2009, Domonkošová Tibenská et al. 2010).

The Sr concentrations and Sr/Zn and Sr/Ca ratios were significantly higher in males. Within the comparison of adults and non-adults, significantly higher Zn content and Zn/Ca ratio were found in adult individuals. Comparison of individuals with and without burial furnishings did not show any significant differences (Table 4). The results indicate that adult individuals consumed more animal protein than non-adults and the women's diet contained even more protein than the men's diet. The Sr values have a greater variability, in contrast with the values of Zn concentration, which is probably due to the lowest and the highest values of Sr (71.3 mg/kg in a female from grave CH 7 and 291.2 mg/kg in a male from grave CH 16) mentioned above. These outlining concentrations of Sr indicate that the diets of the individuals were, respectively, richer and poorer in animal proteins. This could be associated with their health conditions or social status in the population.

Table 4. Results of two-sample testing of the intra-population differences in mean trace-element values – females v. males, non-adults v. adults, and burials with v. without furnishing.

		Males	Females	Non-adults	Adults	With burial furnishing	Without burial furnishing
	N	7	3	4	8	4	8
Ca	**Mean**	31.49	30.50	30.90	31.41	31.01	31.36
	t-statistics	1.0644		–0.5205		–0.2853	
	***p*-value**	0.3225		0.6153		0.7869	
Sr	**Mean**	153.20	89.90	113.20	139.30	115.40	138.20
	t-statistics	2.3796		–0.8637		–0.7581	
	***p*-value**	*0.0489*		0.4102		0.4678	
Zn	**Mean**	131.60	132.20	110.00	138.20	125.40	130.50
	t-statistics	–0.6775		–3.9255		–0.4391	
	***p*-value**	0.5352		*0.0035*		0.6722	
Sr/Zn	**Mean**	1.19	0.68	1.04	1.02	0.94	1.07
	t-statistics	2.7732		0.0497		–0.4819	
	***p*-value**	*0.0323*		0.9617		0.6446	
Sr/Ca	**Mean**	4.80	2.94	3.63	4.37	3.68	4.34
	t-statistics	2.59		–0.8745		–0.7970	
	***p*-value**	*0.04*		0.4073		0.4460	
Zn/Ca	**Mean**	4.19	4.33	3.57	4.41	4.08	4.15
	t-statistics	–0.3247		–3.3568		–0.1711	
	***p*-value**	0.7585		*0.0084*		0.8725	

N – number of individuals, significant *p*-values are written in italics.

The results obtained are compared with those from the analyses performed by Domonkošová Tibenská et al. (2010) and Bodoriková et al. (2009) on individuals from the cemetery of Gáň dated in the Migration Period (5th–6th c. AD), labeled Gáň A. This comparison is interesting because these two populations lived in the same locality with a time difference of 400 to 500 years. Statistically significant differences between examined populations were found in the Sr and Sr/Ca values: higher concentrations were detected in the population from the Migration Period (Table 5). The Zn concentrations were similar in both populations. We can assume that while the population from 9th–10th c. AD consumed food with similar proportion of plants and meats, a diet of plant origin had probably predominated in the population from the Migration Period.

Similar results were obtained by Šefčáková (2003) and Šefčáková & Krištín (2001a, 2001b) who had reconstructed dietary habits in individuals from the Early Medieval cemetery in Borovce (Slovakia) dated in 8th–12th c. AD. Borovce village is located only 50 km north of the locality Gáň. It can be supposed that the conditions of environment, in which both populations lived, had been very similar. On the basis of wider spectrum of trace elements (P, Sr, Si, Mg, Zn, Cu, Fe, Mn, Ti, and Ba), the population from Borovce consumed a mixed diet with a lower proportion of plant food.

Microwear analysis

Dietary habits were also reconstructed on the basis of dental buccal microwear. In nine teeth of nine individuals (Table 1), the density (numbers) and the length of four types of scratches according to the orientation were observed (Table 6). Overall, 684 scratches were considered in the examined population. Vertical scratches, numbering 473 (61.7 %), were the most frequent and a higher density was found in all individuals examined (on average, 46.9 vertical scratches per individual). The horizontal scratches were least frequent, only 19 (2.8 %) scratches were observed, and in two individuals none of them was present.

The method of dental buccal microwear in recent populations was developed on the basis of the research of Lalueza et al. (1996). These authors examined 10 recent

Table 5. Results of two-sample testing of inter-population differences between two populations from Gáň in mean trace-element values.

	Cemetery	N	Mean	t-statistics	*p*-value
Ca	Gáň A	13	30.49	–1.0098	0.3236
	Gáň C	12	31.24		
Sr	Gáň A	13	204.83	2.9397	*0.0101*
	Gáň C	12	130.59		
Zn	Gáň A	13	135.12	0.8774	0.3937
	Gáň C	12	128.80		
Sr/Zn	Gáň A	13	1.53	2.0360	0.0567
	Gáň C	12	1.02		
Sr/Ca	Gáň A	13	6.51	3.4719	*0.0034*
	Gáň C	12	4.18		
Zn/Ca	Gáň A	13	4.45	1.3042	0.2063
	Gáň C	12	4.12		

Significant *p*-values are written in italics, Gáň A – the cemetery dated to the Migration Period (5th–6th century AD), Gáň C – the Early Medieval cemetery dated to 9th–10th century AD

Table 6. Descriptive characteristics of location and variability of the number (NH, NMD, NV, NDM, and NT) and the length (XH, XMD, XV, XDM, and XT) of microwear defects of individuals from Gáň.

	N	n	Mean	SD	Median	Minimum	Maximum
NH	9	19	2.11	2.09	3	0	5
NMD	9	85	9.44	7.78	9	1	27
NV	9	422	46.89	11.40	43	29	67
NDM	9	158	17.56	9.42	18	4	34
NT	9	684	76.00	16.43	76	58	113
XH	9	19	149.82	45.20	203.33	60.75	391.96
XMD	9	85	126.78	58.01	110.63	24.90	227.70
XV	9	422	207.81	24.02	149.37	41.97	360.67
XDM	9	158	144.12	49.24	121.88	25.82	312.92
XT	9	684	197.89	27.71	138.70	24.90	335.99

N – number of individuals, n – number of analyzed scratches, SD – standard deviation, the values of the number of scratches are stated in pieces (frequencies); the values of the length are stated in μm.

populations – Inuits, "Fuegian" Indians, Bushmen, Australian Aborigines, Andaman Islanders, Indians from Vancouver Island, Veddahs, Tasmanians, Lapps, and Hindus (Table 7). According to the dietary type and the ways of obtaining food, the authors

Table 7. Comparing populations, their diet strategy, locality, and the number of individuals (Lalueza et al. 1996).

Group	Population	Diet strategy	Locality	N	Identification
1	Hindus	agriculturalists tropic area	Bihar and Orissa, Central India	20	Hind
2	Andaman Islanders	hunter-gatherers tropic area	Andaman Islands, Gulf of Bengal	18	Andam
	Veddahs	hunter-gatherers tropic area	Sri Lanka	9	Ved
3	"Fuegian" Indians	hunter-gatherers carnivora	Tierra del Fuego, Argentina and Chile	20	Ind.Fe
	Inuit	hunter-gatherers carnivora	Greenland	20	Inuit
	Indians from Vancouver	hunter-gatherers carnivora	Island of Vancouver, Canada	17	Ind.Ve
	Lapps	nomadic pastors carnivora	Norway, Finland and Russia	5	Lap
4	Bushmen	hunter-gatherers arid area	Kalahari Desert, South Afrika	15	San
	Australian Aborigines	hunter-gatherers arid area	Central, north and south of Australia	18	Austral
	Tasmanians	hunter-gatherers arid area	Tasmania	11	Tas

N – number of individuals

established four broad dietary categories from the original 10. The first group is comprised of agriculturalists – Hindus; the second group is comprised of hunter-gatherers from a tropical forest environment who fed on mixed food – Andaman Islanders and Veddahs; the third group: carnivorous hunter-gatherers and pastors –"Fuegian" Indians (hunting and fishing), Inuit (hunting), Indians from Vancouver (hunting and fishing), and Lapps (reindeer herding); and the fourth group consists of hunter-gatherer populations from arid and mesothermal environments, including Bushmen, Australian Aborigines, and Tasmanians (Domonkošová Tibenská et al. 2010).

According to Peréz-Peréz et al. (1994), a higher ratio of vertical scratches, and low scratch density, are indicators of mixed plant and meat food, while a higher ratio of horizontal scratches and their higher density dominate in agricultural populations with a higher content of plant food. The average scratch density in recent populations of hunters and gatherers that consume the mixed food and meat is between 32.0 and 74.8 scratches for the observed area (Lalueza et al. 1996). In medieval agricultural populations, the average number of 83.1 scratches was found (Polo-Cerdá et al. 2007). The average density of 87.11 scratches observed in the population of Gáň indicating that the diet consisted of a higher ratio of abrasive elements. The average length of scratches in recent hunters and gatherers varies from 152.0 μm to 235.2 μm, while the mean length in agricultural populations is maximally 151.2 μm (Lalueza et al. 1996). The average length of all scratches in Gáň was 160.72 μm which indicates that the diet of individuals was of mixed character.

Comparing the scratch density (Table 8), in the number of vertical scratches, the population of Gáň is different from all the recent populations evaluated by Lalueza et al. (1996). In the number of horizontal scratches, the population of Gáň is different from all populations except Fuegian Indians and Indians of Vancouver. When comparing the total number of scratches, the greatest similarity was observed with group 2 (Andaman Islanders, and Veddahs), which includes hunters and gatherers from the tropical areas and with the group 4 (Bushmen, Tasmanians, and Australian Aborigines) consisting of hunters and gatherers from the arid areas. The lowest similarity was found with the group 1, made up of farmers from the tropical areas (Hindus; Fig. 4).

Regarding (Table 8), the length of horizontal scratches, the population of Gáň differs from the population of Hindus and Veddahs. In the length of the vertical scratches, the population of Gáň is different from Hindus, Andaman Islanders, and Australian Aborigines. When comparing the length of all types of striations, the greatest similarity was observed with group 3 ("Fuegian" Indians, Inuit and Indians from Vancouver) comprised of carnivore hunters and gatherers. The lowest similarity was found with group 4 (Australian Aborigines, and Tasmanians), which consists of hunters and gatherers from the arid areas (Fig. 4).

Comparing the population of Gáň C with the population of Gáň A (Domonkošová Tibenská et al. 2010), a significantly higher scratch density, mainly of the horizontal variety, was found in individuals from the Migration Period (Table 8). This suggests that individuals from the cemetery Gáň A consumed food with a higher proportion of abrasive particles, which are typical for a diet of plant character. Regarding the length of scratches, significant differences were found only in the length of total scratches. However, individuals from the cemetery dated to 9th–10th c. AD had shorter horizontal scratches and longer vertical scratches than individuals from Gáň A. This indicates that individuals from Early Medieval period could have consume more meat food than individuals from the Migration Period.

Table 8. Results of testing mean difference of buccal dental microwear density by orientation and length between Gáň cemetery (control population) and 11 selected populations.

Variable	Groups															
	Hind[1]		Andam[1]		Ved[1]		Ind.Fe[1]		Inuit[1]		Ind.Ve[1]		Lap[1]		San[1]	
	t-stat	*p*-val	t-stat	*p*-val	t-stat	*p*-val	t-stat	*p*-val	t-stat	*p*-val	t-stat	*p*-val	t-stat	*p*-val	t-stat	*p*-val
NH	–7.18	*<0.001*	–9.98	*<0.001*	–8.64	*<0.001*	–2.18	0.022	–2.28	*0.017*	–1.22	0.121	–2.90	*0.004*	–4.23	*0.006*
NMD	1.08	0.152	0.12	0.452	0.09	0.466	1.16	0.135	0.67	0.257	0.68	0.253	0.84	0.211	–0.79	0.223
NV	7.41	*<0.001*	4.84	*<0.001*	4.31	*<0.001*	7.04	*<0.001*	7.08	*<0.001*	5.63	*<0.001*	7.18	*<0.001*	5.30	*<0.001*
NDM	2.72	*0.010*	–0.36	0.360	–0.20	0.423	3.41	*0.004*	2.10	0.026	1.78	0.049	2.98	*0.005*	0.53	0.303
NT	2.43	*0.013*	0.17	0.434	0.21	0.419	2.73	*0.006*	5.33	*<0.001*	4.49	*<0.001*	6.33	*<0.001*	0.87	0.203
XH	–2.88	*0.005*	0.03	0.489	–2.57	*0.008*	–1.33	0.099	0.46	0.324	–1.21	0.124	0.21	0.418	–0.63	0.272
XMD	–0.99	0.170	–0.07	0.471	–0.98	0.173	–2.11	0.026	–0.56	0.294	–3.21	*0.003*	–1.90	0.036	–1.28	0.113
XV	3.69	*0.001*	4.00	*<0.001*	0.17	0.432	–2.18	0.019	–0.09	0.464	–2.37	0.019	0.10	0.459	0.39	0.357
XDM	0.11	0.459	–0.09	0.466	–0.75	0.236	–3.04	*0.003*	–0.03	0.489	–2.40	0.014	–4.70	*0.001*	–1.28	0.115
XT	0.69	0.249	4.34	*<0.001*	0.57	0.285	–1.36	0.093	0.78	0.221	–1.72	0.055	–1.72	*0.048*	1.79	0.054

Variable	Groups					
	Austral[1]		Tas[1]		Gáň A[2]	
	t-stat	*p*-val	t-stat	*p*-val	t-stat	*p*-val
NH	–8.03	*<0.001*	–7.00	*<0.001*	–3.99	*<0.001*
NMD	–1.05	0.157	–0.75	0.234	–1.93	0.034
NV	5.50	*<0.001*	4.03	*<0.001*	–0.78	0.221
NDM	0.19	0.427	–0.17	0.434	–1.40	0.089
NT	1.62	0.066	0.28	0.391	–3.44	*0.001*
XH	0.66	0.259	–0.64	0.265	–0.60	0.278
XMD	–0.04	0.484	–0.39	0.354	0.06	0.478
XV	3.25	*0.002*	1.64	0.058	1.72	0.050
XDM	–0.27	0.396	–0.18	0.430	–0.78	0.226
XT	4.56	*<0.001*	2.48	*0.011*	2.11	*0.024*

[1] – Lalueza et al. (1996), [2] – Domonkošová Tibenská et al. (2010), t-stat – t-statistics, *p*-val – *p*-value. Significant *p*-values are written in italics.

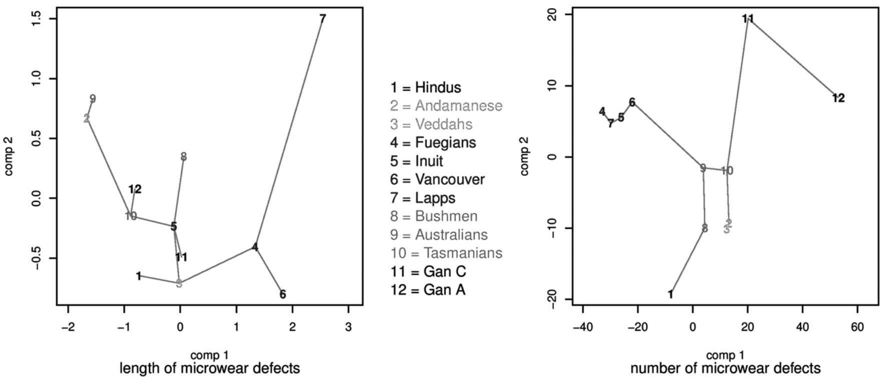

Fig. 4. Results of multidimensional scaling method and minimum spanning tree of standardized effect sizes using first two components describing more than 90 % of the variability (left: length of microwear defects, right: number of microwear defects).

In connection with the lowest Sr value in dental tissues, we wrote about a female from grave CH 7. As regards the density and orientations of scratches in this female, we determined a total of 77 scratches; 41 scratches with average length of 132.42 µm were oriented vertically (Fig. 5). Only two scratches with an average length of

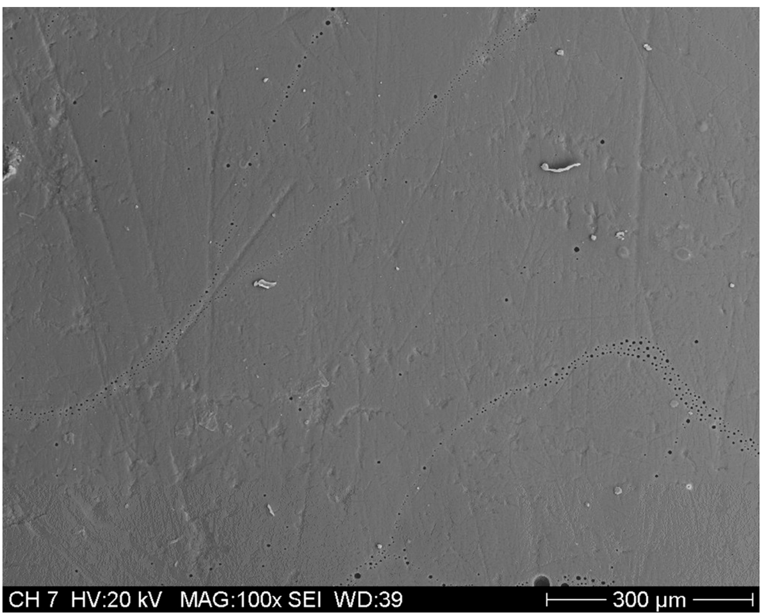

Fig. 5. SEM image of buccal microwear in the lower right second molar of a non-adult female from the grave CH 7. (Air bubbles are not microwear changes; they were formed during preparation of the positive casts).

62.58 μm had a horizontal orientation. The density and orientation of scratches indicate that this female consumed food with a higher portion of meat, which corresponds with the results of the trace-element analysis.

In connection with the highest Sr value, we referred to a male from grave CH 16. It is interesting that this male had also the highest number of scratches in the whole population. Out of 124 scratches, 65 were oriented vertically and only two scratched were horizontal. The average length of vertical scratches was 167.44 μm (Fig. 6). The results suggest the consumption of mixed to meat food and do not correspond with the results of the trace elements analysis. It is therefore possible that the high Sr concentration in dental tissues in this individual was caused by factors other than diet.

On the basis of scratch density in examined individuals from Gáň, we can predict that the most significant components of their diet were plants. The high number of vertical scratches (more than 60 % of all scratches) and their great average length indicate that individuals also consumed a notable proportion of meat.

Conclusions

The population from the cemetery of Gáň dated to the 9th–10th c. AD probably consumed mixed food according to the results obtained on the basis of the trace elements analysis and buccal microwear. The results also indicated that there were also individuals whose diets were, respectively, richer and poorer in animal proteins that could be

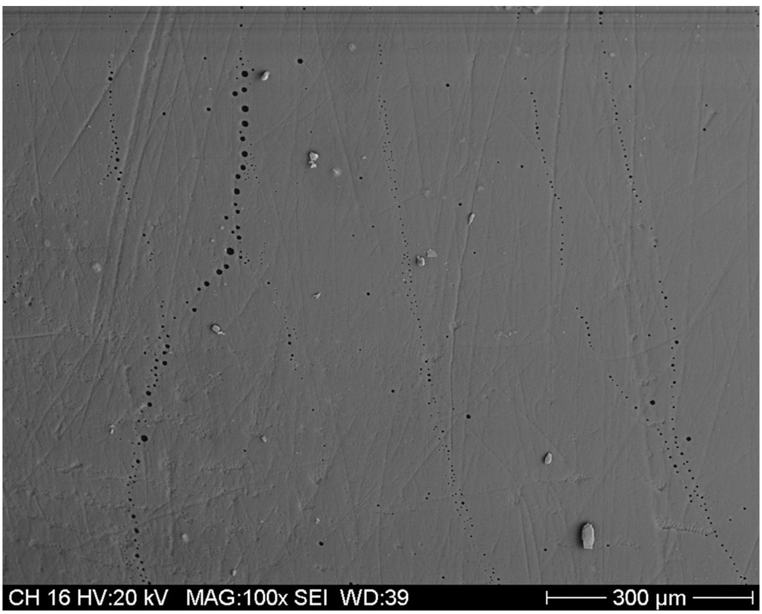

Fig. 6. SEM image of buccal microwear in the lower left first molar of an adult male from grave CH 16. (Air bubbles are not microwear changes; they were formed during preparation of the positive casts).

related to their health conditions or social status in the population. Comparison of individuals from Early Medieval cemetery with individuals from the Migration Period showed that although both populations came from the same locality, particular differences in their dietary habits existed. The diet of the Early Medieval population probably contained a higher ratio of animal protein, while plant food predominated in the population from the Migration Period. The cemetery of Gáň is located on the Danube river basin which is the most fertile region in Slovakia, because it is covered with loess soils and river silts (Hók et al. 2001). Since both populations inhabited the same locality, we can assume that their environmental conditions were approximately the same. Considering the ecological conditions in this area, agriculture was the main source of livelihood. For most people in the Early Middle Ages, grain cultivation and production of domestic livestock, mainly cattle and pigs, were the main and the most important sources of food. In addition to grain cultivation, they grew fruit trees, gathered honey from wild bees (later they also kept bees in hives), gathered medicinal plants, fished and sometimes hunted wild animals (Beranová 1988). We assume that the diet of the population from 9th–10th c. AD was more colourful than food of the population from the Migration Period that corresponds with results obtained by the trace elements analysis and buccal microwear.

Acknowledgements

This study was supported by the projects VEGA 2/0038/12, VEGA 1/0442/13, VEGA 1/0887/13, and UK/90/2012. The authors are grateful to the anonymous reviewers for their valuable comments that improved the manuscript. We would like to thank to Maca and Ivo Vaskovic and, specially, to Professor James Bernard Thomson for language correction.

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Submitted: 2012-April-27;
accepted: 2013-February-25.

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