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Exceptionally high $\delta^{15}\text{N}$ values in collagen single amino acids confirm Neandertals as high-trophic level carnivores

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Isotope and archeological analyses of Paleolithic food webs have suggested that Neandertal subsistence relied mainly on the consumption of large herbivores. This conclusion was primarily based on elevated nitrogen isotope ratios in Neandertal bone collagen and has been significantly debated. This discussion relies on the observation that similar high nitrogen isotope values could also be the result of the consumption of mammoths, young animals, putrid meat, cooked food, freshwater fish, carnivores, or mushrooms. Recently, compound-specific C and N isotope analyses of bone collagen amino acids have been demonstrated to add significantly more information about trophic levels and aquatic food consumption. We undertook single amino acid C and N isotope analysis on two Neandertals, which were characterized by exceptionally high N isotope ratios in their bulk bone or tooth collagen. We report here both C and N isotope ratios on single amino acids of collagen samples for these two Neandertals and associated fauna. The samples come from two sites dating to the Middle to Upper Paleolithic transition period (Les Cottés and Grotte du Renne, France). Our results reinforce the interpretation of Neandertal dietary adaptations as successful top-level carnivores, even after the arrival of modern humans in Europe. They also demonstrate that high $\delta^{15}\text{N}$ values of bone collagen can solely be explained by mammal meat consumption, as supported by archeological and zooarcheological evidence, without necessarily invoking explanations including the processing of food (cooking, fermenting), the consumption of mammoths or young mammals, or additional (freshwater fish, mushrooms) dietary protein sources.

compound-specific isotope analyses | diet | late Neanderthals | Paleolithic | stable isotopes

Since the first publication of bulk bone collagen carbon and nitrogen stable isotope ratios of Neandertals, (e.g., refs. 1–3), supported by more recent studies (4–8), an intriguing pattern has been observed: Neandertals are characterized by relatively high nitrogen isotope values, often higher than the carnivores from the same sites (Fig. 1). Nitrogen isotopes are usually employed as a tracer of trophic level (9), and this pattern was therefore, at first, interpreted as indicative of the top-level trophic position (TP) of Neandertals in Paleolithic food webs (2, 3, 10), specifically focused on the consumption of large herbivorous mammals (11). Early modern humans have a wider range of isotopic ratios (especially in carbon), and some individuals exhibit higher N isotope ratios than of the fauna associated with them and above the range generally observed in Neandertals (refs. 10 and 12–14 and Fig. 1). These elevated values in Pleistocene modern humans have been interpreted as the signature of freshwater fish consumption (10, 13) as N isotope ratios ($\delta^{15}\text{N}$) in fish or shell consumers are usually higher than in pure terrestrial carnivores (10). Recently, an exceptionally high N isotope ratio has been measured

in an infant Neandertal (AR-14) from the site of Grotte du Renne (Arcy-sur-Cure, France; *SI Appendix, Supporting Information 1*). This individual had a $\delta^{15}\text{N}$ value 3–5‰ above the associated carnivores (ref. 6 and Fig. 1). In this case, this very elevated ratio is likely to be due to breastfeeding (15), as the infant was ~1 y old.

Over the last few years, several studies have challenged the interpretation of high Neandertal bone collagen nitrogen isotope values as indicative of large herbivore consumption. It has been suggested that the systematic slightly higher $\delta^{15}\text{N}$ values observed in Neandertal collagen, especially relative to that of the associated carnivores, could be explained by mammoth consumption (4), as mammoths generally exhibit higher $\delta^{15}\text{N}$ values than other associated herbivores. Furthermore, recent studies carried on dental calculus highlight the existence of plant consumption (16–20), challenging the interpretation of a purely carnivorous diet of Neandertals. Other factors such as the consumption of putrid meat (21), mushrooms (16, 22, 23), freshwater fish (24), or cooked food (25, 26) have also been suggested as explanations for the elevated $\delta^{15}\text{N}$ values of Neandertal collagen. The selective hunting of young animals by Neandertals and older animals by carnivores is another factor that would increase $\delta^{15}\text{N}$ values, but the zooarcheological data generally

Significance

Identifying past hominin diets is a key to understanding adaptation and biological evolution. Bone collagen isotope studies have added much to the discussion of Neandertal subsistence strategies, providing direct measures of diet. Neandertals consistently show very elevated nitrogen isotope values. These values have been seen as the signature of a top-level carnivore diet, but this interpretation was recently challenged by a number of additional theories. We here apply compound-specific isotope analysis of carbon and nitrogen in bone collagen single amino acids of two Neandertals. These Neandertals had the highest nitrogen isotope ratios of bulk collagen measured so far, and our study confirms that these values can be most parsimoniously explained by a carnivorous diet.

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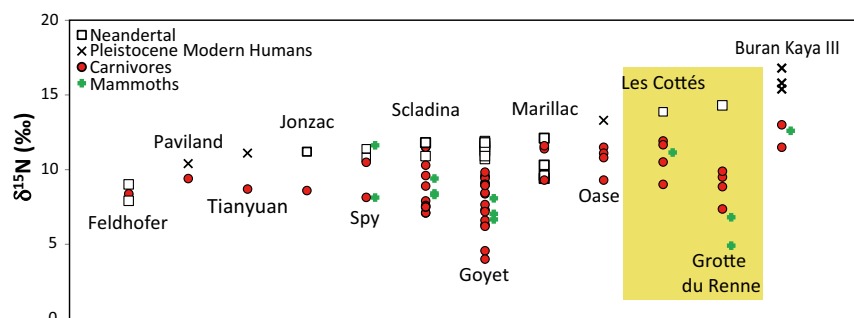


Fig. 1. $\delta^{15}\text{N}$ values of Neandertals, Pleistocene modern humans, and fauna from different Paleolithic European and Asian sites for which analyses on the associated fauna was performed. Data are from (Feldhofer) Richards and Trinkaus (10), (Paviland) Richards et al. (3) and Deviese et al. (56), (Tianyuan) Hu et al. (13), (Jonzac) Richards et al. (8), (Spy) Naito et al. (37), (Scladina) Bocherens et al. (2), (Goyet) Wissing et al. (7), (Marillac) Fizet et al. (1, 57), Bocherens et al. (5), (Oase) Trinkaus et al. (12), (Les Cottés) this study, (Grotte du Renne) Welker et al. (6), and (Buran Kaya III) Drucker et al. (30).

suggest the opposite pattern (27). Bocherens et al. (28) also suggested that the difference between Pleistocene modern humans and Neandertals would not be explained by distinctive diets, but by a climate change during the Middle to Upper Paleolithic transition causing the elevation of the N isotope signature of the local plants, impacting the values in the whole food web and, therefore, temporally distinct populations. Most of the modern humans analyzed so far are indeed from Upper Paleolithic contexts while the Neandertals (24, 28), with the exception of the Grotte du Renne (AR-14) (6) and the Spy Cave individuals (29), were found in Middle Paleolithic contexts. Finally, Drucker et al. (30) demonstrated, using compound-specific isotope analyses (CSIA), that some elevated $\delta^{15}\text{N}$ values of Paleolithic modern human bone collagen could mainly be explained by mammoth consumption, rather than freshwater fish. The above-mentioned factors (Table 1) are all likely to increase the $\delta^{15}\text{N}$ of body tissues without dramatically influencing the $\delta^{13}\text{C}$ values. It is therefore very difficult to estimate which one accounts for the pattern observed in bulk Neandertal bone collagen.

Novel isotope techniques, such as compound-specific isotope analyses performed on single amino acids, could help identify the factor explaining this pattern. The CSIA is a very powerful technique, which consists in establishing the C and N isotope composition of the various amino acids in bone or tooth collagen. Carbon isotope values of individual amino acids can clearly distinguish terrestrial from freshwater food sources (31, 32). Honch et al. (31) demonstrated that animals from food webs based on terrestrial plants usually exhibit similar $\delta^{13}\text{C}_{\text{Phe}}$ and $\delta^{13}\text{C}_{\text{Val}}$ (Phe: Phenylalanine, Val: Valine) in their collagen, with higher values in C_4 plant-based food webs than in C_3 plant-based ones. Fish consumers have $\delta^{13}\text{C}_{\text{Val}}$ overlapping with the animals from C_3 plants (for freshwater environment) and C_4 plants (for marine environment)-based food webs, but much lower $\delta^{13}\text{C}_{\text{Phe}}$ values (31). Nitrogen isotope values of individual amino acids reveal TP in more detail than bulk isotopic data, especially using the data obtained on phenylalanine (Phe) and glutamic acid (Glu). $\delta^{15}\text{N}_{\text{Phe}}$ reflects the local baseline without being really impacted by the trophic level of the animal (trophic ^{15}N -enrichment of $0.4 \pm 0.4\text{‰}$) (33) whereas the

glutamic acid N isotope ratio ($\delta^{15}\text{N}_{\text{Glu}}$), in addition to the local baseline, is strongly impacted by the TP (trophic ^{15}N -enrichment of $8.0 \pm 1.1\text{‰}$) (34–36). The combination of the $\delta^{15}\text{N}$ of these two amino acids therefore allows interpretations free of local baseline bias and allow more precisely assessing the TP of a specimen. For example, Naito et al. (37) documented the $\delta^{15}\text{N}$ values of amino acids for the Spy Cave Neandertals (Fig. 1), revealing a possible contribution of plants into their diet, up to 20%. The use of CSIA of carbon and nitrogen can therefore potentially provide very detailed information on the diet of Neandertals and directly address the debate on the cause of the elevated nitrogen isotope signatures of their collagen.

Recently, we extracted the collagen of a Neandertal tooth from les Cottés (France; *SI Appendix, Supporting Information 1 and Fig. S1*, and ref. 38) for radiocarbon dating (ref. 39, *SI Appendix, Supporting Information 2*). In the frame of the assessment of collagen preservation, we measured the C and N isotope ratios in bulk collagen and found the second highest N isotope ratio ever seen in a Neandertal sample (*SI Appendix, Table S1*), knowing that the first one was the above mentioned breastfeeding infant from Grotte du Renne, AR-14 (6) (Fig. 1). The $\delta^{15}\text{N}$ value of the Les Cottés Neandertal is about $\sim 7\text{‰}$ higher than those of the associated herbivores previously measured in Talamo et al. (40), expected one-step trophic level enrichment (9). As there are a number of suggested reasons for the high Neandertal nitrogen isotope values (we list 10 possibilities in Table 1), we aimed to investigate which ones could account for the elevated value of the Les Cottés Neandertal. The tooth root analyzed belonged to a permanent maxillary lateral incisor, and thus, the dentine formed between the fourth and eighth years of age (*SI Appendix, Supporting Information 3*). If explained by breastfeeding, the $\delta^{15}\text{N}$ value associated to the Les Cottés Neandertal would indicate a very late age of weaning, which would contradict previous findings (41, 42). At Les Cottés, hyena, wolf, and fox bones with cut-marks have been found in layer 06, layer 04/upper, and layer 02. Also, mammoth ivory transformed into beads have been found into layer 04/upper (43). Carnivore and mammoth consumption are two hypotheses that have to be

Table 1. List of factors likely to cause elevated nitrogen isotope ratios in tooth collagen of the Les Cottés Neandertal

Dietary adaptation	Output of the present study
Top-level carnivory	Yes
Mammoth consumption	Unknown
Young animal consumption	No/Minor
Freshwater fish consumption	No
Breastfeeding	Unlikely
Environmental factors triggering an elevation of the whole food web isotope values	No
Mushroom consumption	Unknown
Consumption of putrid meat	Unknown
Cooking	Unknown
Carnivore consumption	No/Minor

The second column lists the relevant factors according to the results of this study. See text for more details.

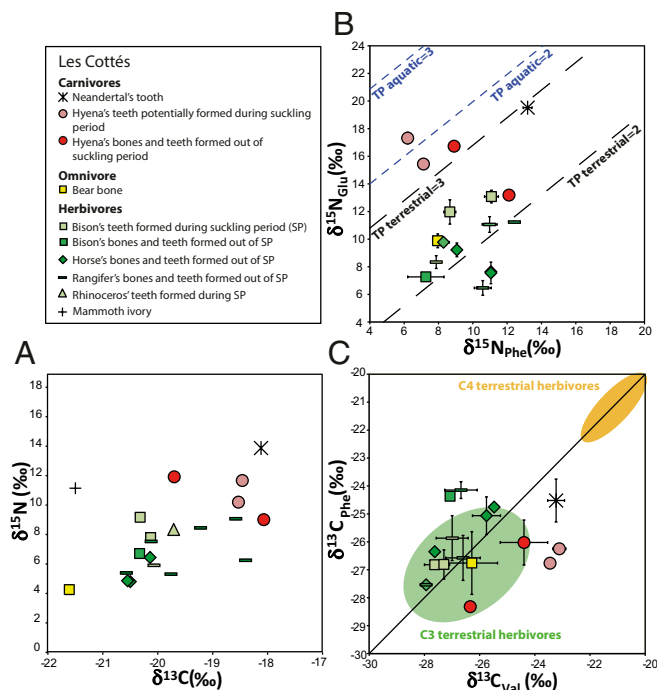


Fig. 2. Isotope results for the site of Les Cottés. (A) C and N isotope ratios in bulk collagen. (B) N isotope ratios in phenylalanine and glutamic acid. The TP lines (B) are defined according to Chikaraishi et al. (35, 36), (C) C isotope ratios in valine and phenylalanine. The green and yellow areas (C) are defined according to Honch et al. (31).

and one hyena. This could be explained by the consumption of plants with a peculiar β factor by these three herbivores. Most plants (50%) have a β factor of $-8 \pm 1\text{‰}$, yet some reach a value of -12‰ and represent 3% of the reported values (45).

However, only 64 β values of plants have been analyzed so far, and there is therefore a need to better document those β factors in plants and the species associated to specific values (e.g., 3‰ in the case of tree flowers). With such a β value, the trophic level of the three herbivores goes up to 2.1–2.2 and to 2.8 for the associated hyena. In Grotte du Renne, this β moves back these herbivores (two Equidae and one Cave bear) as well as the Pantharinae to their expected TP. With the same β factor, the Neandertal from Grotte du Renne would then have a TP of 3.8, one trophic level above the carnivores. Such a TP could easily be explained by breastfeeding.

We observed difference between $\delta^{13}\text{C}_{\text{Val}}$ and $\delta^{13}\text{C}_{\text{Phe}}$ values, which can be explained by the existence of a trophic level effect, mostly affecting the $\delta^{13}\text{C}_{\text{Val}}$ (SI Appendix, Supporting Information 4) rather than freshwater fish consumption. This accounts for the fact that the Neandertal from Les Cottés and the hyenas are falling slightly off the range defined for terrestrial animals. As a result, the difference between $\delta^{13}\text{C}_{\text{Val}}$ and $\delta^{13}\text{C}_{\text{Phe}}$ ($\Delta^{13}\text{C}_{\text{Val-Phe}}$) values correlate to the estimated trophic level (Fig. 4), and the $\Delta^{13}\text{C}_{\text{Val-Phe}}$ is systematically positive in carnivores and negative for herbivores. The report of this trophic level effect is unprecedented, since no other carnivore data are currently available for $\delta^{13}\text{C}_{\text{Val}}$ and $\delta^{13}\text{C}_{\text{Phe}}$ in mammal collagen. We also observed it for $\delta^{13}\text{C}_{\text{Leu}}$ and $\delta^{13}\text{C}_{\text{Gly}}$ values (Gly = Glycine, Leu = Leucine; SI Appendix, Supporting Information 4). The combined use of carbon and nitrogen single amino acid isotope values could therefore help to account for the uncertainty on the TP estimation based on $\delta^{15}\text{N}$ values only.

Neandertal Diets at Les Cottés and Grotte du Renne. Compound-specific isotope analyses conducted on amino acids provide information on the sources of dietary protein over a number of

years of life with a greater level of detail compared with bulk isotope analysis. However, as these CSIA studies are in their infancy, the exact relationship of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of amino acids to TPs is not yet fully understood. Despite these caveats, these analyses shed light on the interpretation of Neandertal diets in Europe. We demonstrate here that exceptionally high $\delta^{15}\text{N}$ values in bulk collagen of the Les Cottés Neandertal can still be explained by a carnivorous diet solely relying on herbivore meat without any need to call upon additional explanations such as consumption of putrid meat or cooked food. These types of consumption might, however, have existed but would mean that fermentation and cooking equally impact $\delta^{15}\text{N}_{\text{Phe}}$ and $\delta^{15}\text{N}_{\text{Glu}}$ values (Table 1). Freshwater fish and carnivore meat were not—or rarely—eaten by these Neandertals. Neandertals at les Cottés could have mostly hunted reindeer from arid environments (showing elevated $\delta^{15}\text{N}_{\text{Phe}}$ and $\delta^{13}\text{C}_{\text{bulk}}$) or horses (also characterized by elevated $\delta^{13}\text{C}_{\text{Asp}}$, SI Appendix, Supporting Information 4), whereas hyenas would have been less specific in terms of their environments and prey. This is a similar interpretation to that proposed by the only other Neandertal CSIA study by Naito et al. (37), which also suggested different ecological niches between hyenas and Neandertals. The $\delta^{13}\text{C}_{\text{bulk}}$ value of the Neandertal tooth does not support here the specific hunting of mammoths, which would generally yield low $\delta^{13}\text{C}$ (as observed in Grotte du Renne, although only one individual could be analyzed). According to CSIA analysis, the Spy Neandertals (Neandertals dated circa 41,000 BP; ref. 46) show different hunting strategies: Spy 430-a and Spy 92b hunted reindeer, horse, and bovines, while Spy 92a seemed more opportunistic (37). Neandertal hunting of reindeer, rather than mammoths, is also generally supported by zooarchaeological data (27, 47) including at Grotte du Renne and Les Cottés (38, 48, 49), even if some exceptions exist for Middle Paleolithic sites (47). In Grotte du Renne, both $\delta^{15}\text{N}_{\text{Phe}}$ and $\delta^{13}\text{C}$ values of AR-14 bulk collagen

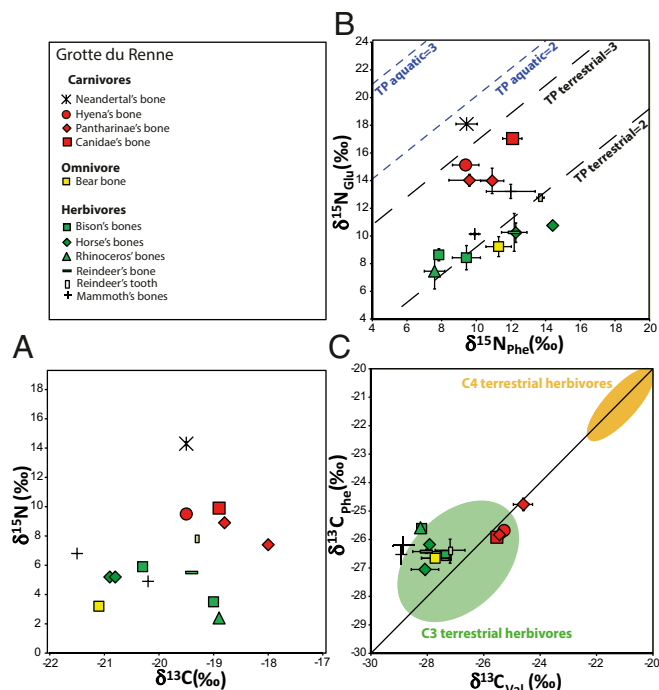


Fig. 3. Isotope results for the site of Grotte du Renne (Arcy-sur-Cure). (A) C and N isotope ratios in bulk collagen; data from Welker et al. (6). (B) N isotope ratios in phenylalanine and glutamic acid. The TP lines (B) are defined according to Chikaraishi et al. (35, 36), (C) C isotope ratios in valine and phenylalanine. The green and yellow areas (C) are defined according to Honch et al. (31).

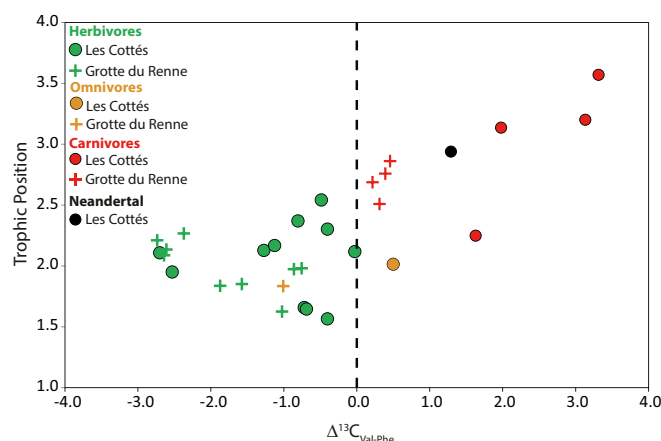


Fig. 4. Difference (%) between $\delta^{13}\text{C}_{\text{Val}}$ and $\delta^{13}\text{C}_{\text{Phe}}$ ($\Delta^{13}\text{C}_{\text{Val-Phe}}$) in bone and tooth collagen and associated TP. The TP was estimated from the $\delta^{15}\text{N}_{\text{Phe}}$ and $\delta^{15}\text{N}_{\text{Val}}$ values. The C isotope ratios in amino acids of the Grotte du Renne Neandertal were not measured because not enough material was available. See text for more details.

falls in the variability of the other carnivores. The TP of the AR-14 Neandertal is clearly above that of the other carnivores, whatever the β factor is. This finding fits with the hypothesis of a suckling infant previously suggested by Welker et al. (6). This infant was therefore not weaned but could possibly already have eaten solid food, as its TP is below 4. According to the isotope data performed on bulk collagen and amino acids, the mother was probably sharing the same ecological niche as the other carnivores.

When we then look at all of the bulk collagen isotope analyses of the 29 Neandertals that have been published so far [ranging in radiometric age from ~90,000 BP (Scladina; ref. 46) to 36,840 BP (Grotte du Renne; ref. 6)], we can see a similar and very stable diet over time. This situation could be readily explained by Neandertals being top-level carnivores consuming herbivore meat, and this pattern even continues after the arrival of modern humans in Europe (50). Our work shows that even exceptionally high $\delta^{15}\text{N}$ bulk collagen values of two late Neandertals still result from a terrestrial meat-based diet, the same diet that was interpreted for the other 27 Neandertals that have had bulk collagen C and N isotope measurements. Despite changes in climate, environment and associated faunal spectra, throughout the Middle Paleolithic, the focus remains on large herbivores.

Concluding Remarks. Compound-specific isotope analyses are a very promising method for reconstructing aspects of past diets, especially by combining nitrogen and carbon isotope analyses on single amino acids to clearly distinguish aquatic and terrestrial sources of proteins, and to estimate with more precision the contribution of plants in individual diets. Our application of CSIA to two Neandertals has shown that both were high trophic level consumers, with large herbivores being the main protein source. Therefore, there is no reason to invoke myriad dietary interpretations such as the consumption of mushrooms, putrid animal flesh, mammoths, or freshwater fish to explain their high bulk bone collagen nitrogen isotope values of Neandertals in relation to carnivores from the same site. However, we acknowledge that our isotopic study does not rule out the occasional consumption of these foods. Instead, the differing high N values between Neandertals and associated carnivores is likely due to the consumption of different herbivores from different environments. When we conduct CSIA, which is not environmentally baseline sensitive like bulk collagen N values, Neandertals and carnivores clearly are at the same trophic level.

Methods

The bones and teeth were taxonomically identified using traditional zooarchaeological methods and ZooMS (6, 49, 51) (Dataset S1, Table S5). The collagen extraction was performed using the protocol outlined in Talamo and Richards (44), and the bulk collagen isotope analyses were conducted at the Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany. The collagen samples were then sent to the University of California, Los Angeles Isotope Lab Facility, where the compound-specific isotope analyses were performed using gas chromatography combustion isotope ratio mass spectrometry. The two first batches of samples were prepared with the following protocol: The amino acids were liberated using acid hydrolysis before the derivatization step to produce methoxycarbonyl methyl esters (MOC) for GC analysis, following the protocol established by Yarnes and Herzog (52). The third batch of samples was prepared using the derivatization protocol (53) recommended by O'Connell and Collins (54), producing N-acetyl isopropyl esters, allowing the comparison of isotope signatures in hydroxyproline and proline (SI Appendix, Fig. S2). An external standard was analyzed with both protocols and exhibit similar isotope signatures for both protocols (Dataset S1, Table S10). All samples were analyzed in duplicate by GC-C-IRMS, and additional measurements were performed when the duplicates fell outside expected measurement error (55). Additional information is available in Dataset S1.

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Supporting Information for

Exceptionally high $\delta^{15}\text{N}$ values in collagen single amino acids confirm Neandertals as high-trophic level carnivores

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This PDF file includes:

Supplementary text

Figs. S1 to S7

Tables S1 to S4

SI reference citations

Data table captions S5 to S10

Other supplementary materials for this manuscript include the following:

Data Tables S5 to S10

Supporting Information 1

Site location

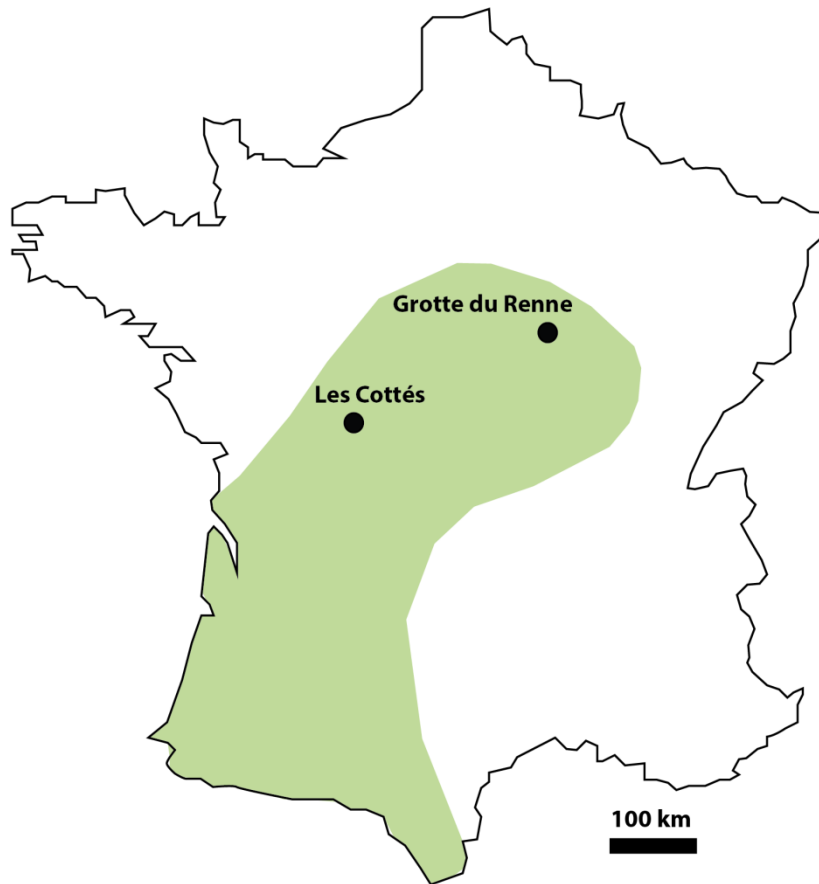


Figure S1: Location of the two French Neanderthal sites (Les Cottés and Grotte du Renne at Arcy-sur-Cure) studied in this paper. The green area represents the geographical extent of Châtelperronian transition sites in France. Map modified from Soressi et al. (1).

Supporting Information 2

Radiocarbon dating

The tooth root sample was pretreated at the Department of Human Evolution at the Max Planck Institute for Evolutionary Anthropology (MPI-EVA) in Leipzig, Germany, using the method described in Talamo and Richards (2). The sample passed the collagen evaluation criteria (see Table S1), and was sent to the Mannheim AMS laboratory (Lab Code MAMS) where it was graphitized and dated (3)

MPI Code	Used mg	Yield mg	% Yield	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	% C	% N	C:N	AMS Lab Code	^{14}C Age	1s Err
R-EVA 1526	461	25.7	5.6	-18.1	13.9	44.0	15.9	3.2	MAMS- 26196	39485	271

Table S1. Radiocarbon dating (uncalibrated) of Les Cottés Z4-1514 Neandertal tooth and collagen evaluation criteria

Supporting Information 3

1. Anatomical determination of the Les Cottés tooth (Z4-1514).

Although the crown was entirely worn away - even beyond the cervix - the Les Cottés tooth (CTS 2008 US 04.35 Z4-1514) can be described as a single-rooted tooth, most likely an anterior permanent tooth (i.e., a central or lateral incisor or a canine). An anatomical determination was conducted by comparing the Les Cottés tooth to the large sample of Neanderthal anterior teeth already published by one of the authors of this manuscript (ALC, 4).

The tooth was scanned by Heiko Temming on the 11th of June 2012, using the Skyscan 1712 micro-CT scanner in the Department of Human Evolution at MPI-EVA (Leipzig, Germany). The scanning parameters used are as follows: 100 kV, 100 μ A, Al+Cu filters, 2 frame averaging, over 360 degrees, 885 ms of exposure time, data were saved as 16-bit tiff files. The μ CT data were reconstructed with a pixel size of 13.04 μ m, using N-Recon. The stack was downsampled to 27 μ m using the Triangle filter (2,2,2) in Avizo 6.3, and then further filtered using a combination of median and Kuwahara filters with a kernel size of 3 (see (4) for details). The dentine and the pulp cavity were segmented in Avizo 6.3 to generate separate 3D models of those dental tissues. Linear, surfacic, and volumetric measurements were taken in Avizo 6.3.

The nine root metrics used in the analysis for the anatomical determination involved the labio-lingual and mesio-distal cervical root diameters, the root length, the root total volume, the root pulp volume, the root surface area, the cervical surface area, and the labial and the lingual root surface areas. The values for Les Cottés tooth and the comparative Neandertal samples are reported in the following table.

Posterior probabilities based on several root metrics (following 4) classify this tooth as a **permanent maxillary lateral incisor** (after abbreviated as: I²) with a 9.4 % chance of misclassification (Table S2).

	Root metrics								
	RMD	RLL	CA	RL	RV	RPV	RSA	RSA-Ling	RSA-Lab
Les Cottés	5.7	8.0	43.1	15.9	415.8	7.3*	305.9	130.7	175.8
Neandertals**									
LI1 (N=18)	4.4 ± 0.4 [3.9-5.7]	7.2 ± 0.4 [6.3-8.0]	26.5 ± 5.0 [20.6-43.1]	17.1 ± 1.9 [13.8-20.9]	335.1 ± 63.6 [200.1-421.7]	12.5 ± 6.7 [5.2-24.9]	300.8 ± 42.3 [195.6-358.9]	147.0 ± 31.3 [70.8-196.3]	153.3 ± 27.1 [92.4-191.2]
LI2 (N=16)	4.6 ± 0.6 [3.3-6.4]	7.5 ± 0.6 [6.1-8.7]	28.4 ± 3.9 [18.0-35.7]	18.0 ± 2.3 [13.4-21.6]	381.4 ± 86.4 [208.9-507.6]	20.7 ± 14.3 [6.6-63.0]	324.4 ± 86.4 [208.9-507.6]	143.0 ± 31.1 [70.3-192.6]	180.3 ± 36.8 [98.5-228.2]
LC (N=16)	5.8 ± 0.5 [4.3-6.4]	8.9 ± 0.7 [7.5-10.5]	42.3 ± 94.5 [25.6-54.5]	20.7 ± 2.9 [16.1-25.6]	595.4 ± 150.5 [339.5-902.3]	31.9 ± 18.8 [11.6-70.8]	410.2 ± 94.5 [221.8-619.7]	190.9 ± 45.9 [78.2-280.3]	236.5 ± 49.1 [150.1-340.8]
UI1 (N=17)	7.0 ± 1.1 [5.4-9.3]	7.6 ± 0.5 [7.0-8.6]	41.9 ± 8.0 [33.2-59.1]	17.2 ± 2.4 [10.5-19.8]	452.0 ± 123.5 [195.8-693.6]	37.2 ± 20.7 [2.0-86.0]	315.3 ± 60.7 [162.9-409.3]	134.8 ± 26.6 [68.5-175.7]	179.6 ± 39.5 [94.5-243.8]
UI2 (N=18)	6.1 ± 0.5 [5.4-7.2]	8.0 ± 0.6 [7.3-9.3]	39.6 ± 6.1 [31.2-53.3]	17.6 ± 1.8 [14.7-20.0]	487.2 ± 91.0 [310.7-633.2]	31.4 ± 18.1 [9.3-81.7]	343.1 ± 42.9 [252.8-409.2]	138.4 ± 20.8 [97.8-183.0]	204.8 ± 32.3 [155.3-269.0]
UC (N=12)	6.2 ± 0.2 [5.8-6.5]	9.3 ± 0.7 [8.7-11.0]	46.2 ± 5.0 [40.0-55.8]	22.5 ± 2.5 [17.7-25.2]	650.6 ± 98.9 [406.9-817.3]	36.7 ± 14.8 [16.4-66.3]	454.3 ± 60.3 [311.2-539.4]	198.3 ± 44.6 [122.2-311.6]	255.4 ± 46.0 [170.4-314.4]

*The very small value of the Les Cottés root pulp volume can be explained by the older age of the individual (evidenced by the filling of the pulp cavity with secondary dentine that occurs with age).

** : Comparative data from (4). “L” stands for “lower”, “U” for “upper”, “I” for incisor, “C” for canine, “1” for “central” and “2” for lateral, so that the UI2 is the upper lateral incisor. Mean ± standard deviation [minimum-maximum]. RMD: mesio-distal root cervical diameter [mm]; RLL: labio-lingual root cervical diameter [mm]; CA: cervical area [mm²]; RL: root length [mm]; RV: total root volume [mm³]; RPV: root pulp volume [mm³]; RSA: root surface area [mm²]; RSA-Ling: lingual root surface area [mm²]; RSA-Lab: labial root surface area [mm²].

Table S2: Root metrics of the Les Cottés tooth (Z4-1514) and comparative Neandertal permanent anterior teeth.

2. Estimation for root formation time of the Les Cottés Neandertal tooth (Z4-1514).

This permanent maxillary lateral incisor (I^2) belongs to an older individual as evidenced by both the heavy degree of wear that reaches the root in its cervical part (the crown is totally worn off; score 8 after 5) and the significant thickness of hypercementosis covering the root dentine. Additionally, some tertiary dentine is visible at the incisal surface and obliterates the exposed pulp cavity, the diameter of which is reduced by the significant apposition of secondary dentine.

This tooth cannot be used to estimate the individual's age at death as it is far too worn and there is no associated permanent first molar, which would give a time of birth (if unworn). Furthermore, neither physical section nor synchrotron imaging were performed on this tooth.

We are left with estimating the time of its root formation using references from other Neanderthals for which an age at death was estimated histologically. This allows us to provide a range of ages for root initiation and completion, as dentine is sampled for collagen extraction.

The I^2 crown from juvenile Neanderthal Engis 2 (6) was nearly complete when the individual died at 3 years of age. The Gibraltar 2 Neandertal was slightly older at death (4.6 years), and its I^2 had already started the development of its root as a spicule (less than 1 mm on the labial side)(6). The dental development of the Scladina child (Scla I-4A) has been extensively investigated (7). Its I^2 root started growing at the age of 3.8 years (7). These comparative observations suggest that the root of the Les Cottés I^2 had started growing at around 4 years of age, which either corresponds to the actual end of the weaning process or shortly after weaning has been completed.

The Scladina child had completed the root of its I^2 by the time of its death at 8 years of age (7). The Spanish juvenile El Sidrón J1 was estimated to have died at 7.7 years (8). The root of its lateral incisor is described as developed at three-quarters of its estimated final length (score F for modern human boys and girls following 8). The primary dentine of Les Cottés I^2 root has likely achieved its growth around ~8 years of age. Overall, this is consistent with the root formation time calculated for Scladina, the I^2 root would have taken 4 years to achieve its development.

In summary, one can hypothesize that **the dentine of the Les Cottés I^2 root formed between 4 and 8 years of age.**

Yearly annulations of cementum were then deposited onto the surface of the root dentine, after ~8 years of age until death, i.e., during adolescence and adulthood. To avoid surface contaminations, and as is routinely done during isotopic analyses, the cementum was removed from the tooth surface to sample only (primary, secondary, and tertiary) dentine. It is of note that the whole root dentine (from cervical area to root tip) was used in the sampling procedure. The amount of tertiary dentine that may have been sampled is negligible as it is restricted to a very small volume of dental tissue formed at the roof of the pulp chamber on the incisal surface. The amount of secondary dentine filling in the pulp cavity is more important, although our sample is dominated by primary dentine which constitutes the core of the tooth root.

Overall, **the root dentine sampled here represents the post-weaning stages (see section 3) of life of the Les Cottés individual.**

39

40 3. Estimation of the weaning age

41 The age of weaning in Neandertals remains intensely debated, and results vary according to the
42 techniques of investigation used and the lines of evidence retrieved.

43 Skinner (9) argued that attrition starts at 3 years of age in Middle Pleistocene infants, citing the
44 initiation of the weaning process by the introduction of solid food into the diet. The pattern of microwear
45 of the deciduous molars show that Gibraltar 2 (dead at 4.6 years) had a diet close to that of Fuegians and
46 Eskimos, i.e., highly carnivorous (10). Similarly, the Neandertal juvenile of the Trou de l'Abîme Cave at
47 Couvin (Belgium) was estimated to have died at 5 or 6 years of age (11). The microwear signature on its
48 lower deciduous second molar suggests that the child was consuming mainly tough food, presumably
49 mostly meat (9). These two examples suggest that by the age of their death, both Gibraltar 2 and Couvin
50 were weaned long enough to record the microwear signature of their diet on their tooth enamel surfaces.

51 Linear enamel hypoplasias (LEH) are non-specific stress markers occurring in enamel during
52 tooth formation. They occur often in Neanderthal teeth, more frequently in the permanent dentition (about
53 41%) than in the deciduous teeth (~4%) (12). This argues in favor of a strong period of stress around 4
54 years of age, potentially related with nutritional pressure associated with the weaning process (12).

55 More recently, using the mapping of barium distribution in dental hard tissues, Austin et al. (13)
56 suggested that breast-feeding had abruptly stopped at 1.2 years of age for the Scladina child. Smith et al.
57 (14) further proposed an age at weaning of 2.5 years for the Payre 6 French Neandertal juvenile.

58 **Provided that the primary dentine of the Les Cottés I² root formed between 4 and 8 years of age,**
59 **the tooth sampling may involve either the very latest stages of the weaning process or concern solely**
60 **the post-weaning stages when the child is feeding autonomously.**

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Supporting Information 4

Additional discussion

Quality of the data

A recent critique of the Naito et al. (16) article was published by O’Connell and Collins (15), revealing the importance of verifying the correlation between the $\delta^{15}\text{N}$ values of proline and hydroxyproline to ensure the quality of the data. Our first batches of samples were prepared by a derivatization method (MOC) (17) which does not allow for the analysis of nitrogen isotope ratios of hydroxyproline. However, our last batch was prepared following the protocol suggested by O’Connell and Collins (15). The data from this protocol is presented in Figure S2 and shows an excellent correlation between $\delta^{15}\text{N}$ values of proline and hydroxyproline. Yarnes and Herszage (17) demonstrated that the two protocols used in this study produce the same values. We monitored it using an external standard, which is also used for C isotope ratio analyses of the amino acids. The results of measurements of this standard are given in Table S3.

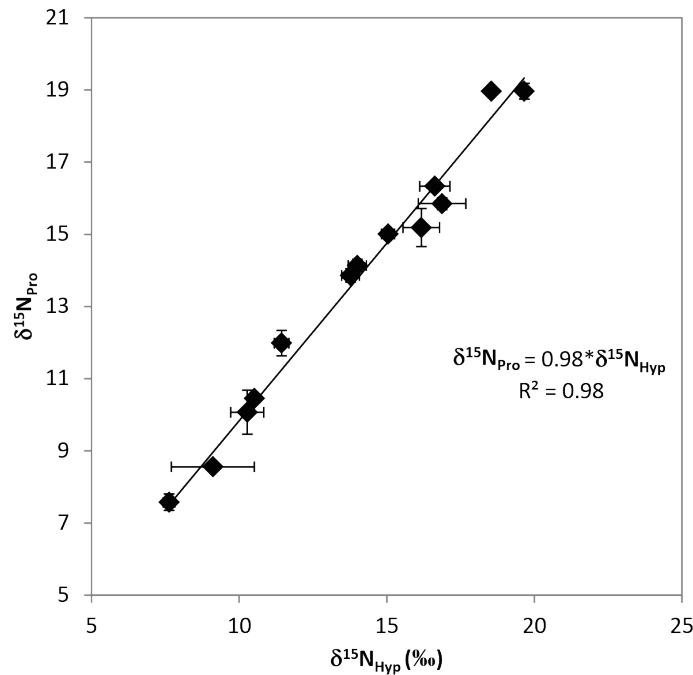


Figure S2: Correlation between $\delta^{15}\text{N}$ values of proline and hydroxyproline of the samples of this study

	$\delta^{15}\text{N}_{\text{Glu}}$	SD	$\delta^{15}\text{N}_{\text{Phe}}$	SD	$\delta^{13}\text{C}_{\text{Phe}}$	SD	$\delta^{13}\text{C}_{\text{Val}}$	SD
Bovine Liver 1577b	10.3	0.4	8.2	0.2	-25.1	0.5	-25.2	0.3

Table S3: Results for the C and N isotope ratios of amino acids (delta values in ‰) discussed in the main text for the external standard, the reference material Bovine Liver 1577b.

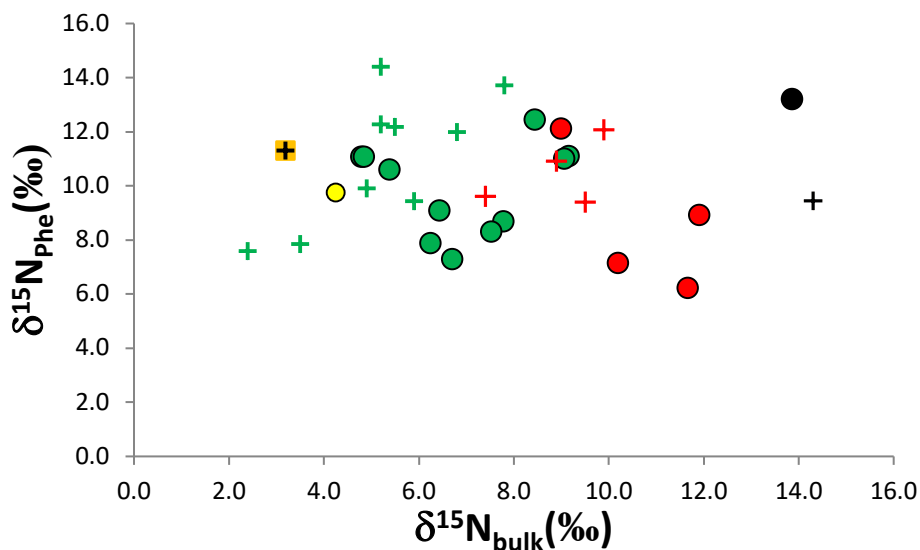


Figure S3: $\delta^{15}\text{N}$ of phenylalanine as a function of bulk values. Circles: Les Cottés samples, crosses: Grotte du Renne samples. Herbivores are in green, carnivores in red, and omnivores in yellow.

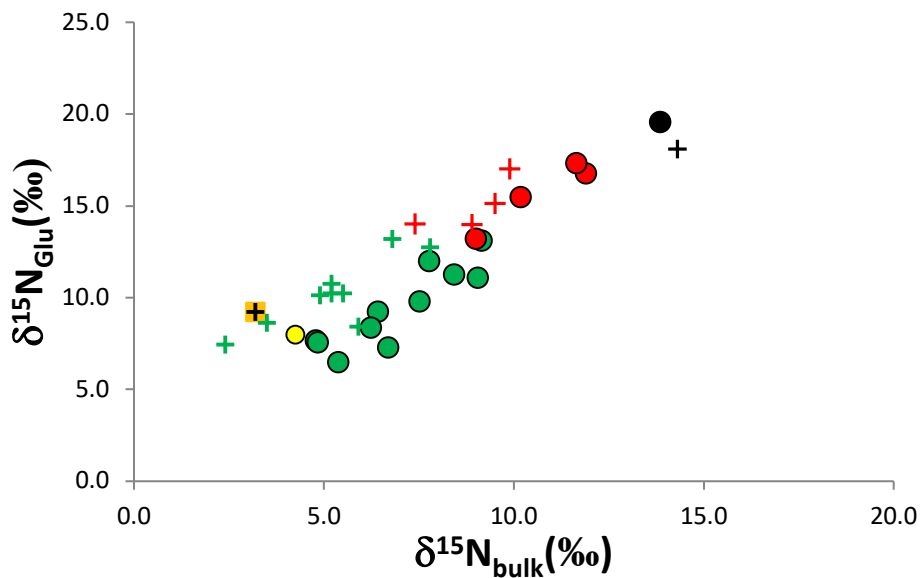
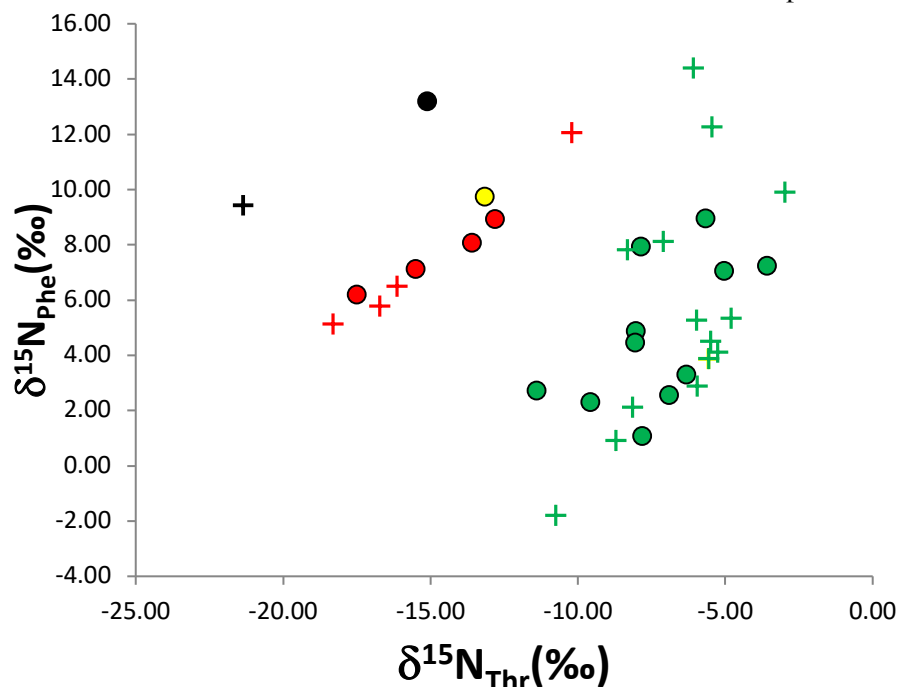


Figure S4: $\delta^{15}\text{N}$ of glutamic acid as a function of bulk values. Circles: Les Cottés samples, Crosses: Grotte du Renne samples. Herbivores are in green, carnivores in red, and omnivores in yellow.

Nitrogen isotope ratios are impacted by the trophic level effect for the following amino acids: Thr (source/metabolic), Val (trophic), Hyp, Leu (trophic), Ala (trophic), and Asp to a lesser extent.

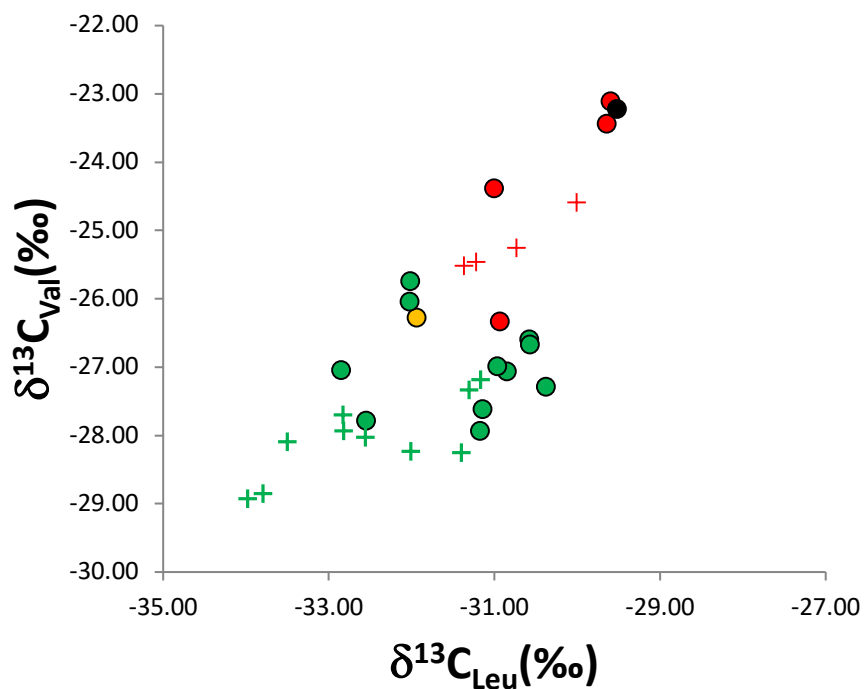
The trophic spacing was previously observed by Fuller and Petzke (18) for Thr and Ala but not for Leu and Ala.

95 The threonine $\delta^{15}\text{N}$ values correlate with the phenylalanine values within each dietary group - with the
 96 exception of the animals with $\delta^{15}\text{N}_{\text{Phe}}$ above 12‰. This suggests that threonine $\delta^{15}\text{N}$ values are both
 97 impacted by local environment and trophic level. This also confirms that the Neandertal from Grotte du
 98 Renne was one trophic level above the carnivores, and therefore probably still breastfeeding at the time of
 99 its death. The Neandertal from Les Cottés is also different from the other carnivores, but its $\delta^{15}\text{N}_{\text{Phe}}$ is
 100 above 12‰, so it is difficult to determine the specific cause of this variation.



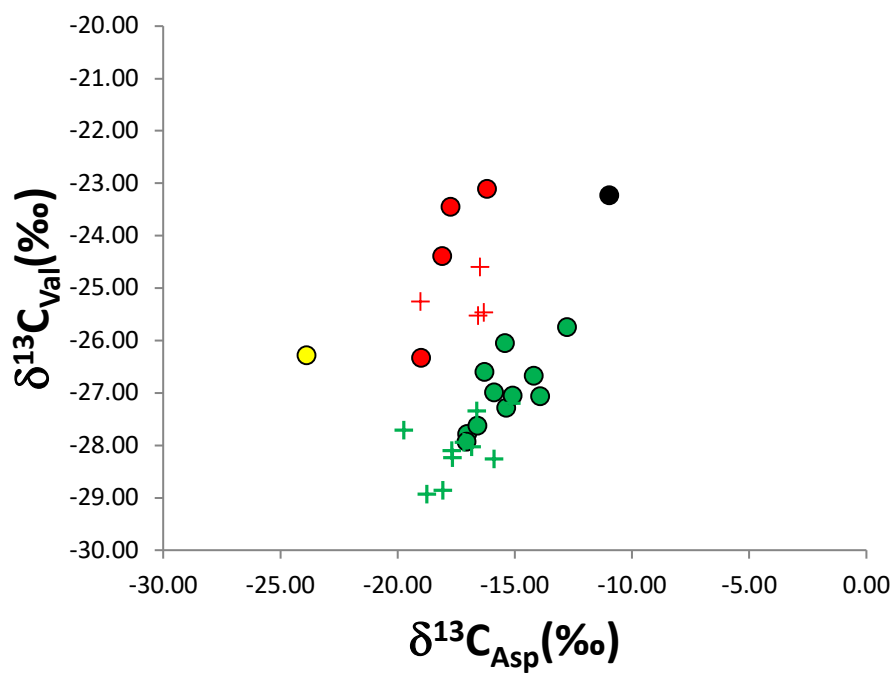
101
 102 **Figure S5: $\delta^{15}\text{N}_{\text{Phe}}$ as a function of $\delta^{15}\text{N}_{\text{Thr}}$ values. Circles: Les Cottés samples, crosses: Grotte du**
 103 **Renne samples. Herbivores are in green, carnivores in red, omnivores in yellow, Neandertals are in**
 104 **black.**

105 Concerning the carbon isotope values of the amino acids, the details of the correlation are given in Table
 106 S2. The values seem to be impacted by trophic level for Val, Leu and Gly (Figure S6), with almost no
 107 overlap between carnivores and herbivores (Figure S7). With each dietary group, the values of $\delta^{13}\text{C}_{\text{Val}}$
 108 correlate to that of Asp (Figure S6), suggesting that this other amino acid could reveal some metabolic or
 109 local environmental information and would explain the rest of the variability of the $\delta^{13}\text{C}_{\text{Val}}$ values. This
 110 would suggest that the Les Cottés Neandertal was hunting animals with elevated $\delta^{13}\text{C}_{\text{Asp}}$ values, such as
 111 horses.



112

113 **Figure S6: $\delta^{13}\text{C}_{\text{Leu}}$ as a function of $\delta^{13}\text{C}_{\text{Val}}$. Circles: Les Cottés samples, crosses: Grotte du Renne**
 114 **samples. Herbivores are in green, carnivores in red, omnivores in yellow, and the Neandertal is in**
 115 **black.**



116

117 **Figure S7: $\delta^{13}\text{C}_{\text{Val}}$ as a function of $\delta^{13}\text{C}_{\text{Asp}}$. Circles: Les Cottés samples, crosses: Grotte du Renne**
 118 **samples. Herbivores are in green, carnivores in red, omnivores in yellow, the Neandertal is in black.**

	Asp	Glu	Gly	Ile	Leu	Phe	Pro	Val
Ala	NC ¹	NC	NC ²	NC	NC	NC	NC	NC ³
Asp		NC	NC ²	NC ⁶	NC ³	SC ⁵	SC ⁵	NC ^{3,4}
Glu			NC ²	NC ⁶	NC ³	SC ⁵	SC ⁵	NC ³
Gly				NC ^{2,6}	C ²	NC ²	NC ²	C ³
Ile					NC	NC ⁶	NC ⁶	NC ³
Leu						NC ³	C ³	C ³
Phe							SC	NC ³
Pro								NC ³
Val								

120 **Table S4: Correlation between $\delta^{13}\text{C}$ values of amino acids. NC: no correlation, C correlation, SC:**
121 **slight correlation**

- 1 The Les Cottés Neandertal is enriched in ^{13}C for Asp compared to other animals.
- 2 Carnivores from Les Cottés are enriched in ^{13}C for Gly but not those of Grotte du Renne.
- 3 Carnivores are enriched in ^{13}C for Val and/or Leu, animals can be grouped depending on their diet regardless of site
- 4 Correlation within dietary group.
- 5 No difference between herbivores and carnivores.
- 6 Herbivores tend to be depleted in ^{13}C for Ile.

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Additional data tables S5 to S10 (separate file)

Table S5: General information on the samples analyzed in this study: layer attribution, dating, sample type, taxonomic identification., 1. Talamo et al. (19), 2. Hajdinjak et al (20), 3. Welker et al. (21) ,4.Welker et al. (22)

Table S6. Summary of isotope data for C and N obtained on bulk collagen ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$), and some amino acids: glutamic acid ($\delta^{15}\text{N}_{\text{Glu}}$), phenylalanine ($\delta^{13}\text{C}_{\text{Phe}}$, $\delta^{15}\text{N}_{\text{Phe}}$) and valine ($\delta^{13}\text{C}_{\text{Val}}$).

TP= trophic position. Bulk collagen data are from this study, from 1. Talamo et al. (19) and 2. Welker et al. (22)

Table S7. Bulk collagen data from this study: Collagen yield (%), C/N ratios and isotope data.

Table S8. Carbon isotope data on amino acids. Average $\delta^{13}\text{C}$ values (top part) and standard deviation on the duplicate measurement.

Table S9. Nitrogen isotope data on amino acids

Table S10. Carbon and nitrogen isotope data on the standard bovine liver 1577b

133 Cited literature:

- 134 1. Soressi M, et al. (2010) *Les Cottés (Vienne). Nouveaux travaux sur l'un des gisements de référence*
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Site	SEVA Id	Id	Layer	Dating	Reference (dating)	Sample	Growth period	Anatomical species determination	Zooms/ADN determination	
Les Cottés	1526	Z4-1514	US04lower		this study	tooth, I2	post weaning	Neanderthal	2	
Les Cottés	2184	X6-727	US06		-	decidual tooth, I3	in utero	hyena	-	
Les Cottés	2185	Z3-688	US06		-	tooth, LP3	possible weaning	hyena	-	
Les Cottés	2183	X7-738	US04lower		-	tooth, UP3	possible weaning	hyena	-	
Les Cottés	13670	Y5-583	US04upper	-	-	tooth, M3	post weaning	hyena	-	3
Les Cottés	1645	Z4-3466	US04lower	43680-44320	1	bone		bison	bison	this study
Les Cottés	1639	Z4-1462	US04lower	39970-40760	1	bone		equidae	equidae	this study
Les Cottés	1641	Z4-1911	US04lower	40870-41360	1	bone		equidae	equidae	this study
Les Cottés	1642	Z4-2245	US04lower	40010-40770	1	bone		equidae	equidae	this study
Les Cottés	1647	Y4-573	US08	45120-45780	1	tooth, UP3	possibl pre weaning	bison	bison	this study
Les Cottés	1648	Y4-425	US08	44200-44750	1	tooth, UPM1/M2	possibl pre weaning	bison	bison	this study
Les Cottés	1651	Z3-170	US04lower	38600-38990	1	tooth, P3 or 4	possible weaning	Rangifer		
Les Cottés	1653	Z4-3398	US06	43570-44200	1	tooth, P4	post weaning	equidae	equidae	this study
Les Cottés	1650	Y6-1960	US04upper	-	-	tooth, Upper M1/M3	post weaning	Rangifer	reindeer	this study
Les Cottés	1652	Z3-781	US04lower	40820-41320	1	tooth, LM2	post weaning	reindeer	reindeer	this study
Les Cottés	13665	S6-557	US04lower	38710-39360	1	bone		rangifer	-	
Les Cottés	2182	R5-1887	US08		-	tooth, LM2	post weaning	bear	-	
Les Cottés	2667	X5-215	US08		-	bone		large herbivore	-	
Les Cottés	2669	Y5-1647	US08		-	tooth	?	rhinoceros	-	
Les Cottés	2670	Y6-2223	US06		-	tooth	?	mammoth	-	
Les Cottés	2671	Z2-76	US08		-	tooth	?	megaceros	-	
Grotte du Renne	32908		Xb2					-	pantharinae	4
Grotte du Renne	32909		Xb2					-	pantharinae	4
Grotte du Renne	32912		Xb2					-	canidae	4
Grotte du Renne	32919		Xc					-	hyena	4
Grotte du Renne	32867		Xb					-	Bos/Bison	4
Grotte du Renne	32874		Xb					-	bison	4
Grotte du Renne	32904		Xb2				possible weaning	-	rangifer	4
Grotte du Renne	32906		Xb2					-	rangifer	4
Grotte du Renne	32907		Xb2					-	equidae	4
Grotte du Renne	32915		Xb					-	equidae	4
Grotte du Renne	32911							-	mammouth	4
Grotte du Renne	32913		Xb2					-	mammouth	4
Grotte du Renne	32916		Xc					-	rhino	4
Grotte du Renne	32886		X					-	hominin	4
Grotte du Renne	32917		Xc					-	Ursidae	4

Table S5: General information on the samples analysed in this study: layer attribution, dating, sample type, taxonomic identification. 1. Talamo et al. (19) 2 Hajdinjak et al (20) 3 Welker et al. (21) 4. Welker et al. (22)

Site	SEVA	Species determination	Diet	C/N	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Ref	$\delta^{15}\text{N}_{\text{Phe}}$	SD	$\delta^{15}\text{N}_{\text{Glu}}$	SD	$\delta^{13}\text{C}_{\text{Phe}}$	SD	$\delta^{13}\text{C}_{\text{Val}}$	SD	TP
Les Cottés	1526	Neanderthal	?	3.0	-18.1	13.9	this study	13.2	0.3	19.5	0.1	-24.5	0.8	-23.2	0.3	2.9
Les Cottés	2184	Hyena	Carnivore	3.1	-19.7	11.9	this study	8.9	0.1	16.7	0.5	-28.3	0.2	-26.3	0.0	3.1
Les Cottés	2185	Hyena	Carnivore	3.1	-18.8	10.2	this study	7.1	0.1	15.4	0.2	-26.2	0.2	-23.1	0.2	3.2
Les Cottés	2183	Hyena	Carnivore	3.1	-18.5	11.7	this study	6.2	0.2	17.3	0.4	-26.8	0.1	-23.4	0.1	3.6
Les Cottés	13670	Hyena	Carnivore	3.2	-18.1	9.0	1	12.1	0.1	13.2	0.3	-26.0	0.8	-24.4	0.9	2.2
Les Cottés	1645	Bison	Herbivore	3.2	-20.3	6.7	this study	7.3	1.1	7.3	1.1	-24.4		-27.1	0.3	2.1
Les Cottés	1639	Equidae	Herbivore	3.2	-20.1	6.4	this study	9.1	0.6	9.2	0.5	-26.4		-27.6		2.1
Les Cottés	1641	Equidae	Herbivore	3.2	-20.5	4.8	this study	11.1	1.4	7.6	0.3	-24.8		-25.5		1.7
Les Cottés	1642	Equidae	Herbivore	3.2	-20.6	4.9	this study	11.1	0.6	7.6	0.8	-25.1	0.5	-25.8	0.7	1.6
Les Cottés	1647	Bison	Herbivore	3.3	-20.3	9.2	this study	11.1	0.4	13.1	0.5	-26.8	0.0	-27.6	0.4	2.4
Les Cottés	1648	Bison	Herbivore	3.2	-20.1	7.8	this study	8.7	0.4	12.0	0.9	-26.8		-27.3	0.2	2.5
Les Cottés	1651	Rangifer		3.2	-18.4	6.3	this study	7.9	0.0	8.3	0.5	-25.9	0.8	-27.0	0.6	2.2
Les Cottés	1653	Equidae	Herbivore	3.3	-20.1	7.5	this study	8.3	0.3	9.8	0.1	-27.5	0.1	-27.9	0.1	2.3
Les Cottés	1650	Rangifer	Herbivore	3.3	-18.6	9.1	this study	11.0	0.4	11.1	0.6	-26.6	0.8	-26.6	0.6	2.1
Les Cottés	1652	Reindeer	Herbivore	3.2	-20.6	5.4	this study	10.6	0.5	6.5	0.5	-27.5	0.1	-27.9	0.1	1.6
Les Cottés	13665	Rangifer	Herbivore	3.2	-19.2	8.5	1	12.4	0.3	11.2	0.1	-24.1	0.3	-26.7	0.6	1.9
Les Cottés	2667	large herbivore	Herbivore	3.1	-19.8	5.3	this study	-	-	-	-	-	-	-	-	-
Les Cottés	2669	rhinoceros	Herbivore	3.1	-19.7	8.3	this study	-	-	-	-	-	-	-	-	-
Les Cottés	2670	mammoth	Herbivore	3.1	-21.5	11.1	this study	-	-	-	-	-	-	-	-	-
Les Cottés	2671	megaceros	Herbivore	3.1	-20.1	5.9	this study	-	-	-	-	-	-	-	-	-
Les Cottés	2182	Bear	Omnivore	3.1	-21.6	4.3	this study	9.7	0.6	8.0	0.1	-26.8	1.1	-26.3	0.9	1.9
Grotte du Renne	32908	Pantherinae	Carnivore	3.2	-18.8	8.9	2	10.9	0.7	14.0	0.9	-25.8	0.1	-25.5	0.0	2.5
Grotte du Renne	32909	Pantherinae	Carnivore	3.2	-18	7.4	2	9.6	1.2	14.0	0.4	-24.8	0.2	-24.6	0.3	2.7
Grotte du Renne	32912	Canidae	Carnivore	3.2	-18.9	9.9	2	12.1	0.6	17.0	0.2	-25.9	0.1	-25.5	0.5	2.8
Grotte du Renne	32919	Hyena	Carnivore	3.2	-19.5	9.5	2	9.4	0.8	15.1	0.2	-25.7	0.1	-25.3	0.2	2.9
Grotte du Renne	32867	Bison	Herbivore	3.2	-20.3	5.9	2	9.4	0.8	8.4	0.9	-26.5	0.1	-27.3	0.1	2.0
Grotte du Renne	32874	Bison	Herbivore	3.2	-19	3.5	2	7.8	0.1	8.6	0.4	-25.5	0.1	-28.2	0.1	2.2
Grotte du Renne	32904	Rangifer	Herbivore	3.2	-19.3	7.8	2	13.7	0.2	12.8	0.2	-26.4	0.4	-27.2	0.6	2.0
Grotte du Renne	32906	Rangifer	Herbivore	3.3	-19.4	5.5	2	12.2	0.7	10.3	1.4	-26.4	0.1	-28.0	0.5	1.9
Grotte du Renne	32907	Equidae	Herbivore	3.2	-20.9	5.2	2	14.4	0.3	10.8	0.3	-27.1	0.1	-28.1	0.5	1.6
Grotte du Renne	32915	Equidae	Herbivore	3.2	-20.8	5.2	2	12.3	0.2	10.2	0.7	-26.1	0.1	-27.9	0.1	1.8
Grotte du Renne	32911	Mammoth	Herbivore	3.3	-21.5	6.8	2	12.0	1.4	13.2	0.5	-26.6	0.4	-28.9	0.1	2.3
Grotte du Renne	32913	Mammoth	Herbivore	3.2	-20.2	4.9	2	9.9	0.2	10.1	0.1	-26.2	0.3	-28.9	0.4	2.1
Grotte du Renne	32916	Rhino	Herbivore	3.2	-18.9	2.4	2	7.6	0.6	7.5	1.3	-25.6	0.1	-28.3	0.4	2.1
Grotte du Renne	32886	Neanderthal	?	3.2	-19.5	14.3	2	9.4	0.6	18.1	0.1					3.2
Grotte du Renne	32917	Ursidae	Omnivore	3.3	-21.1	3.2	2	11.3	0.7	9.2	0.7	-26.7	0.1	-27.7	0.5	1.8

Table S6. Summary of isotope data for C and N obtained on bulk collagen ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$), and some amino acids : glutamic acid ($\delta^{15}\text{N}_{\text{Glu}}$) Phenylalanine ($\delta^{13}\text{C}_{\text{Phe}}$, $\delta^{15}\text{N}_{\text{Phe}}$) and valine ($\delta^{13}\text{C}_{\text{Val}}$).

TP= trophic position. Bulk collagen data are from this study, from 1. Talamo et al. (19) and 2. Welker et al. (22)

Site	Species determination	Diet	% Collagen	C/N	%C	%N	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Les Cottés	Neanderthal	?	5.6	3.2	44.0	15.9	-18.1	13.9
Les Cottés	Hyena	Carnivore	3.4	3.1	44.1	16.4	-19.7	11.9
Les Cottés	Hyena	Carnivore	7.0	3.1	44.8	16.8	-18.8	10.2
Les Cottés	Hyena	Carnivore	6.0	3.1	44.2	16.4	-18.5	11.7
Les Cottés	Large herbivore	Herbivore	5.7	3.1	44.3	16.6	-19.8	5.3
Les Cottés	Rhinoceros	Herbivore	2.9	3.1	44.0	16.6	-19.7	8.3
Les Cottés	Mammoth	Herbivore	1.2	3.1	41.5	15.7	-21.5	11.1
Les Cottés	Megaceros	Herbivore	4.5	3.1	45.2	17.1	-20.1	5.9
Les Cottés	Bear	Omnivore	1.5	3.1	38.4	14.4	-21.6	4.3

Table S7. Bulk collagen data from this study: Collagen yield (%), C/N ratios and isotope data.

Table S8. Carbon isotope data on amino acids. Average $\delta^{13}\text{C}$ values (top part) and standard deviation on the duplicate measurement.

	Ala	Asp	Glu	Gly	Ile	Leu	Lys	Met	Phe	Pro	Thr	Val
1639	-23.47	-17.01	-19.65	-16.90	-21.89	-32.55			-26.23	-19.77		-27.80
1641	-20.26	-15.40	-19.04	-12.57	-21.61	-32.01			-24.56	-18.78		-26.05
1642	-20.49	-12.74	-20.67	-10.26	-21.62	-32.01			-25.06	-19.30		-25.75
1645	-23.38	-13.88	-16.50	-14.01	-25.72	-30.85			-23.98	-18.16		-27.07
1647	-23.38	-15.33	-20.22	-13.39	-26.28	-30.37			-26.81	-18.09		-27.29
1648	-24.32	-16.56	-20.86	-15.08	-25.63	-31.14			-26.82	-18.84		-27.63
1650	-22.83	-16.27	-19.57	-11.93	-25.47	-30.57			-26.58	-18.45		-26.60
1651	-23.08	-15.87	-19.66	-12.91	-24.93	-30.96			-25.87	-18.00		-26.99
1652	-24.61	-15.06	-21.78	-14.12	-21.71	-32.84			-25.67	-19.89		-27.05
1653	-25.86	-17.03	-20.85	-16.93	-26.07	-31.17			-27.54	-19.47		-27.94
1526	-22.94	-10.96	-22.06	-9.89	-21.45	-29.51			-24.52	-17.71		-23.23
13665	-23.85	-14.17	-19.59	-13.09	-24.48	-30.57			-24.15	-17.60		-26.67
13670	-21.46	-18.06	-19.45	-10.50	-21.23	-31.00			-26.02	-18.18		-24.39
32867	-24.98	-16.64	-19.89	-17.56	-24.80	-31.30			-26.47	-19.57		-27.34
32874	-24.59	-17.67	-18.05	-13.14	-26.40	-32.01			-25.49	-19.36		-28.23
32904	-21.76	-15.14	-18.16	-11.32	-25.57	-31.17			-26.43	-19.02		-27.19
32906	-24.56	-16.86	-20.26	-12.79	-26.40	-32.56			-26.45	-20.07		-28.02
32907	-24.17	-17.68	-21.25	-15.25	-23.00	-33.50			-27.07	-20.40		-28.10
32908	-23.73	-16.32	-21.42	-11.40	-21.96	-31.22			-25.77	-19.46		-25.46
32909	-23.14	-16.49	-20.20	-10.98	-22.67	-30.01			-24.80	-18.53		-24.58
32911	-25.71	-18.75	-22.90	-17.32	-24.14	-33.98			-26.56	-21.06		-28.93
32912	-22.24	-16.57	-21.07	-14.13	-22.14	-31.37			-25.91	-19.73		-25.52
32913	-24.61	-18.07	-18.16	-16.53	-24.04	-33.80			-26.24	-20.57		-28.85
32915	-24.85	-17.17	-20.67	-17.36	-22.07	-32.82			-26.06	-20.40		-27.93
32916	-24.29	-15.88	-18.32	-14.35	-25.49	-31.40			-25.61	-18.91		-28.25
32917	-24.32	-19.73	-23.56	-15.58	-23.12	-32.83			-26.69	-22.48		-27.70
32919	-25.59	-19.04	-20.54	-16.82	-22.08	-30.73			-25.71	-19.51		-25.25
2182	-25.74	-21.10	-23.86	-11.38	-21.19	-31.93	-21.67	-26.20	-26.76	-22.10	-13.94	-26.28
2183	-25.49	-17.72	-22.00	-9.28	-21.60	-29.64	-21.54	-25.34	-26.76	-18.70	-11.88	-23.45
2184	-25.72	-18.97	-23.12	-8.46	-24.13	-30.93	-23.79	-25.94	-28.32	-20.63	-15.41	-26.34
2185	-20.18	-16.16	-23.03	-5.53	-21.50	-29.59	-20.03	-23.26	-26.25	-18.10	-10.14	-23.11

	Ala	Asp	Glu	Gly	Ile	Leu	Lys	Met	Phe	Pro	Thr	Val
1639	0.06	0.32	0.49	0.30	0.04	0.24			0.18	0.09		0.24
1641	0.81	0.59	0.74	0.48	0.96	0.19			0.27	0.47		0.82
1642	0.80	0.63	0.63	1.96	0.55	0.29			0.51	0.74		0.67
1645	0.21	1.15	0.01	0.13	0.10	0.15			0.55	0.18		0.28
1647	1.05	0.81	0.75	0.98	0.42	0.56			0.52	0.53		0.21
1648	0.16	0.12	0.26	0.39	0.56	0.36			0.04	0.12		0.37
1650	0.01	1.15	0.85	0.24	0.27	0.61			0.81	0.04		0.63
1651	0.00	1.19	0.54	0.05	0.13	0.35			0.80	0.13		0.58
1652	0.41	0.79	0.94	0.26	0.29	0.34			0.78	0.20		0.15
1653	0.13	0.20	0.07	0.35	0.13	0.11			0.10	0.02		0.15
1526	0.12	1.22	0.19	0.41	0.07	0.40			0.77	0.16		0.30
13665	0.12	0.85	0.00	0.48	0.50	0.42			0.30	0.29		0.59
13670	0.42	1.30	1.00	1.19	0.76	0.43			0.81	0.56		0.86
32867	0.09	0.25	0.05	0.63	0.10	0.40			0.11	0.15		0.10
32874	0.43	0.41	0.03	1.58	0.12	0.02			0.06	0.06		0.08
32904	0.10	0.09	0.23	0.25	0.06	0.20			0.40	0.08		0.56
32906	0.01	0.25	0.40	0.79	0.02	0.18			0.08	0.31		0.51
32907	0.13	0.13	0.53	0.34	0.24	0.21			0.09	0.30		0.47
32908	0.17	0.42	0.42	0.32	0.04	0.13			0.11	0.18		0.01
32909	1.00	0.19	0.99	0.70	0.52	0.22			0.21	0.27		0.33
32911	0.10	0.39	0.13	0.57	0.68	0.64			0.36	0.03		0.05
32912	0.31	0.20	0.24	0.48	0.35	0.48			0.15	0.07		0.49
32913	0.41	0.60	0.24	0.55	0.06	0.06			0.30	0.38		0.38
32915	0.47	0.30	0.18	0.28	0.69	0.02			0.13	0.22		0.05
32916	0.01	1.58	0.38	0.99	0.78	0.36			0.12	0.13		0.41
32917	0.61	0.90	0.18	0.27	0.14	0.06			0.10	0.31		0.51
32919	0.69	0.29	0.17	0.17	0.70	0.90			0.08	0.90		0.18
2182	0.15	0.23	0.30	0.11	1.19	1.24	0.27	1.35	1.12	0.54	0.67	0.93
2183	0.12	0.07	0.07	0.01	0.12	0.14	0.19	0.39	0.05	0.07	0.22	0.08
2184	0.22	0.55	0.08	0.58	0.18	0.03	0.80	0.23	0.19	0.29	0.13	0.05
2185	0.31	0.53	0.26	0.35	0.24	0.37	0.30	0.02	0.18	0.42	0.67	0.24

Table S9. Nitrogen isotope data on amino acids

	Ala	Asp	Glu	Gly	Hyp	Leu	Phe	Pro	Ser	Thr	Val	
std-c	10.91	8.98	9.85	12.20			2.91	8.11	13.15	9.37	-6.36	11.10
1526	15.50	18.38	19.52	13.97	18.55	17.60	13.19	18.97	11.23	-15.08	20.32	
32874	4.02	8.85	8.64	1.13	7.62	7.58	7.84	7.57	0.58	-8.32	10.04	
32907	6.68	11.32	10.76	3.42	11.44	6.40	14.41	11.99	3.30	-6.08	12.12	
32912	14.72	16.11	17.04	9.17	15.04	16.41	12.08	15.01	9.60	-10.20	18.22	
32913	7.07	10.20	10.14	2.11	10.52	8.04	9.91	10.45	2.86	-2.97	11.60	
32915	5.74	9.76	10.24	1.29	10.27	6.56	12.28	10.07	4.31	-5.45	11.71	
32919	11.14	14.24	15.13	8.11	13.77	11.20	9.39	13.86	8.36	-16.79	16.41	
2182	9.80	8.20	7.95	-1.39	9.11	6.97	9.73	8.56	-3.00	-13.14	10.55	
2183	18.44	16.60	17.32	9.10	16.63	13.93	6.20	16.33	7.99	-17.47	16.62	
2184	18.00	16.04	16.73	9.15	16.87	16.21	8.91	15.85	7.23	-12.79	19.13	
2185	17.17	13.65	15.44	10.19	14.00	13.73	7.12	14.14	8.06	-15.48	17.91	
32886	17.01	15.90	18.09	14.35	19.66	16.51	9.44	18.96	12.31	-21.36	19.18	

	Ala	Asp	Glu	Gly	Hyp	Leu	Phe	Pro	Ser	Thr	Val	
std-c	0.21	0.65	0.36	0.28			0.08	0.04	0.14	0.81	0.63	0.66
1526	0.09	0.19	0.09	0.15	0.03	1.84	0.27	0.09	1.05	0.20	1.18	
32874	0.03	0.05	0.43	0.15	0.19	1.12	0.07	0.23	0.11	1.12	0.42	
32907	0.64	0.74	0.33	0.01	0.25	0.24	0.26	0.35	0.45	0.65	0.18	
32912	0.44	0.30	0.22	0.37	0.22	0.17	0.56	0.07	0.34	0.14	0.46	
32913	0.12	0.06	0.07	0.09	0.12	0.96	0.22	0.12	0.41	0.08	0.49	
32915	0.67	0.24	0.71	0.98	0.56	0.27	0.25	0.61	0.48	0.72	0.48	
32919	0.12	1.24	0.20	0.13	0.30	1.16	0.76	0.18	0.19	0.63	1.38	
2182	0.33	0.31	0.07	0.58	1.40	0.12	0.62	0.00	0.46	0.64	0.98	
2183	0.99	0.52	0.41	0.31	0.51	0.12	0.19	0.01	0.09	1.20	0.23	
2184	0.86	0.82	0.55	1.30	0.81	0.75	0.05	0.16	0.63	0.47	0.53	
2185	0.92	0.07	0.16	1.32	0.31	1.22	0.10	0.17	0.95	1.60	0.20	
32886	0.65	0.34	0.09	0.19	0.12	0.00	0.62	0.22	0.49	0.30	0.58	

	Ala	Asp	Glu	Gly	Ile	Leu	Lys	Phe	Pro	Thr	Val
1577b-a	9.71	10.61	10.60	9.10	7.68	5.37	3.53	8.36	13.66	-6.19	10.04
1639	6.88	11.13	9.23	2.07	8.29	6.25	2.55	9.07	8.60	-6.89	8.76
1641	4.90	10.61	7.64	0.24	7.19	5.24	1.06	11.05	7.52	-7.80	7.59
1642	5.05	11.04	7.56	-0.02	7.51	5.49	3.29	11.05	7.73	-6.31	7.79
1645	4.83	8.34	7.27	1.39	8.44	5.82	2.30	7.26	6.25	-9.56	8.24
1647	8.43	12.92	11.97	4.83	11.66	9.60	8.95	8.66	9.46	-5.65	12.03
1648	9.64	14.06	13.08	6.59	12.63	9.99	7.05	11.08	10.79	-5.01	12.75
1650	8.95	12.97	11.06	8.97	11.69	9.26	4.88	10.98	10.06	-8.02	12.88
1651	6.15	9.85	8.34	6.32	7.74	5.51	2.71	7.87	6.07	-11.38	9.88
1652	5.43	10.28	6.47	0.63	8.54	5.47	4.44	10.58	7.31	-8.03	7.18
1653	7.31	10.97	9.78	6.18	9.80	7.48	7.93	8.29	7.68	-7.85	10.35
1526	16.84	18.98	18.38	12.12	17.76	15.54	7.00	12.61	16.76	-17.16	17.98
13665	8.98	13.27	11.24	4.94	12.61	10.49	7.24	12.42	10.95	-3.56	13.34
13670	12.54	15.16	13.19	4.70	13.16	11.67	8.05	12.11	11.92	-13.57	14.99
32867	6.18	11.11	8.43	3.26	9.85	7.22	8.14	9.43	6.84	-7.11	9.16
32874	4.45	8.71	8.26	0.57	8.38	6.28	0.92	8.76	6.22	-8.70	9.25
32904	8.87	12.35	12.77	5.55	12.16	10.15	4.52	13.71	10.89	-5.49	13.01
32906	6.45	10.02	10.26	2.84	10.35	8.25	5.27	12.19	9.72	-5.97	12.14
32907	5.62	10.35	10.28	1.53	8.56	6.76	2.90	13.51	9.79	-5.96	9.32
32908	12.54	13.95	13.98	4.81	13.97	12.48	5.79	10.92	14.17	-16.72	14.73
32909	10.98	11.92	14.03	3.74	13.70	11.60	5.14	9.60	12.38	-18.30	13.57
32911	5.29	8.99	13.22	8.14	8.61	7.01	4.05	11.99	10.36		11.00
32912	13.61	15.10	17.22	7.50	15.44	14.18	5.33	11.68	13.25	-12.95	17.23
32913	5.66	9.30	9.62	0.23	8.13	6.59	5.36	10.54	8.51	-4.80	10.41
32915	5.91	10.64	10.60	1.78	7.82	6.31	4.12	12.72	10.32	-5.27	9.75
32916	3.11	6.86	7.45	-0.91	6.32	4.74	-1.78	7.59	5.40	-10.75	9.01
32917	6.95	8.14	9.23	-3.65	8.63	7.21	3.88	11.29	8.03	-5.57	10.05
32919	12.14	15.06	14.93	7.86	13.47	12.54	6.51	8.51	14.26	-16.14	14.86
1577b-b	10.49	11.20	10.46	10.87	8.61	6.57	1.97	8.27	14.30	-4.75	11.18

	Ala	Asp	Glu	Gly	Ile	Leu	Lys	Phe	Pro	Thr	Val
1577b-a	1.04	0.43	0.14	1.40	0.84	0.97	1.00	0.41	1.09	0.41	0.10
1639	0.13	0.48	0.50	0.02	0.03	0.01	0.82	0.55	0.02	0.92	0.04
1641	0.18	0.65	0.34	0.14	0.38	0.12	0.30	1.38	0.10	0.74	0.18
1642	0.05	0.28	0.78	0.15	0.99	0.56	1.49	0.55	0.06	0.52	0.14
1645	0.14	0.05	0.07	0.18	0.51	0.45	0.11	1.07	0.31	0.07	0.35
1647	0.10	0.45	0.88	0.10	0.01	0.06	0.90	0.35	0.03	0.58	0.17
1648	0.03	0.42	0.46	0.33	0.10	0.15	0.13	0.41	0.19	0.57	0.67
1650	0.28	0.17	0.57	0.32	0.14	0.05	0.64	0.44	0.09	0.14	0.41
1651	0.04	0.01	0.46	0.06	0.06	0.17	0.01	0.02	0.01	0.72	0.01
1652	0.05	0.53	0.53	0.10	0.18	0.08	1.62	0.50	0.04	0.75	0.35
1653	0.12	0.36	0.08	0.19	0.22	0.27	2.26	0.35	0.26	0.29	0.48
1526	0.20	0.42	0.91	0.19	0.35	0.25	0.27	1.28	0.05	0.06	0.47
13665	0.24	0.21	0.06	0.33	0.81	0.82	2.51	0.34	0.22	0.05	0.51
13670	0.08	0.06	0.25	0.11	0.52	0.35	1.45	0.11	0.22	0.57	0.17
32867	0.01	0.08	0.88	0.06	0.00	0.16	0.13	0.81	0.12	0.38	0.09
32874	0.14	0.24	0.22	0.18	0.13	0.17	1.10	0.87	0.02	0.36	0.32
32904	0.09	0.10	0.25	0.06	0.68	0.55	0.14	0.21	0.12	0.54	0.27
32906	0.19	0.01	1.37	0.34	0.18	0.14	0.84	0.74	0.33	1.45	1.27
32907	0.41	0.35	0.74	0.47	0.21	0.04	2.24	1.14	0.52	0.43	0.38
32908	0.06	0.07	0.92	0.05	0.14	0.04	2.04	0.67	0.14	0.64	0.18
32909	0.09	0.61	0.43	0.13	0.15	0.22	1.21	1.18	0.33	1.27	0.65
32911	0.14	0.84	0.51	0.09	1.32	1.13	0.81	1.41	0.32		0.98
32912	0.28	0.03	1.34	0.30	1.10	1.02	0.49	0.28	0.19	0.41	0.86
32913	0.23	0.04	0.55	0.13	0.41	0.05	0.76	1.06	0.07	0.49	0.14
32915	0.26	0.07	0.40	0.00	0.79	0.81	2.00	1.29	0.02	0.67	0.17
32916	0.47	1.12	1.29	0.50	0.11	0.09	2.08	0.59	0.62	1.51	0.45
32917	0.27	0.23	0.72	0.20	1.19	0.67	0.94	0.73	0.24	1.05	0.61
32919	0.11	0.02	0.43	0.21	0.20	0.20	1.30	1.41	0.21	0.13	0.35
1577b-b	0.26	0.13	0.23	0.07	0.28	0.33	0.05	0.40	0.10	0.46	0.21

Table S10 Carbon and nitrogen isotope data on the standard bovine liver 1577b

Carbon isotope ratios

	Ala	SD	Asp	SD	Glu	SD	Gly	SD	Ile	SD	Leu	SD	Lys	SD
1577b-a	-19.52	1.48	-13.04	0.52	-16.86	0.35	-8.01	0.04	-24.06	0.61	-29.22	0.22		
1577b-b	-20.36	0.39	-12.55	0.03	-16.10	0.85	-9.30	0.39	-22.45	0.03	-29.29	0.13		
1577b-c	-19.59	0.23	-12.83	0.46	-16.73	0.94	-7.00	0.76	-24.51	0.21	-30.48	0.12	-15.89	1.20

Nitrogen isotope ratios

	Ala	SD	Asp	SD	Glu	SD	Gly	SD	Ile	SD	Leu	SD	Lys	SD
1577b-a	9.71	1.04	10.61	0.43	10.60	0.14	9.10	1.40	7.68	0.84	5.37	0.97	3.53	1.00
1577b-b	10.49	0.26	11.20	0.13	10.46	0.23	10.87	0.07	8.61	0.28	6.57	0.33	1.97	0.05
1577b-c	10.91	0.21	8.98	0.65	9.85	0.36	12.20	0.28			2.91	0.08		

Met	SD	Phe	SD	Pro	SD	Thr	SD	Val	SD
		-25.16	0.00	-18.48	0.40			-25.54	0.91
		-24.51	0.09	-18.86	0.03			-24.86	0.58
-23.05	0.84	-25.55	0.57	-18.69	0.94	-7.23	0.25	-25.28	0.06

Phe	SD	Pro	SD	Ser	SD	Thr	SD	Val	SD
8.36	0.41	13.66	1.09			-6.19	0.41	10.04	0.10
8.27	0.40	14.30	0.10			-4.75	0.46	11.18	0.21
8.11	0.04	13.15	0.14	9.37	0.81	-6.36	0.63	11.10	0.66