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### Stable Isotope Evidence for Early Modern Human Diet in Southeastern Europe: Peștera cu Oase, Peștera Muierii and Peștera Cioclovina Uscată<sup>\*</sup>

## ERIK TRINKAUS, ANDREI SOFICARU, ADRIAN DOBOŞ, SILVIU CONSTANTIN, JOÃO ZILHÃO and MICHAEL RICHARDS

**Key-words:** Radiocarbon dating, Early Upper Paleolithic, Peștera cu Oase, Peștera Muierii, Peștera Cioclovina Uscată, Middle Paleolithic, Early Upper Paleolithic.

Abstract: During the process of direct radiocarbon dating of four Early Upper Paleolithic modern humans from the Romanian sites of Peştera cu Oase, Peştera Muierii and Peştera Cioclovina Uscată, carbon and nitrogen stable isotope ratios ( $\delta^{13}$ C and  $\delta^{15}$ N) were measured from their bone collagen. These individuals have  $\delta^{13}$ C values similar to other Late Pleistocene humans. Their  $\delta^{15}$ N values are well within the carnivore trophic level range, among the higher of the Middle Upper Paleolithic values, and significantly greater than those of preceding Middle Paleolithic and Initial Upper Paleolithic Neanderthals. These data suggest a shift towards a broader faunal dietary spectrum among these early modern humans, despite western and eastern European archeological evidence and human functional morphology indicating little change in faunal exploitation from the Middle Paleolithic to the Early Upper Paleolithic.

**Cuvinte cheie:** Datări cu radiocarbon, Peștera cu Oase, Peștera Muierii, Peștera Cioclovina Uscată, Pleistocen superior, Paleolitic mijlociu, Paleolitic superior.

**Rezumat**: Cu ocazia datării cu radiocarbon a patru fosile de oameni moderni de la începutul paleoliticului superior vechi, descoperite la Peștera cu Oase, Peștera Muierii și Peștera Cioclovina Uscată, din colagenul oaselor au fost măsurate și proporțiile izotopilor stabili de carbon și azot ( $\delta^{13}$ C și  $\delta^{15}$ N). Valorile  $\delta^{13}$ C sunt similare cu cele ale populațiilor din Pleistocenul superior, în vreme ce valorile  $\delta^{15}$ N indică o dietă perponderent carnivoră. Aportul de carne în cazurile studiate este printre cele mai ridicate din Paleoliticul superior mijlociu, și în același timp mult mai însemnat decât al neandertalilor din Paleoliticul mijlociu și de la începutul Paleoliticului superior vechi. Aceste date arată că primii oamenii moderni din această regiune au inclus în dietă o gamă mai variată de animale, deși datele arheologice și morfologia funcțională a scheletelor umane din vestul și estul Europei indică schimbări minore în exploatarea faunei pentru Paleoliticul mijlociu și Paleoliticul superior vechi.

#### Introduction

Insight into the dietary spectra of Late Pleistocene humans, at least in Europe, is primarily based on the associated faunal remains, especially for assemblages in which the primary accumulating agent appears to have been humans, organic preservation is good, and the excavation has been sufficient to permit detailed zooarcheological analysis. However, not all sites have sufficient preservation, many were excavated in the past with less rigorous methods (including industrial exploitation of the site), and/or represent complex palimpsests of human and carnivore activities variably combined with geological disturbances of the sediments. As a result, stable isotope analysis of human (and faunal) remains from the Late Pleistocene has been increasingly undertaken to provide further insight into Late Pleistocene human dietary spectra, especially for well-dated (and in many cases, directly-dated) human remains.

These latter conditions variably apply to three of the European early modern human samples, those from the Peştera cu Oase, Peştera Muierii and Peştera Cioclovina Uscată, all in southwestern Romania (Rainer and Simionescu 1942; Gheorghiu

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<sup>&</sup>lt;sup>\*</sup> Erik Trinkaus: Department of Anthropology, Campus Box 1114, Washington University, Saint Louis MO 63130, USA; Andrei Soficaru: Institutul de Antropologie "Fr. J. Rainer," Eroii Sanitari 8, P.O. Box 35-13, 050471 Bucureşti, Romania; Adrian Doboş: Institutul de Arheologie "Vasile Pârvan," Henri Coandă 11, Sector 1, 010667 Bucureşti, Romania: Silviu Constantin: Institutul de Speologie "Emil Racoviță", Str. Frumoasă 31, 010986 Bucureşti, Romania; João Zilhão: Department of Archaeology and Anthropology, University of Bristol 43 Woodland Road, Bristol BS8 1UU, UK; Michael Richards: Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, 04103 Leipzig, Germany; Department of Anthropology, University of British Columbia, Vancouver BC V6T 1Z1, Canada.

and Haas 1954; Trinkaus et al. 2003; Soficaru et al. 2006, 2007; Rougier et al. 2007). These samples represent a substantial portion of the more complete remains for early modern humans >29 ka <sup>14</sup>C BP; only the Moravian Mladeč sample is larger, the western European remains (such as those from Brassempouy, La Quina-Aval and Les Rois) consist principally of partial mandibles and isolated teeth (Martin 1936; Gambier and Houët 1993; Henry-Gambier et al. 2004; Teschler-Nicola 2006), and further eastern European remains (from Kostenki) consist of undescribed and/or undiagnostic teeth and postcrania (Sinitsyn 2004; Anikovich et al. 2007). The Romanian human remains are, however, approached in time by the (probably) early Gravettian remains from Cro-Magnon and Paviland (Henry-Gambier 2002; Jacobi and Higham 2008).

The oldest of these remains, from the Peştera cu Oase (Anina, Caras-Severin) at ~35 ka <sup>14</sup>C BP, were water-transported surface finds within a karstic system that served primarily as a hibernation locale for large cave bear (Ursus spelaeus) with fossil and taphonomic evidence for some wolf (Canis lupus) activity (Zilhão et al. 2007). There are no archeological remains or other evidence of Pleistocene human activity within the cave system, and the human fossils appear to be secondary intrusions into the system. The isotopic data (Table 1) derive from the direct <sup>14</sup>C dating of the Oase 1 mandible (Trinkaus et al. 2003); repeated attempts to directly date the Oase 2 cranium provided only a minimum age for the specimen due to poor collagen preservation (Rougier et al. 2007).

The next oldest specimens, from the Peştera Muierii (Baia de Fier, Gorj; aka Peștera Muierilor) at ~29-30 ka <sup>14</sup>C BP, were found during excavations in the Galeria Musteriană (Gheorghiu and Haas 1954), probably secondarily mixed with Middle Paleolithic lithic remains (Soficaru et al. 2006). Although there is evidence for an earlier Upper Paleolithic occupation of the adjacent Galeria Principală and especially Middle Paleolithic activity in both galleries, the site contains an abundance of U. spelaeus remains, combined with those of other carnivores and herbivores. Given the use of the cave system as a carnivore den, partial excavation recovery of faunal remains, and uncertainties regarding the association of the human remains with specific archeological assemblages, the only dietary data available for the Muierii humans derives from their stable isotope values, also obtained as part of the <sup>14</sup>C dating process.

The youngest specimen, slightly more recent than the Muierii remains at  $\sim 28,500^{-14}$ C BP, is the isolated human neurocranium from Pestera Cioclovina Uscată (Bosorod, Hunedoara) (Rainer and Simionescu 1942; Soficaru et al. 2007). The cranium was found during phosphate mining, and its original position in the cave is unknown. Although Middle Paleolithic and Early Upper Paleolithic assemblages have been found in the cave (Dobrescu 2008), the cave served principally as a hibernation den for U. spelaeus, and remains of other species are rare. The human fossil is therefore without stratigraphic, paleontological or archeological context (cf., Rainer and Simionescu 1942; Păunescu 2001; Soficaru et al. 2007), and both its age and any dietary inferences must be based on its bone chemistry.

#### Stable Isotopes and Diet

The measurements of the stable isotope ratios of carbon ( ${}^{13}C/{}^{12}C$ , the  $\delta^{13}C$  value) and nitrogen ( ${}^{15}N/{}^{14}N$ , the  $\delta^{15}N$  value) in bone collagen extracted from human and animal hard tissue has become a well established method for determining past diets (Sealy 2001; Lee-Thorp 2008). Through a number of lines of research, including empirical observations and controlled experiments on living organisms, it has been determined that the carbon and nitrogen isotope ratios in mammal bone collagen reflect the isotope ratios of dietary protein consumed over the last years of the life of that mammal (Wild *et al.* 2000).

Nitrogen in bone can only come from dietary protein, so mammal bone collagen  $\delta^{15}$ N values indicate the main sources of dietary protein in a mammal's long-term diet, and it is enriched by approximately 2-4‰ over dietary protein  $\delta^{15}$ N values (Schoeninger and DeNiro 1984; Jenkins *et al.* 2001). In temperate environments such as Europe, strict herbivores tend to have low  $\delta^{15}$ N values, generally <7‰, whereas dedicated carnivores have higher  $\delta^{15}$ N values, generally >8‰, and omnivores have intermediate values (*cf.*, Bocherens 2002).

There is variation between sites, and ideally one should evaluate human  $\delta^{15}$ N values with respect to values from animals of known diet from the same levels of the same sites, something that is not always possible (*e.g.*, Richards *et al.* 2000; Bocherens *et al.* 2005; Beauval *et al.* 2006). Such

correction is partly feasible for Peştera cu Oase (Richards *et al.* 2008a) and, to a lesser extent, for Peştera Muierii (Doboş *et al.* 2009). However, dietarily significant differences in  $\delta^{15}N$  values, given within population variation and within dietary category variation, should mitigate intersite variation in values.

Carbon isotope ratios ( $\delta^{13}$ C) do not change, or change only slightly, between trophic levels, and they therefore reflect variation in plant carbon isotopes (C<sub>3</sub> versus C<sub>4</sub> plants, with the former having lower or more negative  $\delta^{13}$ C values), access to marine resources (which have higher  $\delta^{13}$ C values), and possibly climate (van Klinken *et al.* 1994, Richards and Hedges 2003). In Late Pleistocene Europe, C<sub>4</sub> plants were not present, so  $\delta^{13}$ C variation should reflect minor paleoecological differences and, to some extent, access to marine resources (Richards 2000; Pettitt *et al.* 2003).

#### Materials and Methods

The stable isotope data for the Oase, Muierii and Cioclovina human remains was generated through the direct AMS radiocarbon dating of the specimens, principally by the Oxford Radiocarbon Accelerator Unit (ORAU); in addition three corroborative AMS dates derive from the Centrum voor Isotopen Onderzoek in Groningen and the Laboratoriet för <sup>14</sup>C-datering i Lund (Table 1). The dates and available chemistry have been published previously (Trinkaus et al. 2003; Soficaru et al. 2006, 2007); all of the samples have  $\delta^{13}$ C values and C:N ratios (DeNiro 1985) within acceptable ranges; the initial ORAU Oase 1 date was a minimum age, so further dating was done through Groningen to produce a combined age of ~35 ka <sup>14</sup>C BP. The ORAU dates were all run using ultrafiltration (Brown et al. 1988; Higham et al. 2006a).

Oase 1 sample derives from the posteroinferior mandibular corpus. The Muierii 1 sample is from the zygomatic bone, and the Muierii 2 one is from the squamous temporal. The Cioclovina 1 sample is from a detached portion of the inferior occipital bone. The relevant faunal remains from Oase, the wolf (*C. lupus*), red deer (*Cervus elaphus*) and ibex (*Capra ibex*) (Figure 2) bracket the Oase 1 mandible in time (Richards *et al.* 2008a). The moose (*Alces alces*) sample from Muierii (an M<sup>1</sup>) is radiometrically the same age as the human remains, but the cave lion (*Panthera spelaea*) is older (Soficaru *et al.* 2006; Doboş *et al.*, 2009). The other fauna providing stable isotopes from Oase and Muierii, and all of the fauna providing such data from Cioclovina, are cave bears (Richards *et al.* 2008a; Doboş *et al.* 2009). Cave bears, as with modern brown bears (Mowat and Heard 2006), appear to have potentially broad dietary spectra (Richards *et al.* 2008a) and therefore cannot be used as a baseline for evaluating the human isotopic results.

Comparative Late Pleistocene human stable isotope values derive from published analyses of European late archaic (Neandertal) and early modern (Early and Middle Upper Paleolithic) humans, many of which were generated in the context of direct AMS radiocarbon dating of the human remains (Table 2). Eight of the Neandertals with stable isotope data are Late Pleistocene (OIS 4 and 3) Middle Paleolithic in context; the other three Neandertals (Saint-Césaire 1, Spy 572a and Vindija 208) providing data are from the Initial Upper Paleolithic (Lévêque et al. 1993; Janković et al. 2006; Semal et al. 2009). In cases in which specimens have been redated (e.g., Higham et al. 2006b; Jacobi and Higham 2008), values from the more recent analyses are employed. Variation in values from repeated measures of the same specimens and from different sample preparation protocols tend to be modest, close to  $\pm 1.0\%$ .

Most of the human and faunal data derive from fully mature bone. However, four of the specimens are immature (the Middle Upper Paleolithic Kostenki 4 and Sunghir 2 and 3 late juveniles and the Neandertal Engis 2 infant) and one (the Jonzac 1 Neandertal) is a premolar root. In addition, among the comparative faunal remains, the Peştera Muieri A. alces data are from the root of an  $M^1$ . It appears that nursing immature individuals should be considered to be a trophic level higher than adults due to lactation. As a result, bone or dentin formed postnatally and prior to weaning may have  $\delta^{15}$ N values a few per mil higher than that of the mother, depending on the duration of nursing and the degree to which the diet of the infant is supplemented with other foods (Jenkins et al. 2001; Fuller et al. 2006; Jay et al. 2008). This should not pose a problem for the bone samples from the older juvenile Kostenki and Sunghir remains, nor for the Jonzac premolar given late juvenile root formation for human premolars (Smith 1991). However, the Engis 2  $\delta^{15}$ N value of 12.6‰ (Bocherens *et al.*  2001) may be elevated relative to its population average, and the *A. alces*  $\delta^{15}$ N value of 7.3‰ (Doboş *et al.* 2009) may be similarly elevated given early postnatal formation of cervid first molar roots (Brown and Chapman 1991).

Given the non-parametric nature of the stable isotope distributions, sample comparisons are made using Wilcoxon P-values. Significance levels are adjusted using a sequentially reductive multiple comparison corrections within sets of tests (Proschan and Waclawiw 2000).

#### Results

Among Late Pleistocene humans, there is a shift in average  $\delta^{13}$ C and  $\delta^{15}$ N values from the Neandertals to the Middle Upper Paleolithic modern humans (Fig. 1). The latter sample has significantly higher (less negative)  $\delta^{13}$ C values (P = 0.001). This is due in part to the high values for Arene Candide 1 and La Rochette 1 and the low values for Feldhofer 1 and 2. The Arene Candide 1  $\delta^{13}$ C value, as well as the slightly lower Paviland 1 one, are apparently due to the consumption of significant amounts of maritime resources (Richards 2000; Pettitt *et al.* 2003). It is not clear why Feldhofer 1 and 2 have very low  $\delta^{13}$ C values, but they are close to those of cervids from the same site (Richards and Schmitz 2008).

In  $\delta^{15}$ N values, there is a modest but nonsignificant shift between the adults of the Neandertal and Middle Upper Paleolithic samples (P = 0.129), despite the low values for the Feldhofer specimens; including the value for the Engis 2 provides less of a difference (P = 0.236). The  $\delta^{15}$ N values for the Initial Upper Paleolithic Saint-Césaire 1, Spy 572a and Vindija 208 (11.4‰, 11.0‰ and 10.3‰ respectively) fall among the Middle Paleolithic Neandertal values.

The  $\delta^{15}$ N values for Peştera cu Oase and Peştera Muierii carnivores and herbivores are within expected values for these dietary groups, with the wolves and the cave lion being ~8‰ up to ~12‰ and the herbivores being below 7.5‰ (Fig. 2); reducing the *A. alces* value slightly, given the young age of its M<sup>1</sup> formation, would place it closer to the other herbivores. The four Romanian early modern humans are in the middle of the faunal range in  $\delta^{13}$ C values, but they are at the top of the range and above the carnivores in  $\delta^{15}$ N. When considered among other Late Pleistocene humans (Fig. 1), these Early Upper Paleolithic humans are among the highest of the Middle Upper Paleolithic humans, being exceeded only by Barma Grande 6 and Kostenki 4 in their  $\delta^{15}$ N values. The very high  $\delta^{15}$ N Early Upper Paleolithic value is provided by Kostenki 8, an undescribed tibia and fibula dated to ~32.6 ka <sup>14</sup>C BP (Richards *et al.* 2001; Sinitsyn 2004). At the same time, these four human  $\delta^{15}$ N values are above those of all of the mature Neandertals, but matched by the Engis 2 infant's value. As a group they are significantly (P = 0.006) higher than those of the adult Neandertals (P = 0.003 with Kostenki 8; P = 0.005 with both Kostenki 8 and Engis 2 included).

#### Discussion

The modest shift in  $\delta^{15}$ N values between the Neandertal and Middle Upper Paleolithic samples, in combination with the generally higher more recent  $\delta^{13}$ C values, has been attributed to a broader food spectrum and a greater reliance on aquatic resources with their longer food chains among some of the Middle Upper Paleolithic populations (Richards *et al.* 2001). The growing evidence for some degree of maritime resource exploitation among Neandertals (Stiner, 1994; Stringer *et al.* 2008), however, suggests that any dietary contrasts may be more subtle than originally suggested and/or more regionally variable.

The addition of data from the earliest modern humans in Europe, now represented isotopically by those from Oase, Muierii and Cioclovina, as well as the undescribed Kostenki 8 specimen, extends any isotopic contrasts further back in the Upper Paleolithic. The significant increase in  $\delta^{15}$ N values for these early modern humans relative to the Neandertals implies an increase in dietary protein from animals and/or in aquatic resources with long food chains relative to the Neandertals. Yet, any such differences are likely to be in degree, given the proximity of most of the isotopic values between the Neandertals and these early modern humans, all of them well within the "carnivore" range.

At the same time, the available archeological and human paleontological evidence does not imply a major dietary difference between Neandertals and Early Upper Paleolithic modern humans. There is archeological evidence for improved weaponry in the Aurignacian (Knecht, 1993; Liolios, 2006; Bolus and Conard, 2006), and similar evidence for effective spears is known from the Galeria

Principală in the Peștera Muierii (Gheorghiu et al. 1954; Păunescu 2000) and occurs in other Romanian Aurignacian assemblages (Chirica et al. 1996; Dobrescu 2008). Yet, Aurignacian faunal assemblages, to the extent that they have been appropriately analyzed zooarcheologically (albeit not in southeastern Europe [cf., Dobrescu 2008]), show little difference with earlier Middle Paleolithic or Initial Upper Paleolithic ones (Grayson and Delpech 2002, 2003, 2008; Morin 2008). Organic residue analysis of eastern European Middle Paleolithic and Early Upper Paleolithic lithics (Hardy et al. 2001) shows similar patterns of exploitation in both groups of a broad spectrum of plant and animal resources. In addition, functional analyses of the few known Early Upper Paleolithic human upper limb remains (including the Muierii 1 scapula) show little of the anatomical change associated with habitual spear throwing (Trinkaus et al. 2006; Trinkaus 2008; cf., Churchill and Rhodes 2006).

It also remains to be assessed whether some degree of the isotopic contrast may be related to variation in geographic and/or climatic milieu. The Neandertal data derive from central and western Europe, whereas the Early Upper Paleolithic data are all eastern European (Tables 1 and 2). Yet, the Middle Upper Paleolithic human sample derives from sites across Europe, and the few herbivore and carnivore isotopic values for Peştera cu Oase and Peştera Muierii are similar to those for western European Late Pleistocene mammals.

Clearly, more sampling of both Early Upper Paleolithic and preceding humans in diverse ecozones of Europe is needed to resolve this isotopic contrast, in addition to zooarcheological data from across Europe and including species other than ungulates.

#### Conclusion

The addition of carbon and nitrogen stable isotopic data for four Early Upper Paleolithic modern humans from Romania adds significantly to our dietary record for the European transitional period from Middle Paleolithic to Middle Upper Paleolithic and from Neandertals to early modern humans. A significant increase in  $\delta^{15}N$  values among the more recent humans implies greater carnivory and/or a broader proteinaceous dietary spectrum for them, a trend apparently present into the Middle Upper Paleolithic. However, current archeological analyses suggest little dietary change from the Middle Paleolithic through the Early Upper Paleolithic, turning this isotopic insight into hypotheses to be, hopefully, tested against additional isotopic and faunal data.

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Table 1

Direct radiocarbon ages and stable isotopes for the Oase, Muierii and Cioclovina human remains. For detailed chemistry, see Trinkaus *et al.* (2003) and Soficaru *et al.* (2006, 2007). The two dates for Oase 1 provide a combined age of 34,950 +990, -890 B.P. (Trinkaus *et al.* 2003). δ<sup>13</sup>C values are measured relative to the VPDB (Vienna Pee Dee Belemnite) standard, and δ<sup>15</sup>N values are measured relative to the AIR (Ambient Inhalable Reservoir) standard

Specimen	<sup>14</sup> C age	δ <sup>13</sup> C (‰)	δ <sup>15</sup> N (‰)
Oase 1	>35,200 (OxA-11711)	-18.7	13.3
	34,290, +970, -870 (GrA-22810)	-19.0	
Muierii 1	30,150 ± 800 (LuA-5228)	-20.0	
	29,930 ± 170 (OxA-15529)	-19.3	12.3
Muierii 2	29,110 ± 190 (OxA-16252)	-19.1	12.4
Cioclovina 1	29,000 ± 700 (LuA-5229)		
	$28,510 \pm 170$ (OxA-15527)	-19.6	12.7

	δ <sup>13</sup> C (‰)	δ <sup>15</sup> N (‰)	Source
Middle Paleolithic Neandertals			
Engis 2 (child), Belgium	-19.6	12.6	Bocherens et al. 2001
Feldhofer 1, Germany	-21.6	7.9	Richards and Schmitz 2008
Feldhofer 2, Germany	-21.5	9.0	Richards and Schmitz 2008
Jonzac 1, France	-20.7	10.6	Richards et al. 2008b
Les Pradelles 64801, France	-19.1	11.6	Bocherens et al. 2005
Les Pradelles M300, France	-19.1	11.5	Bocherens et al. 2005
Les Pradelles M400, France	-19.5	11.4	Bocherens et al. 2005
Rochers-de-Villeneuve 1, France	-19.0	11.6	Beauval et al. 2006
Initial Upper Paleolithic Neandertals			
Saint-Césaire 1, France	-19.8	11.4	Bocherens et al. 2005
Spy 572a, Belgium	-19.8	11.0	Bocherens et al. 2001
Vindija 208, Croatia	-20.2	10.3	Higham et al. 2006b
Early Upper Paleolithic			
Kostenki 8, Russia	-18.2	15.3	Richards et al. 2001
Middle Upper Paleolithic			
Arene Candide 1, Italy	-17.6	12.4	Pettitt et al. 2003
Barma Grande 6, Italy	-19.7	12.9	Formicola et al. 2004
Brno-Francouzská 2, Czech Rep.	-19.0	12.3	Richards et al. 2001
Dolní Věstonice 35, Czech Rep.	-18.8	12.3	Richards et al. 2001
Eel Point 1, UK	-19.0	10.9	Schulting et al. 2005
Kostenki 4, Russia	-19.1	13.1	Richards et al. 2001
Paviland 1, UK	-18.2	10.35	Jacobi and Higham 2008
La Rochette 1, France	-17.1	11.2	Orschiedt 2002, per. comm.
Sunghir 1, Russia	-19.2	11.3	Richards et al. 2001
Sunghir 2, Russia	-19.0	11.2	Richards et al. 2001
Sunghir 3, Russia	-18.9	11.3	Richards et al. 2001

Table 2. Comparative Middle and Initial Upper Paleolithic Neandertal and Early and Middle Upper Paleolithic modern human stable isotope data from Europe

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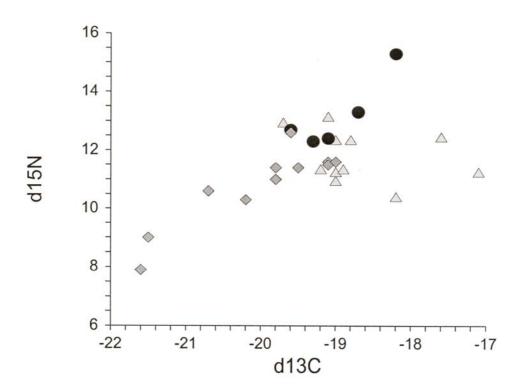
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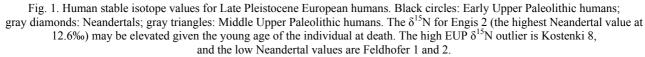
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#### CAPTIONS TO THE FIGURES

- Fig. 1. Human stable isotope values for Late Pleistocene European humans. Black circles: Early Upper Paleolithic humans; gray diamonds: Neandertals; gray triangles: Middle Upper Paleolithic humans. The  $\delta^{15}N$  for Engis 2 (the highest Neandertal value at 12.6‰) may be elevated given the young age of the individual at death. The high EUP  $\delta^{15}N$  outlier is Kostenki 8, and the low Neandertal values are Feldhofer 1 and 2.
- Fig. 2. Human stable isotopes (black circles) from the Peştera cu Oase (O1), Peştera Muierii (M1, M2) and Peştera Cioclovina (C1), plotted against carnivores (gray diamonds) and herbivores (gray triangles) from the first two sites. The human isotopic values are in Table 1, and the faunal values are in Richards *et al.* (2008) and Doboş *et al.* (2008) respectively for Peştera cu Oase and Peştera Muierii. The high herbivore  $\delta^{15}$ N value is the Muierii *A. alces* (7.3‰), which is probably elevated since it derives from an M<sup>1</sup> root. The Oase herbivores consist of *Cervus elaphus* and *Capra ibex*, the Oase carnivores are *Canis lupus*, and the Muierii carnivore is *Panthera spelaea.*





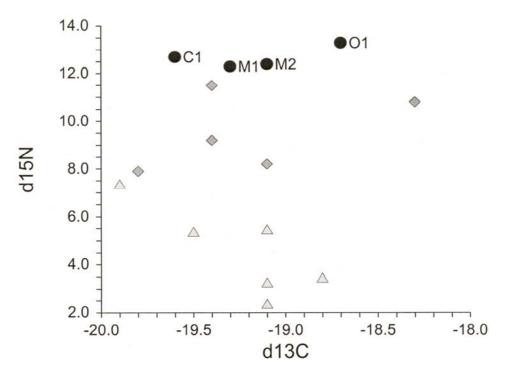


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