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STABLE ISOTOPES AS A FINGERPRINT OF HUMAN BEHAVIOUR – ANALYSIS OF HUMAN ARCHAEOLOGICAL CREMAINS: PROBLEMS AND PERSPECTIVES

ABSTRACT: One of the basic methods used in investigating migration processes is the analysis of stable oxygen and strontium isotopes. This method was introduced to archaeo--anthropological studies from zoological research, who first applied it to tracing animal migrations. Stable isotope analyses in origin and migration studies are based on the premise that the isotope ratios in osteological material almost precisely reflect the isotope composition of the environment (water and soil) inhabited by the group. If the bone material of a given individual reveals any departure from oxygen and strontium isotope ratio relative to the local level, it is possible to speak of its allochthonic origin. In addition, the discrepancy between the isotope ratio in bone tissue and tooth enamel allows us to isolate from the group individuals who had spent childhood in a place different from where the remains were found. Isotope studies using cremated remains are still a largely unexplored field. The fact that high temperature causes changes in the isotopic composition of the bone tissue unconditionally excludes incinerated bone material from such analyses. This problem has only recently been addressed once again and more and more works, usually model studies, attempt to explain how heat and duration of the incineration affect final concentrations of stable isotopes in bones, which may result in designing a standardised method for analysing stable isotopes in cremated material, e.g. from the Bronze Age cemeteries, in the future.

STRESZCZENIE: Analiza stabilnych izotopów tlenu i strontu w archeologicznym materiale kostnym jest metodą badania procesów migracyjnych, zaczerpniętą z prac zoologów, śledzących szlaki wędrówek zwierząt. Podstawą do wykorzystania analizy stabilnych izotopów w badaniach pochodzenia i migracji jest obserwacja, iż proporcje izotopowe tlenu i strontu w materiale osteologicznym niemal dokładnie odzwierciedlają skład izotopowy środowiska (przede wszystkim wody i gleby), które badana grupa zamieszkiwała. Odstępstwa proporcji izotopowych tlenu oraz strontu w materiale kostnym danego osobnika w stosunku do lokalnego poziomu świadczą o jego allochtonicznym pochodzeniu. Dodatkowo różnica w składzie izotopowym pomiędzy tkanką kostną a szkliwem zębów pozwala na wyśledzenie w grupie osobników, którzy spędzili dzieciństwo w innym miejscu niż znaleziono ich szczątki.

Badania izotopowe z wykorzystaniem szczątków ciałopalnych są nadal gałęzią słabo rozwiniętą. Wiedza, iż wysoka temperatura powoduje zmiany w składzie izotopowym tkanki kostnej, niejako bezwarunkowo wykluczała materiał kostny spalony z tego typu analiz. Stosunkowo niedawno problem ten został na nowo podjęty i coraz częściej można spotkać się z pracami, w większości modelowymi, które próbują ustalić, w jaki sposób temperatura oraz czas spalania wpływają na ostateczne koncentracje stabilnych izotopów w kościach. Być może w przyszłości zaowocuje to opracowaniem ustandaryzowanej metody analizowania stabilnych izotopów w materiale ciałopalnym i pozwoli na pełniejsze poznanie populacji np. z epoki brązu, charakteryzujących się ciałopalnym obrządkiem pogrzebowym.

KEYWORDS: stable isotopes, migration, diet, cremation.

INTRODUCTION

The mobility of historical and prehistoric human groups as a consequence of (among others) the overpopulation of the area of settlement, searching for better food sources, marriages, conquests and colonization of territories, still remains a subject of interest for anthropologists and archaeologists. The arrival of new communities in a given area is frequently an argument in attempts at explaining the appearance of innovative 'inventions' in archaeological finds, accompanying the spread of agriculture in the Neolithic or the use of metals for making tools. The discussion of the character and consequences of human migrations in the prehistoric period takes place on many levels and the adaptation of diverse and innovative methods (including chemical and genetic) is meant to facilitate studying and understanding this issue.

Inter- and intra-population comparative analyses concerning the quality of nutrition, social paleostratification or the origin, direction and dynamics of the spread of historical human populations may be carried out without considerable methodological problems by analysing stable isotopes or studying trace element concentrations in remains from skeletal burials. Obviously, the cultural diversity and changeability of the *Homo* species is immense both in time and space. Along with such diversity we observe a striking diversification of the manner of burying the dead, with two prominent types of burial: inhumation or cremation. The universality of cremation and the related occurrence of exclusively or almost exclusively crematory prehistoric burial grounds is characteristic of the period from Middle/Late Bronze Age to early Middle Ages. This nearly 2500-year period, in which multiple human populations co-existed, was a watershed in terms of bio-cultural and nutritional changes, as well as on the one hand the intensification of settlement, and relocation to other areas on the other. A subject of particular interest – from an archaeological point of view – are necropoleis of biritual nature, in which both skeletal and cremation burials dating back to the same period coexist. According to Rysiewska (1996) the presence of such burial grounds was connected with family structure of Upper Silesian/Lesser Poland group of the Lusatian culture. The quoted author makes a conjecture that the burial rites (inhumation or cremation) reflected membership in a specific family group. A probable

consequence of endogamy was the appearance of cemeteries with 'burned' and 'unburned' bodies alike.

Biritual cemeteries are unique and include an enormous variety of isochronous burials: skeletal graves, cinerary urn graves, cremation burial pits, cremated bones laid in an organic container, layered and ditch burials. Inhumation rites in the history of development of our species underwent a long, nearly 2500 year period of cremation of cadavers. The remainders of this period are almost exclusively limited to cemeteries of cremation graves of various cultures of the Bronze, Iron and early Middle Ages (Śliwa ed. 2005; Kaczanowski, Kozłowski 1998).

For archaeologists and anthropologists, the epochs which used cremation are rather challenging, due to methodological inadequacies in designing and interpreting studies of human remains subjected to incineration. The Polish school of anthropology has designed a range of tested methods for evaluating cremated material. Still, there is a conspicuous shortage of interpretation and comparison of results from individual sites obtained from various anthropological and archaeological centres in Europe (Strzałko et al. 1972; Piontek 1976; Malinowski 1969; Dzierżykray-Rogalski 1967; Gładykowska-Rzeczycka 1971; Wrzosek 1928). In particular, there are doubts stemming from the assessment of sex, age, height (Piontek 1976), pathological changes and the number of individuals in a grave (Strzałko et al. 1973). Variations in the type of crematory burials – from pits to ditches to cinerary urn graves – pose additional problems. The latter create most favourable opportunities for interpretation, as the material is contained within a single vessel and generally quite well-equipped with archaeological indexes. At high temperatures of the funeral pyre, the skeleton undergoes radical deformations, shortening and noticeable fragmentation. In consequence, this may lead to misinterpretations of demographic structure and mortality in individual age categories. Note that cremation of cadavers in times of cultural and demographic transformations in Europe, which are of such a great interest to anthropologists, ethnologists and archaeologists, is one of the many causes of the (so far) unexplained origin of the Slavs.

As can be seen, the convenient position of a researcher analysing the osteological material from skeletal graves changes unfavourably when studying burned or incinerated bones and teeth. The possibilities of using standard osteometric methods and chemical analyses are radically limited, since the unearthed bone fragments after cremation are considerably changed by temperature, both in morphological and chemical terms. Consequently, this clearly impedes the evaluation of a research problem by means of chemical analyses of isotopes and elements.

Burning bone tissue involves the overheating of its organic elements, containing mainly collagen. It has a form of fibrous bundles, out of which osteons are built. The elasticity and flexibility of osteons makes the bone extensible and resistant to fracture (Sandford 1992). High temperature alters the structure of osteons and their dimensions, resulting in changes in the size, mass and shape of the burned bone. Microscopic studies have shown that at high temperature the bone undergoes certain chemical changes of its mineral components, e.g. calcium compounds become dehydrated. Experimental studies (Fig. 1) have shown that bone dimensions after burning were 10–17% smaller in relation to measurements made before incineration (Strzałko, Piontek 1974).

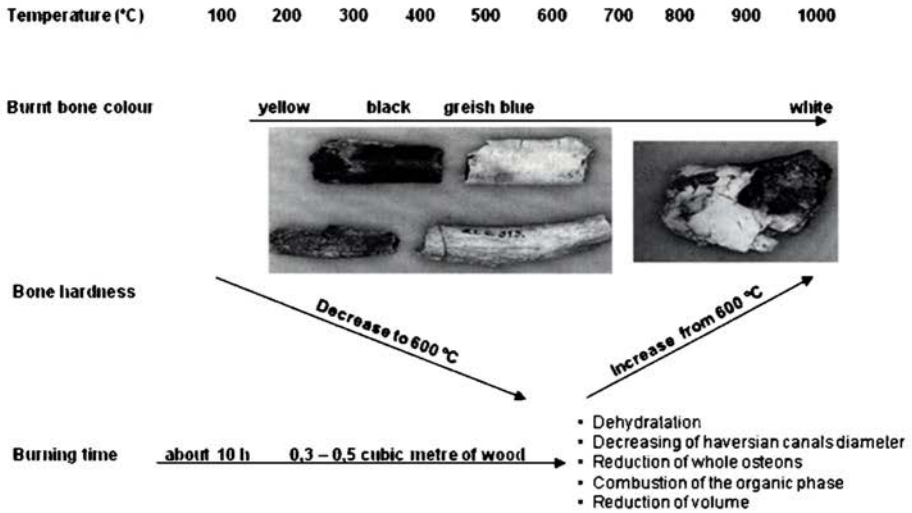

Fig. 1. Morphological changes in bone during cremations

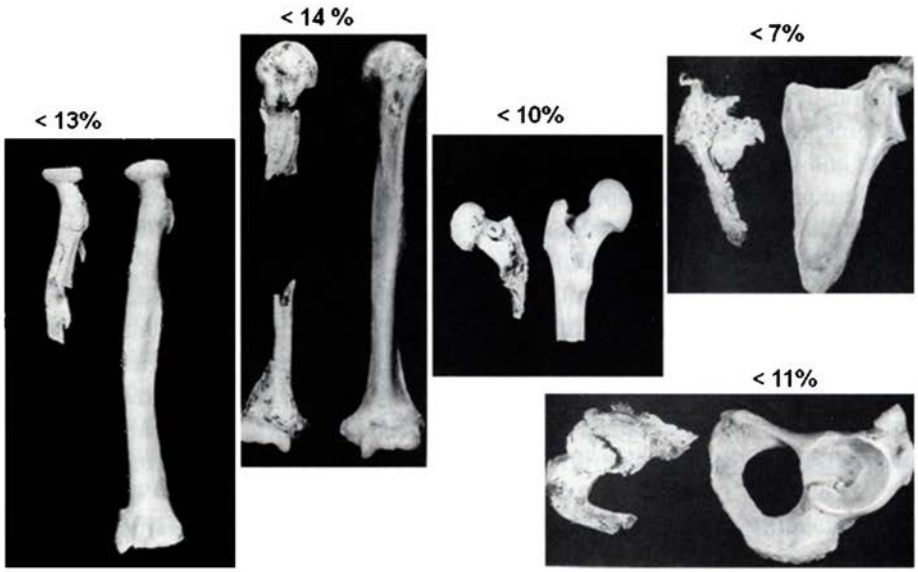

Fig. 2. Influence of high temperatures on bone morphology

The incineration ratio of bone material depends largely on temperature of the pyre on which the cremation took place and the time of incineration. Bones have different colours and hardness depending on the temperature of the pyre (Fig. 2). 650°C is the lower limit of temperature at which cremations took place, being a temperature at which enamel starts cracking. Judging by the state of metal objects, which were sometimes

put in graves, the temperature of the pyre could be as high as 1000°C. The diversity of colours of the burned bones, their shape, degree of cracking and fragmentation suggest that pyre temperatures varied throughout the discussed range.

DIRECT METHODS – BIOARCHAEOLOGICAL SIGNALS

The present section contains a synthesis of current state-of-the-art on studies of stable isotopes in terms of reconstructing diet, origin and migrations of human populations. In addition, it presents the benefits, but also limitations and interpretative pitfalls, of adapting this technique to the analysis of cremated material.

Bone oxygen and migration

Human being constitutes an integral part of the inhabited environment, which provides nutrients necessary for proper development and functioning of the body. It transpires that the isotope and element profile characteristic of the environments (flora and fauna as well as geological features) corresponds to tissue proportions of living organisms (including bone and teeth), leaving its trace even after many thousands of years. A staggering development in the field of chemistry and physics makes it possible to reconstruct the habitats (climate, temperature), diet, origin as well as migration directions of animals and people in historical and prehistoric times on the basis of chemical analyses of plant, animal and human remains.

One of the basic methods used in investigating migration processes is the analysis of stable oxygen isotopes. This method was introduced to archaeo-anthropological studies from zoological research, which first applied it to tracing animal migrations (Hobson 1999; Rubenstein, Hobson 2004; Amiot et al. 2008). Stable isotope analyses in origin and migration studies are based on the premise that the oxygen isotope ratios in osteological material almost precisely reflect the isotope composition of the environment (mainly water) inhabited by the group. If the bone material of a given individual reveals any departure from oxygen isotope ratio relative to the local level, it is possible to speak of its allochthonic origin. In addition, the discrepancy between the isotope ratio in bone tissue and tooth enamel (which does not undergo the process of *intra vitam* remodelling of elements) allows us to isolate from the group individuals who had spent childhood in a place different from where the remains were found (Longinelli 1984; White et al. 2004).

There are three stable isotopes of oxygen in nature: ^{16}O (99.759%), ^{18}O (0.204%) and ^{17}O (0.037%). However, only the heavier-to-lighter isotope ratio is used for the purpose of isotope analyses of osteological material: $^{18}O/^{16}O$, excluding the ^{17}O isotope, which is present in nature only in trace quantities. A slight disproportion of masses of various isotopes of the same element resulting from the number of neutrons in the nucleus is the cause of different physical and chemical properties of isotopes. Lighter isotopes create weaker bonds, more liable to breaking. Thus they do not require supply of such a large amount of energy during the reaction as in the case of heavier isotopes (e.g. $H_2{}^{18}O$ water evaporates more slowly than $H_2{}^{16}O$). This leads to the so-called isotope fractionation, i.e. different separation of isotopes in individual elements of the environment (Katzenberg 2000).

Stable oxygen isotopes originate from mineral fraction of bones or teeth: phosphates (~35% O by weight) and carbonates (~3,3% O by weight) (Cerling, Sharp 1996). Mineral compounds constitute approx. 70% of dry bone mass and dentin, as well as 98% of enamel, the remaining part being the organic fraction – mainly collagen. The inorganic part is present in crystalline form, predominately as hydroxyapatite, with a conventional structural formula – $Ca_{4,5}[(PO_4)_{2,7}(HPO_4)_{0,2}(CO_3)_{0,3}](OH)_{0,5}$ (Kohn et al. 1999). By physiological substitution, carbonate radicals may replace PO_4^{3-} groups – this is type B substitution (the so-called B-carbonates) – and/or OH of hydroxyapatite – type A substitution (the so-called A-carbonates). Carbonates may also form bonds on the crystalline surface of the hydroxyapatite (Wright, Schwarcz 1996; Sponheimer, Lee-Thorp 1999).

The results of spectrometric isotope analyses are presented in the form of isotope ratio (δ) deviation in the sample against laboratory standards, such as VSMOW (Vienna Standard Mean Oceanic Water) or VPDB (Vienna Pee Dee Bellemnite) (Benson et al. 2006), and expressed in per mills according to the following formula:

$$\delta^{18}O(‰) = \frac{^{18}O/^{16}O_{sample} - ^{18}O/^{16}O_{standard}}{^{18}O/^{16}O_{standard}} \times 1000$$

$\delta^{18}O$ level is analysed mainly in bone or tooth phosphates, since the covalent P-O bond formed as a result of enzyme-catalysed *in vivo* reaction is very strong. Phosphates retain their *post mortem* integrity for a long time, also in low temperature conditions. Below 100°C the exchange of oxygen between phosphates and water is very slow compared to the rate of this process for bone carbonates (Kohn, Cerling 2002; Lee-Thorp 2002). Carbonates are also exposed to diagenetic contamination by geological carbonate ions included in sedimentary calcium carbonate. Very frequently they accumulate in free spaces and on the surface of crystalline structure of bones, thus distorting measurement results of isotope ratio of oxygen (Wright, Schwarcz 1996; Katzenberg 2000).

Local level of stable oxygen isotopes is determined by spectrometric studies of isotope composition of water. As mentioned before, environmental water (rivers, reservoirs or precipitation) from different areas assumes $^{18}O/^{16}O$ ratios unique for a given area (Fig. 3), which depend largely on local air temperature and humidity, distance from a larger reservoir and altitude above sea level (White et al. 1998; Dupras, Schwarcz 2001; McGlynn 2007). Oxygen is mostly absorbed by the body with drinking water and – to a lesser extent – with food. Oxygen is also inhaled in the form of molecular atmospheric oxygen, but its isotope composition in the air remains at a fixed level of approx. 23.5‰, so it is not a differentiating factor (Benson et al. 2006). Oxygen is released with urine, sweat or exhaled CO_2. Consequently, body water displays homeostasis between absorbed and excreted oxygen (Bryant, Froelich 1995; Prowse et al. 2007). Oxygen is incorporated during the formation and rebuilding of body tissues, including bone and tooth tissues. Thus $\delta^{18}O$ of the bone mineral depends almost exclusively on the level of stable oxygen isotopes in drinking water, namely local environmental water. For phosphates it is: $\delta^{18}O_p = 0.78\ \delta^{18}O_{(water)} + 22.7$; for carbonates: $\delta^{18}O_{carb} = 0.78\ \delta^{18}O_{(water)} + 31,2$ (Luz et al. 1984; Dupras, Schwarcz 2001). A further linear relationship is observed between $\delta^{18}O_p$ and $\delta^{18}O_{carb}$ level: $\delta^{18}O_p = 0.998\ \delta^{18}O_{carb} - 8.5$ (Iacumin et al. 1996). Determining a local $^{18}O/^{16}O$ ratio in water is supplemented by isotope analysis of animal remains dating back to the same period as the investigated human population.

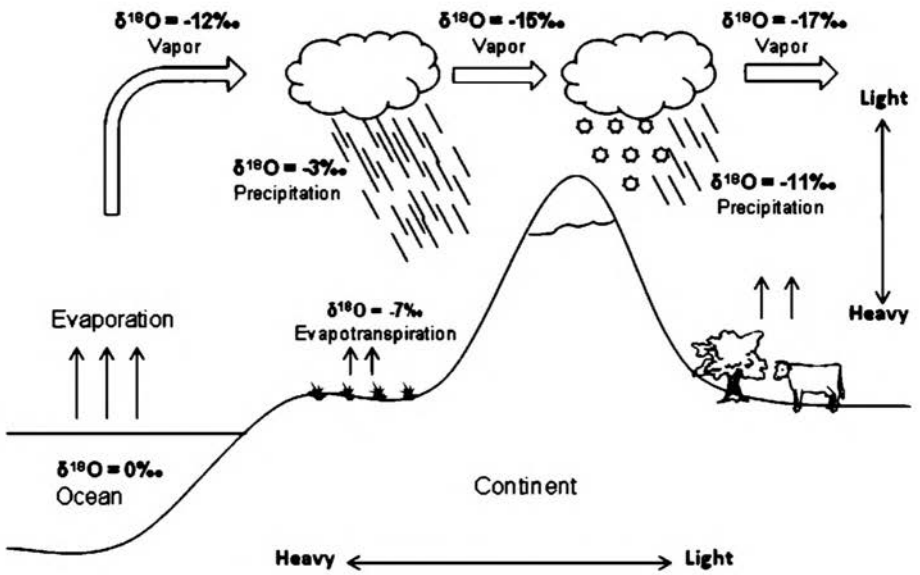

Fig. 3. Stable oxygen isotopes fractionation (after McGynn 2007)

Schwarcz and colleagues (1991, quoted in: McGlynn 2007) were first to determine $\delta^{18}O$ levels in the remains of soldiers fighting in the Anglo-American War of 1812 to identify their geographic location of origin. Analysis of stable oxygen isotopes has become an integral part of paleo-anthropological research ever since, verifying earlier, traditional methods. White et al. (1998) presented the efficiency of determining $\delta^{18}O$ ratio for human osteological tissue in migration studies. Difference in $^{18}O/^{16}O$ ratios of two historical populations from Mexico, populating different geographic areas, were substantial and confirmed by local water isotope levels. Immigrants were also successfully identified by means of stable oxygen isotope analysis in Dakhleh Oasis group (Egypt) (Dupras, Schwarcz 2001); a comparison of oxygen isotope proportions in M1 and M3 teeth in Isola Sacra population (Italy) (1^{st}–3^{rd} century AD) made it possible to isolate individuals who had spent childhood in a place different from the location in which their remains were found (Prowse et al. 2007). Other research provided information about the origin of the 'Amesbury Archer'– the richest burial during the Bronze Age in Britain found near Stonehenge. The $\delta^{18}O$ values calculated for apatite phosphate from his tooth led to the conclusion that in his childhood he lived possibly somewhere in central Europe, near the Alps (Evans et al. 2006).

Bone strontium and migration

Migrations may also be studied by analysing strontium isotope proportions. There are four stable strontium isotopes in nature: ^{84}Sr (~0.56%), ^{86}Sr (~9.87%), ^{88}Sr (~82.53%) and ^{87}Sr (~7.04%), out of which $^{87}Sr/^{86}Sr$ ratio is used in migration studies.

^{87}Sr is a stable isotope, but created as a result of radioactive disintegration of rubidium (^{87}Rb, $t_{1/2}$~4.7×10^{10} years). That is why the concentration of molecular strontium as

well as its isotope composition varies depending on the geological character of bedrock in a given area, mainly on the age and composition of minerals. Old geological units, dating back to > 100 million years, with high level of Rb/Sr, are characterised by high $^{87}Sr/^{86}Sr$ values, fluctuating around 0.720. These include clay slates and granites. In contrast, geologically young rocks (<100 million years) of low Rb/Sr ratio (np. Cenozoic volcanic rocks, basalt, limestone) are characterised by low isotope ratio of strontium: below 0.706. Slight as they seem, differences in isotope levels of strontium are significant from a geological standpoint and the highly sensitive TIMS analyzer (Thermal Ionization Mass Spectrometer) measures $^{87}Sr/^{86}Sr$ ratio accurate to ±0.00001 (Price et al. 2004; Bentley 2006).

Rocks are a source of strontium for the soil and underground water, from where the element is absorbed by plants, animals and humans (Fig. 4). Strontium is incorporated in the mineral fraction of bones and teeth by substituting calcium – a component of hydroxyapatite – and becomes absorbed without isotope fractionation (Hodell et al. 2004; Price et al. 2004; Bentley 2006). Therefore, it almost exactly reflects the isotope composition of bedrock in a specific area. This means that an individual, who for at least 10 last years of his life had inhabited the area in which his remains were found, will represent identical values of isotope level of strontium in his bones in relation to the soil. Any variance in such levels may indicate a foreign origin of the individual. Strontium isotopes in enamel, on the other hand, reveal individual's location during childhood, when enamel was formed and mineralised. As in the case of oxygen isotopes, local level of strontium isotopes is determined by analysing animal remains from the studied area. Particularly accurate data are obtained by analysing strontium isotopes in bone material of farm and domestic animals such as pigs or dogs (Bentley et al. 2004).

On the basis of concentration of strontium isotopes in teeth and bones and analysis of geochemical composition of parent rocks of a given area we may draw conclusions about the dynamics and activity of individuals as well as entire human groups. Price et al. (2001) proposed an outline for interpreting isotope signals which discriminates between local or non-local origin of individuals (Fig. 5).

Many studies demonstrate the application of strontium isotope analysis as an auxiliary tool in investigating migration routes and origins of individuals or entire human populations. Comparisons of isotope composition of strontium in enamel and bones of skeletons from several Neolithic sites in Germany indicates that most individuals in the studied groups changed their location during their lives (Bentley et al. 2004). Analysing isotope proportions of strontium in human remains from Grasshopper Pueblo (AD 1300) in central-north Arizona, Price et al. (1994) and Ezzo et al. (1997) reported a high percentage of migrations in this population. Isotope-based research also confirmed very high mobility of the Bell-Beaker culture in Central Europe, supplying new information on directions and extent of the spread of this culture in Europe. A strontium isotope analysis of people from Bell Beaker graves in southern Bavaria indicated that 18–25% children and adults had come from a considerable distance outside the area. The general direction of the local movement was probably from the northeast to the southwest (Grupe et al. 1997; Price et al. 1998). Recent studies by Price et al. (2004) shed more light on the movements of the Bell Beaker people in southern Germany, Austria, the Czech Republic and Hungary. The strontium isotopes analysis indicated that 63% individuals had moved

during their lifetime. Additionally Grupe et al. (1997) suggest high mobility of females which could be associated with the practice of exogamy characteristic of preindustrial societies.

There is no definite answer to the question which (strontium or oxygen) isotopes are more useful in migration studies. All depends on environmental and geological conditions in a specific area (e.g. whether geological and hydrological isotope profiles of a given area are available). Using both methods appears to be most appropriate, since it allows researchers to draw more complete conclusions.

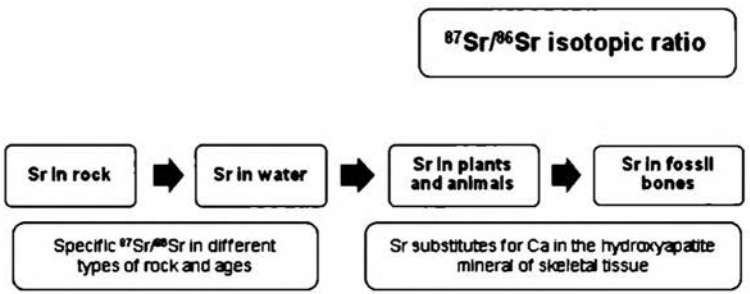

Fig. 4. Conceptual diagram of Strontium isotope analysis in bones for research on ancient migration route

		BONES: local signal	BONES: non-local signal
TEETH	local signal	Either 1/ a lifelong resident 2/ teeth are diagenically contaminated 3/ the region is geologically homogeneous	Unusual: Possibly a locally born individual spent the last years of life in a different region and then returned home shortly before burial.
	non-local signal	Immigration some time after the adult teeth were formed.	Either 1/ a recent migrant 2/ an eater of non-local foods or seafood 3/ lifelong seasonal mobility over different geological areas

Fig. 5. Possible outcomes of the analysis of bone and enamel from a single individual (after Price et al. 2001)

For example, by studying oxygen and strontium isotope ratio Evans et al. (2006) confirmed that a group of individuals buried at Lankhills cemetery in southern England (Late Roman period) according to rites different from the local population did not come from the said location. It was also revealed that the group was heterogeneous and that individuals had arrived from geographically diverse locations. Elsewhere, Evans et al. (2006) carried out isotopic analysis of three adult individuals from a collective burial of Bronze Age Beaker people at Boscombe Down, referred to as the Boscombe Bowmen. Stable strontium and oxygen isotopic evidence suggested that the Boscombe Bowmen had apparently spent their early childhood in Wales or Scotland. Also, a European origin could not be ruled out. They must have migrated to the Stonehenge area of Wiltshire at a later time in their lives. Both methods were used also to recreate the origin of a mummified individual found in the Ötztal valley in Tyrol Alps on the Austrian-Italian border. Tests carried out on water from the nearby rivers both to the north and south of the mountain ridge and their comparison with results of isotope ratio analysis of Ötzi's bone phosphates demonstrated that these proportions were similar to the ones obtained for rivers located on the southern (Italian) side of the Alps. Analysis of strontium isotopes in geological and osteological material from those areas narrowed Ötzi's place of origin down to the area adjacent to the contemporary town of Merano (Hoogewerff et al. 2001; Müller 2005).

Isotopes and diet

Analyses of historical and prehistoric osteological material based on carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratios, aimed at reconstructing diets, were the first studies in which analysis of stable isotopes were used for anthropological and archaeological research. So far they have been the best explored field.

Mechanisms related to preference of the absorbed carbon or nitrogen isotope and its further fractionation during metabolic processes are individual characteristics of groups of living organisms from different ecosystems, which are reflected in their respective isotope proportions. Land plant tissues differ in $^{13}C/^{12}C$ ratio depending on the type of photosynthesis (C_3, C_4 or CAM). $\delta^{13}C$ values for C_4 plants (including millet, maize, millet groats, sorghum and sugar cane) range from -7 to -16‰ and are much higher than for C_3 plants (most plants in moderate climate), which display values from -20 to -35‰, mainly because of lower discrimination of C_4 plants in absorbing the heavier carbon isotope. Moreover, a difference in $\delta^{13}C$ values is observed among organisms inhabiting land and aquatic ecosystems. This difference is great enough to specify whether diet contained land, aquatic organisms or both sources of food (Fig. 6) (Bocherens 1997; Ambrose 1993; Katzenberg 2000).

Analysing stable nitrogen isotopes allows us to reconstruct trophic pyramids of specific groups or populations living in diverse ecological niches and to assign individuals to a given trophic level. Many studies reported that the value of $\delta^{15}N$ increases successively with the growth of trophic level by 3-4‰ (Ambrose 1993; Richards, Hedges 1999; Katzenberg, Weber 1999; Katzenberg 2000). This phenomenon is related to preferential excretion of the lighter nitrogen isotope (^{14}N) in favour of the heavier isotope, which is incorporated in tissues, including also bones and teeth. Along with the step-up of trophic level, the consumer's intake includes food increasingly enriched with the heavier nitrogen isotope, in addition to his own metabolic processes increasing its accumulation in tissues (Katzenberg 2000).

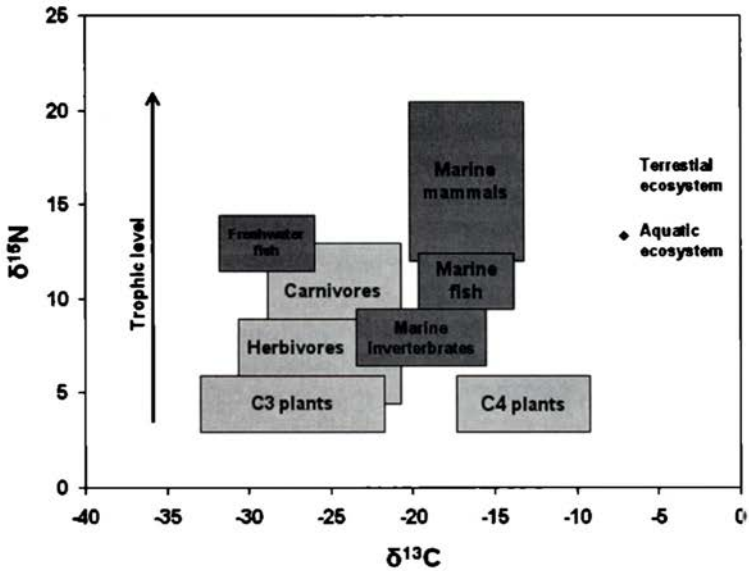

Fig. 6. The distribution of stable carbon and nitrogen isotopes in terrestrial and marine food-webs (after Ambrose 1993)

The carbon absorbed with food can be incorporated into bone or tooth tissue in three ways: as a component of the organic part (primarily of collagen, less frequently fats), structural carbonates of apatite (bioapatite) or soluble unsteady carbonates bonded on the crystalline surface of apatite (Wright, Schwarcz 1996; Gibson, Bonfield 2002). In comparison, nitrogen is a structural material of bones and teeth only in collagen. For a number of reasons, most studies focus of the reconstruction of diet using isotope analysis of collagen. The first and foremost reason is the possibility of a complex analysis of stable isotopes of carbon and nitrogen. Also, collagen is sparingly soluble and highly stable, which makes it rather resistant to diagenetic factors. Therefore, $\delta^{13}C$ measurements in bone or tooth apatites are used less frequently, usually when collagen was totally degraded *post mortem* or as a result of bone material incineration. This raises a discussion on the usefulness of $\delta^{13}C$ markings in carbonates, since they are widely assumed to be the most diagenesis-prone component of apatite (Wright, Schwarcz 1996; Koch et al.1997; Munro et al. 2008).

Note, however, that due to carbon isotope fractionation processes related to metabolism, their level in bone collagen or carbonates is higher than $\delta^{13}C$ from diet by the so-called fractionation factor, which is 5-6‰ for collagen and 12-14‰ for carbon, respectively (Ambrose 1993; Katzenberg 2000).

An analysis of stable carbon isotopes contributed significantly to recreating the stages of introducing maize into diet in successive regions of South America (van der Merwe et al. 1981) and then North America (van der Merwe 1982; Ambrose 1993). It also proved helpful in gaining a broader picture of the nutritional habits of early hominidae by providing evidence that their diet included not only fruit and leaves (as in the case of today's chimpanzees or gorillas), but also $\delta^{13}C$-rich grass and sedges and/or animals consuming

such plants, which suggests that hominidae were moving towards open spaces to search for food (Schoeninger 1995; Lee-Thorp et al. 2003). Analyses of stable carbon and nitrogen isotopes also allowed researchers to study dietary variation (among others) in Bronze Age groups from Crete (Petroutsa, Manolis 2010) or Italy (Tafuri et al. 2009), and to estimate the proportion of aquatic animals in diet of several groups from late Mesolithic period, who inhabited the European coast of the Atlantic Ocean (Richard, Hedges 1999), or a Neolithic group from Lake Baikal (Katzenberg, Weber 1999).

Stable isotopes and burned bone

Isotope studies using cremated remains are still a largely unexplored field. The fact that high temperature causes changes in the isotopic composition of the bone tissue unconditionally excludes incinerated bone material from such analyses. This problem has only recently been addressed once again and more and more works, usually model studies, attempt to explain how heat and duration of the incineration affect final concentrations of stable isotopes in bones, which may result in designing a standardised method for analysing stable isotopes in cremated material in the future and allow us to fully explore the characteristics of populations with cremation burial rites.

As previously mentioned, effects of high temperatures on bones or teeth include cracks and changes in shapes, colour and structure. Different intensity of these changes is a good indication for reconstructing pyre temperatures and bone incineration time (Hanson, Cain 2007). Along with rising temperature, bones and teeth also undergo chemical and microstructural changes. Between 20°C and 300–350°C the bone loses most of unbounded water and the process of carbonizing organic matter starts. Above 400°C the organic fraction is burned, and at 600°C the recrystallization of the bone mineral starts, until at 1600°C it is totally melted (Holden et al. 1995; Quatrehomme et al. 1998; Hanson, Cain 2007). Some authors almost equate successive, temperature-dependent changes in the structure of bone tissue during bone incineration with consecutive stages of diagenesis. Therefore, one needs to be aware of the influence of separate mechanisms responsible for both phenomena, although the sequence seems to be similar (Person et al. 1996).

Cremation and carbon and nitrogen isotopes

First attempt at investigating the effect of temperature on the level of stable carbon isotopes was made by DeNiro et al. in 1985. A series of laboratory experiments on contemporary animal bone subjected to thermal treatment showed an increase in the level of stable isotopes of both carbon and nitrogen in bone collagen by 4–5‰ in comparison to a thermally untreated bone. Those findings were not entirely confirmed by the latest model studies by Schurr et al. (2008), in which $\delta^{13}C$ and $\delta^{15}N$ measurements were made in collagen of bovine bone incinerated at temperature ranging from 150 to 600°C. The results suggest lack of any significant changes in the isotopic composition of carbon in bone collagen in relation to temperature within a given range. Still, the authors support the observations by DeNiro et al. (1985) concerning isotopic disproportions in nitrogen; they obtained a significant, positive correlation between $\delta^{15}N$ level and temperature, whereby the difference between the bone burned at 600°C and the unburned bone was 4–6‰. In addition, a relationship between the level of $\delta^{15}N$ and the time of incineration at the same temperature was reported.

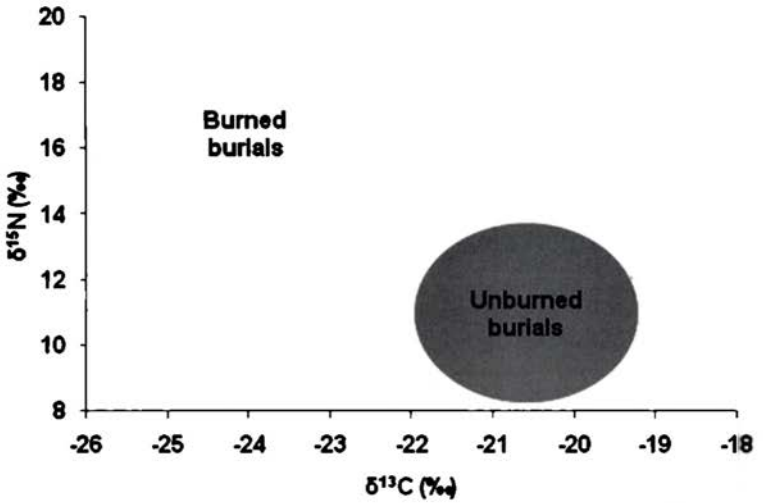

Fig.7. Stable carbon and nitrogen isotopes from the Klunk burials (after Schurr et al. 2008)

Schurr et al. (2008) also performed analyses of stable carbon and nitrogen isotopes in bone material collagen from three sites in North America (Pete Klunk, Yokem site, Angel site; 1500BP–1400AD), where both cremation and skeletal graves were found. $\delta^{15}N$ and $\delta^{13}C$ levels in collagen of burned and unburned bones were compared (Fig. 7). As in the case of model studies, for each studied group an identical ~5‰ increase of $\delta^{15}N$ in burned bones relative to unburned bones from a given site was observed. A comparable difference was reported when analysing $\delta^{13}C$ – distinct from model studies by Schurr et al. (2008) but postulated by DeNiro et al. (1985). Thus, the problem of thermal stability of $\delta^{13}C$ in collagen is yet to be fully explained. Authors of archaeological studies point out the influence of diagenetic factors on isotope level of carbon from collagen in tested investigated bones, which is indicated by C/N values of burned and unburned bones. It seems that the conjecture, that the pyres on which cremations took place reached temperatures higher than 600°C, which may have caused $\delta^{13}C$ to change, seems quite plausible.

As mentioned before, stable carbon isotopes may also be analysed in bone or tooth carbonates, especially following the organic degradation of part of remains occurring also as a result of high temperature during incineration. As proposed above, a higher stability of $\delta^{13}C$ carbonates in comparison to collagen is confirmed by data presented by Person et al. 1996. The first phase of the experiment included measurements of stable carbon isotopes in fresh bovine bone, both in collagen and hydroxyapatite carbonates. This was followed by the incineration of entire bone samples of the same individual (NB. without isolating collagen or carbonates) at various temperatures from 300 to 700°C. As it turned out, the level of $\delta^{13}C$ in the unburned bone was almost identical to the one in the isolated collagen; the higher the incineration temperature was, the more the $\delta^{13}C$ value approximated the isotopic composition of carbon in isolated carbonates of hydroxyapatite (Fig. 8). The findings of a study made using bones of a white-tailed deer (*Odocoileus viginianus*) provide conclusions similar to the research by Person et al. (1996). $\delta^{13}C_{carb}$ remains on almost the same level throughout the range of 25–650°C. Above 650°C there

is a rapid increase of $\delta^{13}C_{carb}$ by 2–6‰ on average, until 725°C, when total disintegration of carbonates takes place (Munro et al. 2008). However, model studies are not backed by any evidence from the analyses of isotopic proportions of archaeological cremated bone material. Notwithstanding the fact that the question of stability of $\delta^{13}C$ collagen is not yet finally resolved (further research is necessary), analyses of $\delta^{13}C$ carbonates seem to be a suitable tool for reconstructing diets of populations with crematory burial rites in cases where pyre temperature did not exceed 700°C. The authors also suggest that isotope analyses should be carried out only on partially burned bones in sections less exposed to temperature (Schurr et al. 2008).

Cremation and oxygen isotopes

Analyses of stable oxygen isotopes in cremated material have been carried out only on some model studies, which explain certain mechanisms accompanying bone burning and changes in isotope ratios. Nevertheless, there are no data from isotope tests of cremated archaeological material whatsoever. The literature of the subject also does not contain any information, even model data, concerning the analysis of stable strontium isotopes in cremated material.

A study by Munro et al. (2007) included of three simultaneous experiments: the first one involved burning contemporary sections of a white-tailed deer's bones (*Odocoileus viginianus*); in the second experiment, fresh bone fragments were boiled at predefined temperatures. Next, levels of stable oxygen isotopes in bone phosphates were measured in the samples. It was reported that an incineration temperature up to 300°C did not cause the $\delta^{18}O_p$ ratio to change significantly. Above 300°C the $\delta^{18}O_p$ ratio falls together with the rise in temperature, so that its value at 700°C is ~3‰ smaller than in the blank test, with a greater, ~5‰ difference at 900°C (Fig. 9). Changes in oxygen isotope ratios probably result in structural and phase changes in the crystalline structure of calcium phosphates and a gradual loss of carbonates and phosphates, as confirmed by FTIR results. Also, above 700°C CaO is formed: its strong hygroscopic properties cause the cooled-off sample (which has assumed the form of structurally modified apatite) to incorporate atmospheric water. A rapid exchange of oxygen isotopes takes place between water and apatite, which affects further spectrometric analyses (Lindars et al. 2001). It is also suggested that phosphates are less susceptible to diagenetic degradation and more resistant to high temperatures than apatite carbonates, in which $\delta^{18}O_{carb}$ distortions occur in temperatures as 'low' as 200°C (Munro et al. 2008).

Consequently, it appears that the analyses of stable oxygen isotopes in burned material should be carried out on material which was subjected to temperatures of no more than 300°C if reliable information on migrations needs to be obtained. As indicated by various archaeological artefacts, temperatures of pyres at which cremations took place ranged from 650°C to as much as 1000°C, a point at which $\delta^{18}O$ distortions are considerable. A solution proposed by some authors (Schurr et al. 2008) is the isotope analysis of the least burned part of the bone or tooth. Apparently, another way is to evaluate the pyre temperatures with high precision and assign to them sequences of shifts in stable isotope proportions. As a result, it will be possible to extrapolate the results of isotope analyses of cremated material on the actual, *intra vitam* values.

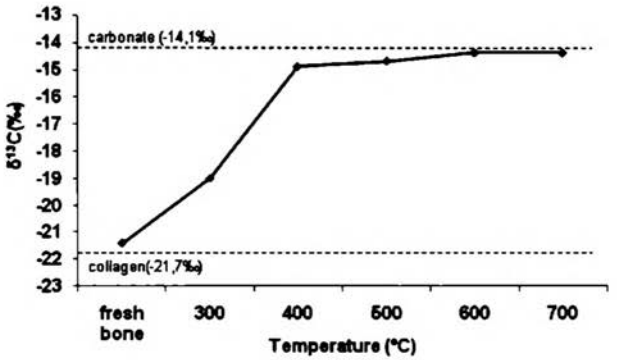

Fig. 8. Changes of $\delta^{13}C$ and % of organic matter in cremated bone

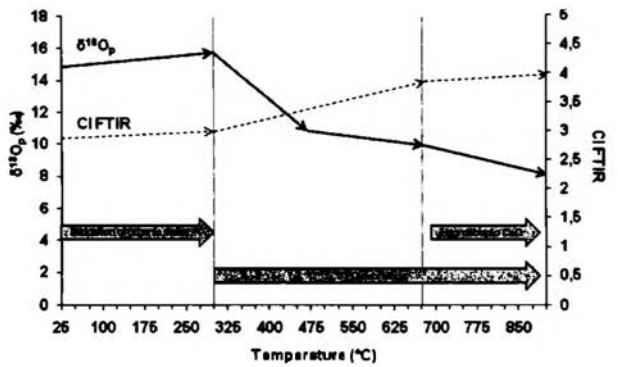

Fig. 9. Changes in stable oxygen isotope and cristallinity index ratio with raising temperature (after Munro et al. 2007)

Cremation and its effect on bones

We are not able to accurately specify the phase of decomposition of an incinerated bone on the basis of its macroscopic characteristics. This limitation plays a crucial role when determining the suitability of material for stable isotope analyses and, as previously discussed, predicting isotope changes. In line with the above, procedures of an accurate assessment of the chemical degradation of incinerated bones or teeth increasingly use parameters which were earlier applied in determining the extent of diagenetic processes in osteological material, such as: C/N ratio, % content of C and N in collagen and crystallinity index (FTIR).

Investigating the purity of a collagen sample is performed by determining several parameters, such as the carbon-oxygen atom ratio (C/N), concentration of collagen in the bone and % content of carbon and nitrogen in collagen by means of mass spectrometer (Pearsall 2000). Collagen is unique amongst animal proteins, since it is the only protein

which contains hydroxyproline; glycine makes up 30% of collagen (Stryer 2005). Selective, *post mortem* loss of any amino acids may have an impact on the proportion of stable isotopes in collagen. Due to its conservative amino-acid structure, the C/N ratio for collagen should be between 2.9 and 3.6 (Jørkov et al. 2007). Any deviations from the above values indicate either contamination of collagen by other substances or its degradation. Furthermore, percentage of collagen in the bone should not be below 2% of the bone or tooth mass and % concentrations should not decrease below 6.6% for C and 1.9% for N (Pearsall 2000). Research has made it possible to investigate changes which occur in collagen in the process of bone-burning, and thus to evaluate the suitability of the isolated collagen for isotope analyses. It was observed that % content of N remains unchanged until the temperature of 200°C is reached, then gradually falling until it reaches 0% when the temperature rises to 350°C, at which point the carbonization process starts (Schurr et al. 2008).

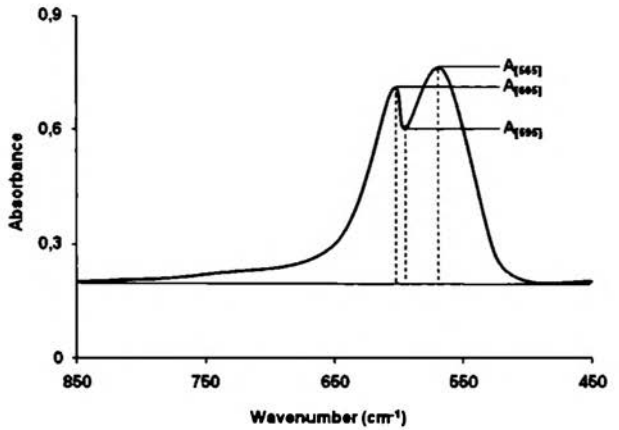

Fig. 10. FTIR spectrum with phosphate double peaks uses for calculating CI value (after Surovell and Stiner 2001)

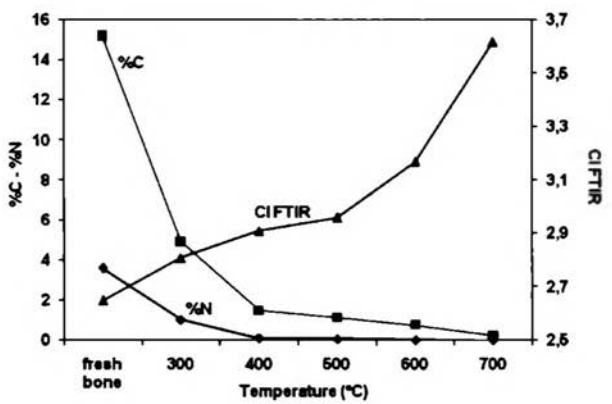

Fig. 11. The influence of temperature on biochemical structure of bone

Changes in the crystalline lattice of bioapatite can be investigated by means of Fourier Transform Infrared Spectrometry (FTIR). An IR spectrometer is used for the analysis of the spectrum of a powdered bone. On the basis of measured data a crystallinity index is determined CI = $(A_{[565]}+A_{[605]})/ A_{[595]}$, where $A_{[565]}$ and $A_{[605]}$ represent band absorbance in the region of 565 and 605 $cm^{-1}x$, characteristic for biogenic phosphates, and $A_{[595]}$ – absorbance of the narrowing between PO_4^{3-} group bands (Fig. 10). For a modern bone, the CI is 3.1. For a prehistoric osteological material these values should not exceed 3.8 (preferably <3.3). Above 3.8 the bones are considered very contaminated and recrystallized (amorphous). If an additional correlation exists between $\delta^{18}O$ and CI, such material is unsuitable for further processing (White et al. 1998; Surovell, Stiner 2001).

The crystallinity index in the burned bone rises, gradually but slightly, along with increasing temperature. At 625°C, an steep rise in the value of CI above 3.8 indicates total recrystallization of the material (Munro et al. 2007). Comparable results were obtained by Person et al. (1996) and Surovell and Stiner (2001) in their own model studies. In addition, a correlation was found between CI and $\delta^{18}O$ level along with temperature, where the increase in the CI was accompanied by falling $\delta^{18}O$ value (Munro et al. 2007). Moreover, Person et al. (1996) observed that growing crystallinity of bones associated with rising temperature is negatively correlated with % content of N and C in collagen, which supports the observation that the degradation of the organic fraction of the bone or dental tissue significantly affects its crystallinity level (Fig. 11).

FTIR results and attempts at specifying pyre temperature should be treated with caution. As reported by Thompson et al. (2009), this method allows researchers to approximately specify whether the temperature of the pyre was low or high. Model tests performed by this author and aimed at accurate specification of pyre temperature on the basis of crystallinity index did not yield unequivocal results.

CONCLUSIONS

The research benefits of the analysis of stable carbon, nitrogen and oxygen isotopes are undeniable if skeletal material is used for the purpose. However, in this article we tried to point out that before such methods become a commonplace tool in analysing cremated material, a substantial amount of knowledge on the mechanisms governing the influence of temperature on the bone must be available and those mechanisms need to be accurately linked to isotope ratios. The key to finding a solution seems to lie in a large-scale model studies on animal material and simultaneous isotope analyses of archaeological material. Analyses of cemeteries containing both skeletal and cremation graves would prove a source of particularly valuable information. Thanks to this, in the foreseeable future scientists will perhaps be less 'inadequate' when studying cultures with cremation burial rites.

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