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ISOTOPIC ANALYSIS OF HUMANS AND ANIMALS FROM VEDROVICE

ABSTRACT: We report here the results of our isotopic analysis of humans from the site of Vedrovice. We undertook carbon and nitrogen analysis to reconstruct human diets and strontium and sulphur isotope analysis to reconstruct human mobility. Our carbon and nitrogen isotope analysis of bone collagen from 57 humans indicated that their diet was largely homogenous, with an average $\delta^{13}C$ value of –19.7±0.4‰ and an average $\delta^{15}N$ value of 9.9±0.6‰. This indicates that the diet was largely C_3 based, and that there was a large component of animal (meat or milk) protein in their diets. The sulphur and strontium isotope analysis showed that most of the humans at the site had values indicating that they spent their childhood (strontium isotope values) and adulthood (sulphur isotope values) at or near to Vedrovice. We did identify four individuals with different sulphur isotope values than the majority, who likely spent there adult lives in different locations, and similarly we found four adults who had different strontium isotope values than the others, indicating they spent at least a portion of their childhood elsewhere. Of these individuals, one had both different sulphur and strontium value than the majority, so they likely spent their childhood and adult lives at a different location and were probably recent immigrants to the site. Future work on establishing the isotopic baseline for this region will allow us to better pinpoint the diets of the Vedrovice humans, as well as better potentially indicate the regions of origin for the 'immigrants.'

KEY WORDS: Isotopes – Diet – Migration – Carbon – Nitrogen – Sulphur – Strontium – Collagen

INTRODUCTION

Isotopic analysis of bones and teeth can tell us about the lifetime diets and migration patterns of humans and animals. In this project we applied a suite of isotopic measurements to humans from the site of Vedrovice to reconstruct diets (carbon and nitrogen isotopes) and to look for potential immigrants (sulphur and strontium). This analysis was undertaken as part of the larger AHRC funded bioarchaeological project on the site, directed by M. Zvelebil and P. Pettitt and was a unique opportunity to look at an early Neolithic population from this region. Below we describe the results of these analyses in turn.

CARBON AND NITROGEN ISOTOPE ANALYSIS

Carbon and nitrogen isotope analysis was undertaken to determine the diets of a selection of the humans buried at Vedrovice. This analysis, now well established in archaeology, provides information on the sources of protein in diets, over the last 10 to 20 years of life (Mays 2000, Sealy 2001). It can identify if the main sources of protein were from marine or terrestrial foods, or the use of C_4 plants, like millet, and the consumption of freshwater fish. Additionally, isotope analysis of juveniles can tell us about the age of weaning in a past society (Herring *et al*. 1998, Schurr 1998, Richards *et al*. 2002).

Not all samples were suitable for analysis, as there was too little material preserved, or the extracted collagen was too poorly preserved for measurement. In total we were able to determine carbon and nitrogen isotope values from bone collagen extracted from 57 individuals. Ideally, it is best to compare the human carbon and nitrogen isotope values to contemporary animals from the same site, as the animals provide a comparative baseline for interpreting the human values. Unfortunately it was not possible for us to obtain animal bones from the site for our analysis, so we are only able to provide a broad interpretation of the human diets.

METHODOLOGY

Samples were first cleaned with an air-abrasive and collagen was then extracted following methods outlined elsewhere (Richards, Hedges 1999). Samples were demineralised in 0.5% HCl at 5 °C for 2–5 days and then gelatinized in pH 3 H_2O for 48 hours. The resulting solution was filtered through a 5 micron filter and then through a 30 kDa ultrafilter, following Brown *et al.* (1988). The resulting collagen was then freeze-dried. The collagen isotope values were measured using a ThermoFinnigan elemental analyzer coupled to a Delta Plus XP mass spectrometer at the Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany. All samples were measured in duplicate, and the measurement errors on the $\delta^{13}C$ values are ±0.1‰ and ±0.2‰ for the $\delta^{15}N$ values.

RESULTS

The isotope data, along with measures of the quality of the collagen are given in *Table 1*. The generally accepted values for the quality indicators of well preserved collagen for palaeodietary studies is a C:N ratio of between 2.9 and 3.6, a %C of >13%, a %N of >4.8%, and a collagen yield of over 1% (DeNiro 1985, Ambrose 1990). As can be seen in *Table 1*, all of the collagen fit within the criteria for acceptable collagen, so we then measured the carbon and nitrogen isotope values of all of these samples.

The isotope data is plotted in *Figure 1*. The average $\delta^{13}C$ value of all of the humans is –19.7±0.4 ‰ and the average $\delta^{15}N$ value is 9.9±0.6‰. The isotopic values of all of the individuals are very similar, indicating that they all likely had a similar lifetime diet. The carbon isotopic values ($\delta^{13}C$) fall in the range expected for a 100% C_3 diet, indicating that there was no consumption of C_4 food either directly or of animals that had consumed C_4 foods such as millet (or seasonal C_4 grasses). The $\delta^{15}N$ value of consumer body tissues (such as collagen) is generally about 3 to 5‰ higher than the protein they consume. Using assumed values for the herbivores at this site, based on measurements of herbivores from other Holocene sites in Europe, as well as from Iron Age sites in the Czech Republic (see below), the Vedrovice human $\delta^{15}N$ values indicate that dietary protein mainly came from animal sources, namely herbivores, and this could have been from either meat or milk. One infant (17/75) had a very high nitrogen isotope value indicating that it had been breastfed before it died. Interestingly, the young juveniles do not show this elevated signal, so weaning must have occurred in the period of time before they died. There are a number of juveniles that have been aged to three years old and they do not have this elevated value, therefore weaning must have occurred well before this age.

COMPARISON WITH OTHER DATA

There are two published studies of isotopic values from Holocene Czech Republic, and both of these are from the Iron Age (La Tène) (Le Huray, Schutkowski 2005, Le Huray *et al.* 2006). There is data from seven sites, all

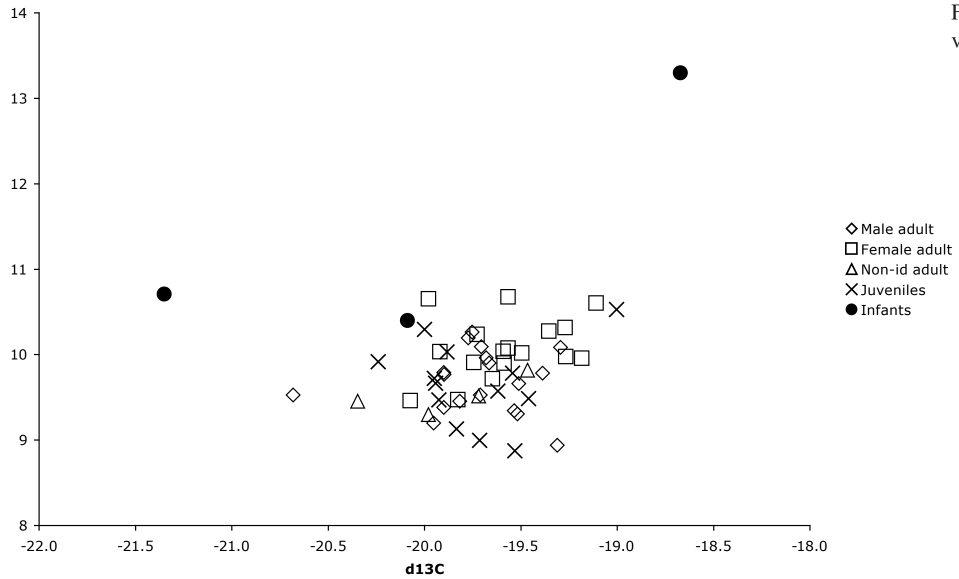


FIGURE 1. Bone collagen $\delta^{13}C$ and $\delta^{15}N$ values for the Vedrovice humans.

TABLE 1. Isotope data.

S-EVA	Individual	%Coll	δ13C	δ15N	%C	%N	C:N	%S	δ34S	87/86Sr	Sr ppm
3224	13/75	3.6	–19.7	10.1	40.5	14.5	3.3	0.2	0.6		
3227	14/75	1.8	–19.3	10.1	42.7	14.8	3.4	0.2	4.0		
3216	15/75	3.0	–19.2	10.0	40.6	14.4	3.3	0.2	–0.1	0.710847	71
3215	16/75	6.1	–19.9	9.7	44.3	15.9	3.2	0.2	–1.4	0.711150	77
3220	17/75	2.6	–18.7	13.3	42.9	14.3	3.5	0.2	4.3		
1306	22/75	1.0	–19.8	9.5	39.0	13.7	3.3				
1298	23/75	3.8	–20.0	10.6	41.4	15.0	3.2	0.2	–1.9		
3221	24/75	4.7	–19.9	9.2	41.3	14.5	3.3	0.2	0.2		
3223	25/75	2.8	–19.5	10.0	41.2	14.4	3.3	0.3	–0.6		
1318	28/76	1.9	–19.9	10.0	33.4	11.7	3.3	0.2	–1.7		
1319	30/76	2.4	–19.5	9.5	32.2	11.0	3.4	0.1	2.6	0.710831	88
1307	31/76	1.7	–20.0	10.3	37.0	12.9	3.3	0.2	1.1		
1299	37/76	0.5	–19.6	9.6	32.1	11.0	3.4				
1325	38/76 bone	2.9	–20.3	9.5	43.5	14.7	3.5	0.2	0.1		
1300	38/76 tooth									0.710407	137
3230	39/80	3.9	–19.0	10.5	43.7	15.4	3.3	0.2	–0.7		
1308	42/77	1.2	–19.5	9.8	38.6	14.0	3.2	0.2	–1.1	0.711011	113
1320	43/77	3.0	–20.0	9.7	42.7	14.9	3.4	0.2	1.1	0.711242	78
1321	44/77	1.9	–19.9	9.5	41.1	13.9	3.4	0.2	–1.0		
3233	48/75	1.5	–19.8	10.3	37.0	12.9	3.3	0.2	–1.2	0.711032	82
1324	50/77	3.2	–19.8	9.5	44.4	15.6	3.3	0.2	–0.4		
3240	51/77	0.5	–20.7	9.5	40.7	11.7	4.0			0.709112	190
3237	54/78	6.5	–19.6	10.1	44.2	15.4	3.3	0.2	0.8	0.711092	58
3232	56/78	3.0	–19.8	9.1	42.6	14.5	3.4	0.2	1.0		
1301	57/78	1.7	–19.6	10.7	37.8	13.6	3.2	0.2	1.0		
1302	59/78	0.7	–19.4	10.3	27.8	9.8	3.3			0.711441	109
1309	62/78	1.0	–19.9	9.4	36.2	12.7	3.3	0.2	–0.3	0.711293	127
3243	63/78	2.7	–19.9	10.0	42.0	14.3	3.4	0.2	4.3		
4011	66/70		n/a							0.711529	104
3234	66/78	3.0	–19.7	9.9	43.5	15.1	3.4	0.2			
1303	71/79	1.5	–19.3	10.3	36.0	12.9	3.3	0.2	1.5		
1310	72/79	2.5	–19.5	9.3	30.3	11.0	3.2	0.1	–0.9		
3217	73/79	4.9	–19.7	10.2	43.9	15.5	3.3	0.2	1.8	0.711009	75
3239	75/79	4.3	–19.5	9.3	44.7	15.3	3.4	0.2	0.9	0.711177	119
1304	77/79	2.4	–19.3	10.0	37.9	13.7	3.2	0.2	–0.8		
3219	79/79	3.9	–19.6	10.0	43.7	15.7	3.3	0.2	–3.4	0.709852	202
1322	81b/79	2.2	–20.1	10.4	44.0	14.8	3.5	0.2	0.4		
3218	82/79	3.8	–19.1	10.6	42.0	15.1	3.2	0.2	–0.6	0.711331	101
3222	84/80	2.2	–20.2	9.9	30.6	10.5	3.4	0.2	–1.4	0.710971	100
3229	86/78	6.3	–19.6	10.0	44.1	16.0	3.2	0.2	–1.1		
1311	86/80	2.0	–19.9	9.8	42.4	14.9	3.3	0.2	–2.0		
3231	87/80	2.4	–19.5	9.7	42.1	14.7	3.3	0.2	–0.6		
1312	89/80	2.0	–20.0	9.3	34.4	11.9	3.4	0.2	–0.8		
1313	90/80	1.6	–19.7	9.5	33.8	12.0	3.3	0.2	0.5		
1314	91/80	4.4	–19.4	9.8	41.5	14.9	3.3	0.2	–0.2		
3228	93/80	4.4	–21.4	10.7	46.7	13.8	3.9	0.2			
3235	93a/80	3.6	–19.8	10.2	41.6	14.3	3.4	0.2	2.4	0.711515	119
3225	95/80	4.5	–19.6	9.7	43.5	15.7	3.2	0.2	4.1		
3226	97/80	3.5	–19.7	9.5	43.2	15.5	3.3	0.2	–0.5		
1323	96/80	5.1	–19.5	9.8	45.6	16.1	3.3	0.2	–0.7		
1305	99/81	0.9	–20.1	9.5	37.7	13.3	3.3				
1315	100/81	3.4	–19.7	10.0	44.9	16.0	3.3	0.2	–0.3		
1316	101/81	3.5	–19.7	9.9	41.1	14.3	3.4	0.2	–3.3		
3244	102/81	4.4	–20.0	9.2	43.6	15.5	3.3	0.2	–0.2	0.712627	101
3236	104/81	2.5	–19.9	9.8	39.5	13.4	3.4	0.2	–1.5	0.711201	98
3242	105/81	7.3	–19.7	9.0	44.9	15.9	3.3	0.2	–0.1	0.711301	91
1317	106/82	1.4	–19.5	8.9	42.2	15.4	3.2	0.2	–0.1		
3238	107/82	4.9	–19.3	8.9	45.4	16.2	3.3	0.2	1.3	0.711373	49
3241	108/84	3.3	–19.6	9.9	45.0	16.1	3.3	0.2	–0.1		

located in Bohemia (Soběsuky, Tišice, Jinonice, Ruzyně, Makotřasy, Kutná Hora and Radovesice). The data from these two previous studies are plotted in *Figure 2* (taken from Le Huray *et al*. 2006). The isotopic data shows large differences in the human isotopic data within and between sites.

Of particular note are the $\delta^{13}C$ values, which indicate that C_4 foods, likely millet, were important in the diets of many of these individuals (i.e. those with the less negative $\delta^{13}C$ values). The Iron Age data is much more diverse than the data from Vedrovice, reinforcing the conclusion that the humans at Vedrovice all shared a similar diet which did not contain any C_4 foods such as millet. Additionally, a number of individuals from the site of Jinonice had very high $\delta^{15}N$ values (i.e. 13 ‰), which can best be explained by the significant consumption of aquatic resources such as freshwater fish. As these values are much higher than those found at Vedrovice it is very unlikely that freshwater fish was a significant component of the diet of the Vedrovice humans.

Additionally, Le Huray *et al*. (2006) measured the animal bones from the La Tène settlement site of Soběsuky, Czech Republic. The data from this study are plotted in *Figure 3*. Although this data is from a different region and time period than the Vedrovice humans, it does show that there are no unusual isotopic effects in the Holocene Czech Republic (e.g. anomalously high or low $\delta^{15}N$ values) since the animals plot as expected. If we were to use those data to interpret the Vedrovice humans then we would conclude that the main source of dietary protein was cattle and sheep meat/milk.

SULPHUR ISOTOPE ANALYSIS

A new method of analysis is the measurement of sulphur isotopes in bone collagen (Richards *et al.* 2001, 2003). This method requires large amounts of collagen for analysis, and we were able to extract sufficient collagen for analysis from

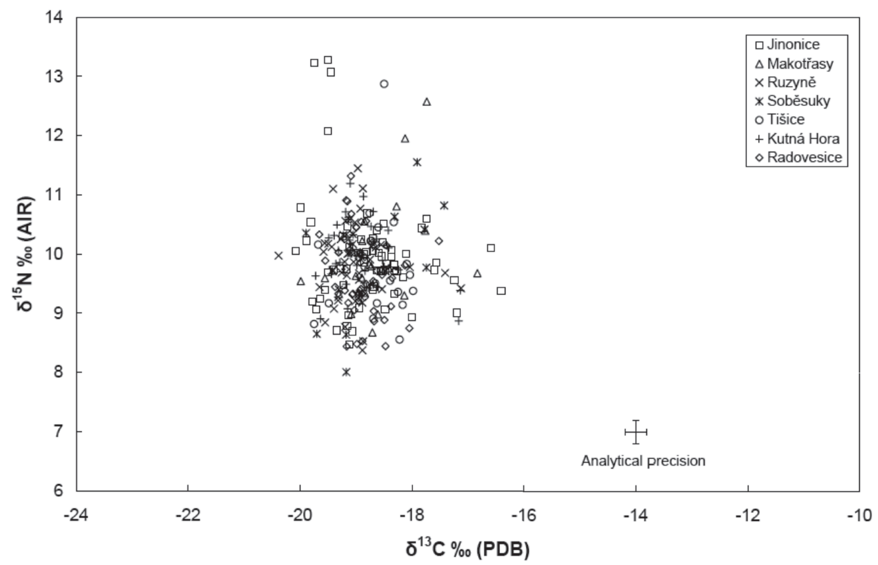


FIGURE 2. Human $\delta^{13}C$ and $\delta^{15}N$ data from various Iron Age sites in the Czech Republic (from Le Huray *et al*. 2006).

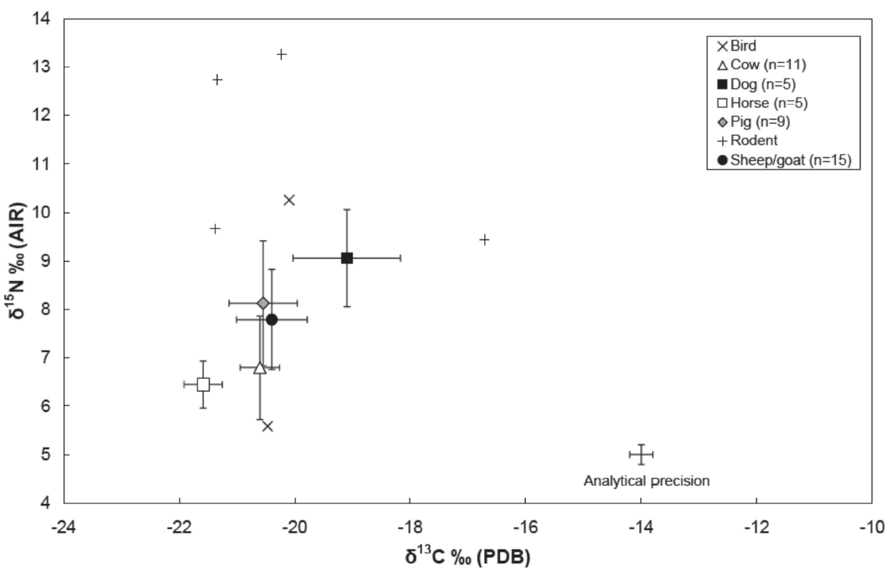


FIGURE 3. Animal $\delta^{13}C$ and $\delta^{15}N$ data from the Iron Age settlement site of Soběsuky, Czech Republic (from Le Huray *et al*. 2006).

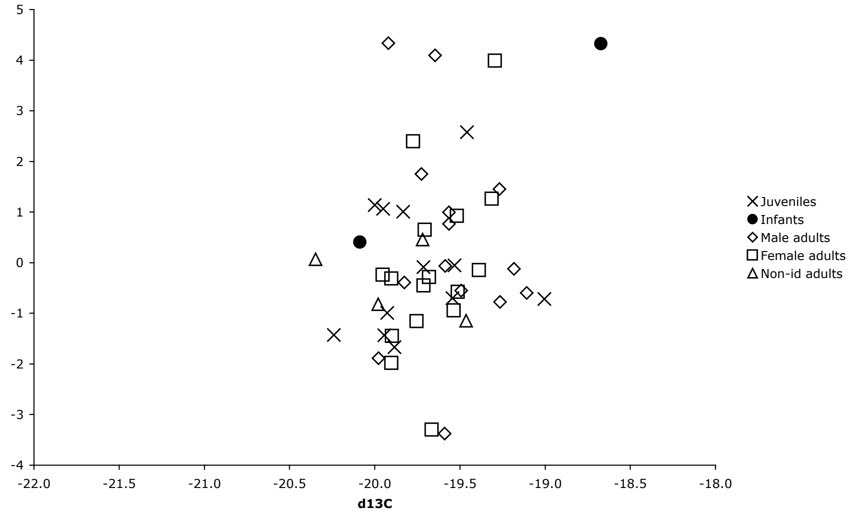


FIGURE 4a. Carbon and sulphur data.

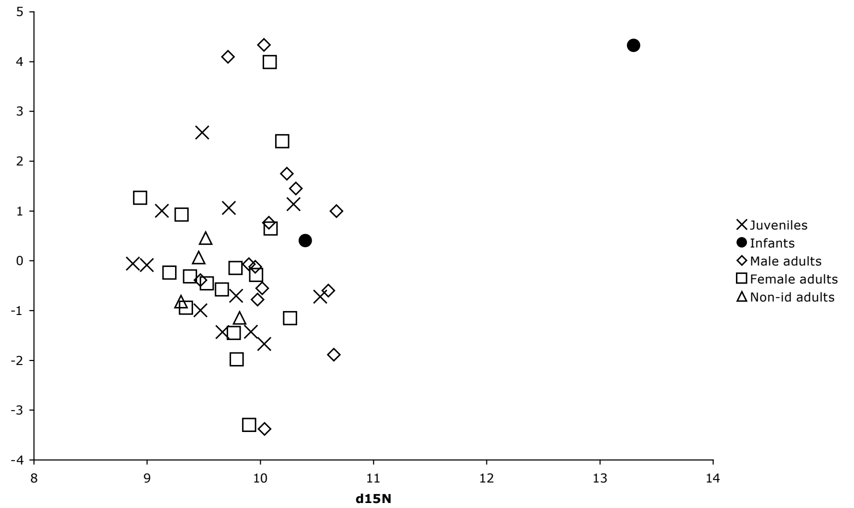


FIGURE 4b. Nitrogen and sulphur data.

50 individuals. These are somewhat indicative of diet, but are best used to determine the geographical location where the individual was living when the collagen was formed. As the collagen was formed over the past years of life this gives a measure of fairly recent locations (compared to strontium, as discussed below). The sulphur isotope data (*Figures 4a* and *4b*) cluster around a value of approximately 0 with a range of –2 to +2. This is consistent with the location of Vedrovice in central Europe (i.e. no sea spray effect). There are a number of individuals with different sulphur isotope values. Two adult males (63/78 and 95/80) and an adult female (14/75) have higher S isotope values than the others, and one adult male (79/79) and one adult female (101/81) have lower sulphur values than most others. These individuals likely lived elsewhere in the past 10 to 20 years of their life. Without comparative sulphur isotope data we cannot, at this stage, give specific locations where they might have lived. The young infant with the high nitrogen isotope value (17/75) also has a high sulphur value, which may be a physiological effect of breastfeeding/growth which was previously unrecognised.

STRONTIUM ISOTOPE ANALYSIS

Strontium isotope ratios and concentrations are measured in tooth enamel, and indicate the location where the individual was living when the enamel was formed during childhood (Price *et al.* 2002, Bentley 2006). Unlike bone, once formed, enamel is not remodelled and, unlike bone and dentine, it is also highly resistant to postmortem diagenesis (Budd *et al*. 2000, Hoppe *et al*. 2003, Trickett *et al*. 2003). The enamel value therefore tells us if any of the adults lived in a different location as children compared to where they were eventually buried as adults.

METHODOLOGY

We were able to undertake this analysis on teeth from 22 individuals from the site. The surface of the tooth was cleaned mechanically with a tungsten carbide dental bur and a small chip of enamel was separated with a circular diamond dental saw. All enamel surfaces were carefully

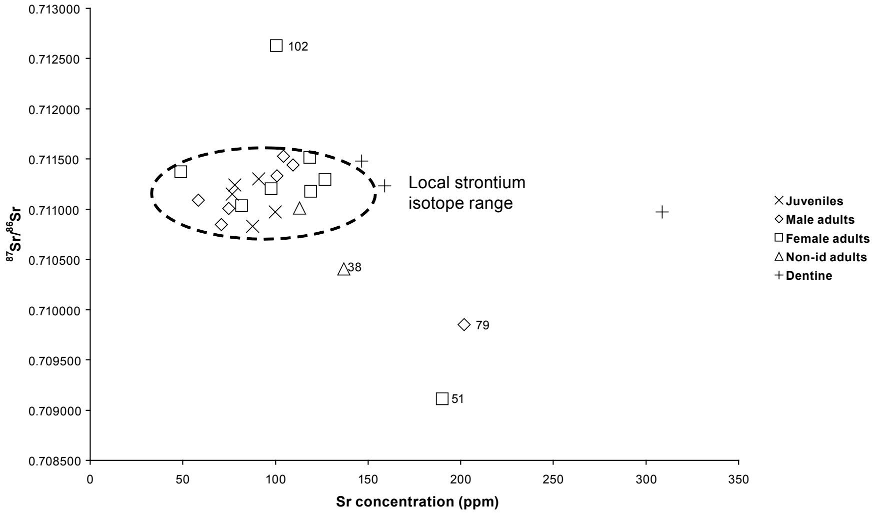


FIGURE 5a. Strontium ratio and concentration data.

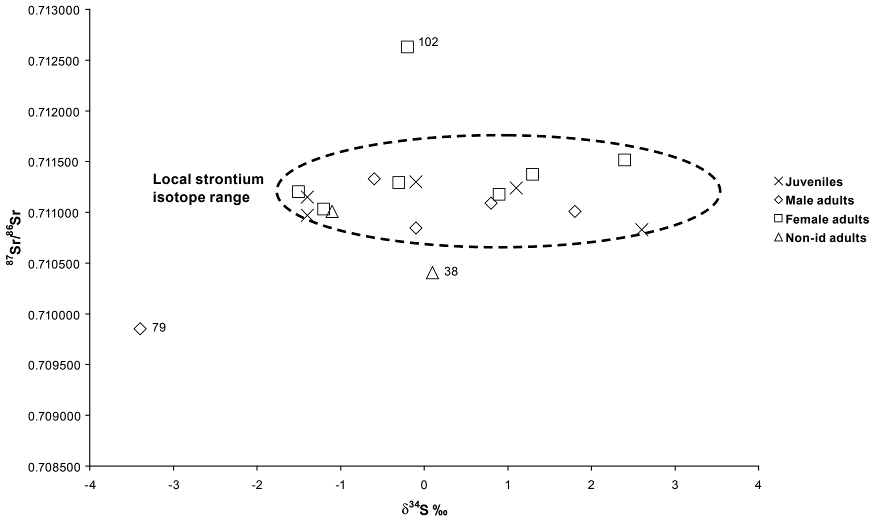


FIGURE 5b. Sulphur and strontium data.

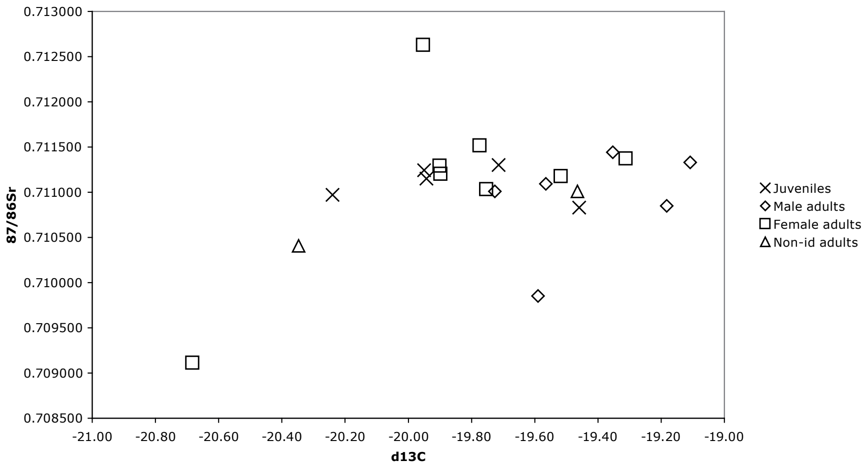
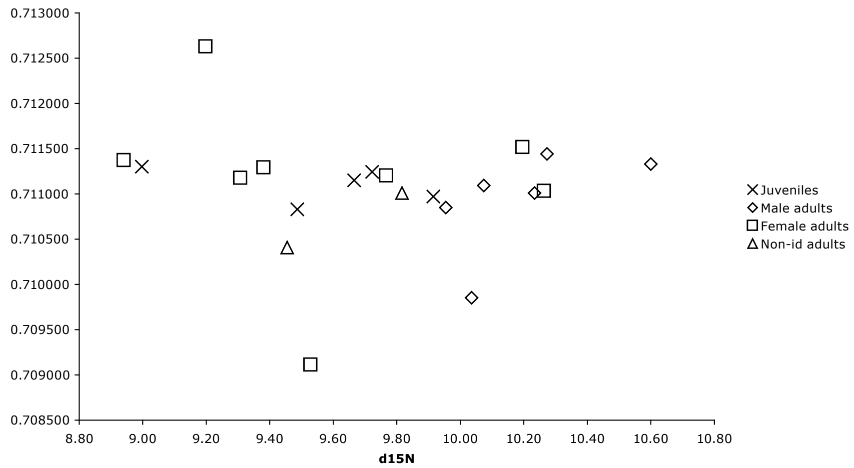


FIGURE 5c. Carbon and strontium data.

abraded to obtain a piece of core enamel, without any micro-cracks or dentine. Dentine samples were taken from the underlying primary crown dentine of three of the teeth and all surface material (soil, enamel, secondary dentine etc.) was discarded. No attempt was made to remove diagenetic strontium from the dentine samples as they were being used as proxies for the mobile soil strontium (Budd *et al.* 2000, Hoppe *et al*. 2003, Montgomery *et al*. 2007, Trickett *et al.* 2003). After mechanical separation, the samples were sealed in containers and transferred to the clean laboratory suite at the Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany for chemical separation and analysis by plasma ionization multicollector mass spectrometry (PIMMS) following the

FIGURE 5d. Nitrogen and strontium data.



method outlined in Copeland *et al.* (2008). In brief, enamel and dentine samples were rinsed twice in Milli-Q deionized (DI) water (18.7MΩ), once with ultrapure acetone (99.99%), and then allowed to dry before being dissolved in 1 ml of hot (100 °C) 14.3M nitric acid (HNO_3) (SupraPur®, Merck). The solutions were evaporated to dryness and the sample residues were resolved in 3M HNO_3 before loading on preconditioned 2 ml columns (EiChroM, Darien, USA) containing clean Sr-Spec resin (EiChroM, Darien, USA). After several successive washes of 3M HNO_3 were passed through the columns, strontium was eluted from the resin in 1 ml of Milli-Q DI H_2O, which was then evaporated to dryness and resolved in 1 ml 3% HNO_3. Dilutions of the 3% HNO_3 were analysed for both Sr isotopes ($^{87}Sr/^{86}Sr$) and Sr concentration (ppm) on a ThermoFisher Neptune™ PIMMS using instrument parameters and methodology for solution analysis given elsewhere (Richards *et al.* 2008, Copeland *et al*. 2008). Measured $^{87}Sr/^{86}Sr$ values from SRM987 (NIST international strontium carbonate isotope standard) analysed concurrently with the samples were 0.71027±0.000015 (1σ, n=7), which is well within its certified value of 0.71034±0.00026.

RESULTS

Enamel strontium concentrations have a mean =105±36 ppm 1s and a range 49–202 ppm, n=23, and are consistent with omnivorous humans in non-coastal, temperate Europe (Montgomery 2002, Montgomery *et al*. 2007). Most of the individuals have a similar strontium isotope value: 18 of the 22 individuals analysed form a cluster with a strontium isotope range of 0.7108–0.7115 and a mean =0.7112± 0.0002 1s, n=18 (*Figures 5a* to *5d*). This group includes all the juveniles and is co-incident with the range of dentine ratios which are indicative of local mobile strontium (Montgomery *et al.* 2007). The dentine range (0.7110–0.7114) corresponds well with values of 0.7110–0.7112 obtained for bone and dentine from the loess LBK site at Asparn-Schletz, in Lower Austria (Prohaska *et al.* 2002). Juveniles have been suggested as a useful way of estimating the local strontium range of values for a community because they have had much less time than adults to undertake a migration (Bentley *et al*. 2007, Evans, Tatham 2004, Montgomery *et al*. 2005, Schutkowski 2002).

There are some exceptions however. One female (102/81) has a higher strontium isotope value than the majority of the others, and two males (38/76 and 79/79) and one female (51/77) have values lower than the majority, although 38/76 has an isotope ratio that is close to the main Vedrovice cluster. These four individuals, which are located in four different regions of the cemetery, appear to have spent a part of their childhoods at a different location than Vedrovice. None of these individuals are consistent with origins in regions of geologically young rocks, such as recent volcanic basalts, or marine sediments such as Cretaceous chalks (McArthur *et al*. 2001, Montgomery *et al*. 2005, Price *et al.* 2004). Neither do they result entirely from origins on Precambrian granites and gneisses of the Bohemian Massif where strontium ratios from pigs have been found to exceed 0.718 (Bentley, Knipper 2005). However, the female 102/81 has a strontium isotope ratio of 0.7126 which is indicative of some dietary contribution from Palaeozoic or Precambrian rocks which are found principally to the north and west in upland regions (Bentley, Knipper 2005). The two individuals with the lowest strontium isotope ratios (79/79 and 51/77) have strontium isotope ratios consistent with younger Mesozoic rocks such as those found to the east and south of Vedrovice.

COMPARISON WITH OTHER DATA

Strontium isotope ratios of the European loess belt range from 0.712963–0.730248 (Gallet *et al.* 1998) which indicates a significant contribution from Precambrian and Palaeozoic rocks to European loess. However, these are whole soil values. Analyses of the more easily leached component, dominated by the carbonate fraction, suggest that the resulting biogenic strontium would be dominated by much lower values, i.e. ~0.710 (Gallet *et al.* 1996). Strontium ratios obtained from prehistoric humans and animals from loess sites in Central Europe would tend to confirm this observation and have been observed to provide biologically available strontium ratios between 0.7086 and 0.7103 (Bentley, Knipper 2005, Bentley *et al*. 2007, Price *et al.* 2001, 2004). Individuals 51/77, 79/79 and possibly 38/76 from Vedrovice would therefore be consistent with such lowland loess regions. The average bone strontium value obtained from the nearest sites to Vedrovice at Alicenhof in Austria and Moravská Nová Ves in the Czech Republic is 0.7104 (Price *et al.* 2004) which is significantly lower than the average of 0.7110 obtained from the enamel samples at Vedrovice but consistent with individual 38/76. The lower strontium ranges obtained for other loess-based sites in Europe suggests there is a greater contribution from Precambrian and Palaeozoic rocks to the loess in the vicinity of Vedrovice which may reflect its position on the edge of the Bohemian Massif. Consequently, it should be possible to identify immigrants to the site from the regions of Tertiary and Quaternary geology to the east and south of Vedrovice, such as the Hungarian Plain, where lower biosphere strontium ratios such as those obtained from individuals 51/77, 79/79 are found (Giblin 2005, Price *et al.* 2004).

SUMMARY AND CONCLUSIONS

We undertook a multi-isotopic study of the humans from the Early Neolithic site of Vedrovice in order to reconstruct the past diets of humans at this site as well as determining if any of the humans buried here had spent part of their lives elsewhere (i.e. immigrants). The carbon and nitrogen data indicate that adults and juveniles all had a similar diet, with most of the dietary protein coming from animal (meat or milk) sources. From the isotopic analysis of the few juveniles sampled we suggest that weaning occurred before the age of three in this population. Finally, although most individuals at the site had sulphur and strontium isotopic values indicating that they likely spent all, or the majority, of their life at or near Vedrovice, we were able to identify a few individuals who lived elsewhere as children or adults. Specifically, three adult males and two adult females had sulphur isotope values indicating that they lived elsewhere relatively recently (i.e. over the past 10 to 20 years of life). Two males and two adult females had strontium isotope values indicating that they lived elsewhere as children. Finally, one of the male adults (79/79) had different sulphur and strontium values than the rest of the population, indicating that this person lived elsewhere as a child and relatively recently, so must have been a recent immigrant to Vedrovice.

In conclusion, this is one of the first multi-isotopic studies of a prehistoric population and the use of this approach has allowed us to reconstruct aspects of the lives of these individuals that we would not have been able to see otherwise. We were able to identify that all of these individuals had a very similar diet, with no evidence of the inclusion of wild foods like fish, and no input from millet in the diet, and instead match well with a Neolithic diet of meat or milk from, likely, domesticated animals. We were also able to identify individual immigrants at the site, which would not have been possible without isotopic analysis. This study is, hopefully, the first of many in this region and future research, especially on the animal remains from the site and region, may allow us to better define early Neolithic diets in this region, as well as pinpoint where the immigrants at Vedrovice originated from.

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