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Stable isotopes and the metabolism of the European cave bear

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Abstract Isotopic analyses of fossil bones of the extinct European cave bear indicate that this animal was a hibernator with the same unusual metabolic processes as some modern bear species. This finding provides useful biological and archaeological information on an extinct species, and the methods themselves may prove generally useful in studies of the metabolisms of modern bears, other hibernators, and perhaps of starving animals.

Key words Carbon isotopes · Nitrogen isotopes · Cave bears · Hibernation

Introduction

The Divje Babe archaeological site in northwestern Slovenia contains a remarkable quantity of fossil bones of the extinct European cave bear (*Ursus spelaeus*) as well as artifactual evidence for occasional human presence during the Middle and Early Upper Palaeolithic periods (Turk 1997). A radiocarbon dating study of these bear bones confirmed that the cave was used by bears over a period starting at least 50,000 years ago (the limit of the ^{14}C method) until about 30,000 years ago (Nelson 1997). The bones dated in this study (Table 1, A-series sample numbers) were very well preserved for samples of this age, and we were tempted to use this ancient material to test the carbon isotope method for dietary reconstruction, even though there was no reason to anticipate new information on cave bear diet from such a study. Measures of the stable carbon isotope ratios of bone protein provide dietary information in those circumstances in which the consumer can choose between marine and terrestrial food chains or between food chains based on C3 or C4 plants. (Ambrose 1993 gives a review and extensive bibliography.) Since cave bears are thought to have been primarily herbivorous (Kurtén 1976), and since one would not expect either C4 plants or marine protein to have been part of the diet of Pleistocene bears in the Slovenian mountains, the carbon isotope method can at best only confirm these suppositions. In fact, recent isotopic measures on fossil cave bear bones from similar sites in France (Bocherens et al. 1990, 1994) do indeed indicate that these bears were eating a C3-based terrestrial diet. However, an isotopic study (Bocherens et al. 1994) of bear teeth shows isotopic signatures which hint at unusual circumstances during the early part of the bear's life.

Materials and methods

To explore this in detail, we selected from the available samples a second set of bones (B-series samples, Table 1) of bears ranging in development from newborn to fully developed adults. With few exceptions, these B samples were tibia from a specific stratigraphic layer in the cave (dated at about 48,000 BP) so that we would obtain measures of different individuals of varying ages from the same approximate time period. The samples were divided into six age classes ranging from fetal/newborn to adult (as given in Table 1) on the basis of their bone size and epiphyseal fusion. High molecular-weight portions of the remnant collagen molecules were extracted using the method of Brown et al. (1988). The concentrations of proteinaceous material in the bones ranged from 3–11%, with substantial fractions (one-third to one-half) of each extract having molecular weight $\geq$30 kD. These observations, and measured carbon/nitrogen concentration ratios of 2.8 to 2.9 wt% strongly indicate (cf. DeNiro1985) that the extracted material provided reliable isotopic measures. Each extract was then combusted by heating it with CuO at 900°C for $\approx$5 h in a sealed,

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Table 1 Sample descriptions and isotopic results for fossil cave bear bones from Divje Babe Cave, Slovenia

Age group	Developmental stage	Sample number	Bone identification	δ^{13}C (‰ PDB)	δ^{15}N (‰ AIR)
I	Neonate/fetus	B1:193	Tibia	−22.5	–
	” ”	B1:188	Tibia	−22.8	–
	” ”	B1:171	Femur	−23.4	6.8
	” ”	B1:173	Ulna	−22.1	6.6
	” ”	B1:163	Unknown	−23.2	7.7
II	Cub	B2:173-1	Tibia	−21.3	3.9
	”	B2:173-3	Tibia	−22.8	7.2
	”	B2:182	Tibia	−22.0	4.0
	”	B2:188	Tibia	−21.5	4.7
III	Yearling	B3:188-1	Tibia	−21.6	2.3
	”	B3:181-2	Tibia	−20.4	3.0
	”	B4:188-1	Tibia	−21.0	3.3
	”	B4:188-2	Tibia	−21.1	3.2
	”	B4:188-3	Tibia	−21.2	2.1
IV	Juvenile	A1	Cranium	−21.5	4.4
	”	A3	Femur	−21.3	4.0
	”	A9	Humerus	−20.1	–
V	Sub-adult	B5:188-1	Tibia	−20.2	1.1
	”	B5:188-2	Tibia	−20.1	2.3
	”	B6:182	Tibia	−20.7	4.1
	”	A7	Femur	−19.9	2.5
VI	Adult	B7:183	Tibia	−21.0	1.4
	”	B8:181	Tibia	−20.4	1.6
	”	B9:173	Tibia	−20.2	1.9
	”	B10:192	Tibia	−20.1	1.6
	”	B11:173	Tibia	−20.6	2.2
	”	A2	Radius	−20.3	2.6
	”	A4	Fibula	−20.4	2.4
	”	A5	Cranium	−21.0	3.3
	”	A6	Radius	−21.0	1.7
	”	A8	Metacarpus	−20.1	–
	”	A10	Metatarsal	−20.5	0.6
	“	A11	Metacarpus	−19.9	1.9

evacuated quartz tube. The δ^{13}C values of the resulting CO_2 were measured using a VG Prism mass spectrometer and are given in the usual format $\delta^{13}C = 1000\ (Ru/Rs - 1)$‰ where *R*u and *R*s are the respective $^{13}C/^{12}C$ ratios for the unknown and the PDB isotopic standard. As discussed below, we later required stable nitrogen isotope ratios (δ^{15} N values) as well. These have an analogous definition, i.e. $\delta^{15}N = 1000\ (Ru/Rs - 1)$‰ where *R*u and *R*s are the respective $^{15}N/^{14}N$ ratios for the unknown and the AIR isotopic standard. These procedures give a measurement accuracy of ≤ 0.1‰ for carbon and ≤0.3‰ for nitrogen, both of which are negligibly small compared to the differences of interest here.

Results and discussion

The data obtained are given in Table 1. The δ^{13}C values for the adult bears are as expected for animals obtaining their protein from a food chain based on C3 plants, in concordance with the results found for cave bear bones in French sites (Bocherens et al. 1990, 1994). Further, the variation about the mean for the adults (0.37‰ at 1 SD) is also very similar to that for the French bears, for other animals (e.g. Angerbjörn et al. 1994) and for humans (Lovell et al. 1986). However, there is a very clear correlation (Fig. 1a) between the animal's δ^{13}C value and its age; the newborns have a very light isotopic value which gradually changes to the adult value as the animal grows. Such age differences have not been observed for other species. As examples, carbon isotopic studies of a prehistoric human population (Lovell et al. 1986), of modern and prehistoric human mother-infant pairs (Fogel et al. 1989), and of modern arctic foxes (Angerbjörn et al. 1994) showed no significant differences for individuals of differing ages. However, these present results are in general accord with those for the teeth of adult cave bears (Bocherens et al. 1994), as the teeth are formed before weaning and so retain the isotopic signature of the young animal.

Since the bears are born with an unusual carbon isotope value, we must postulate an isotopic effect *in utero* that is different in bears from that in other animals. Either the females had an unusual diet while pregnant or they had a metabolism different from that of humans or foxes. The dietary explanation is unlikely, as it is difficult to think of an available diet that could account for the shift. However, the metabolic explanation is in entire accord with present understanding of the remarkable metabolic processes of modern black and grizzly bears (*Ursus americanus* and *A. arctos*) while in hibernation.

These modern bears have an unusual metabolism while hibernating in that they maintain their body temperature but neither defecate nor urinate (Nelson 1973, 1980, 1989; Nelson et al. 1983; Lundberg et al.

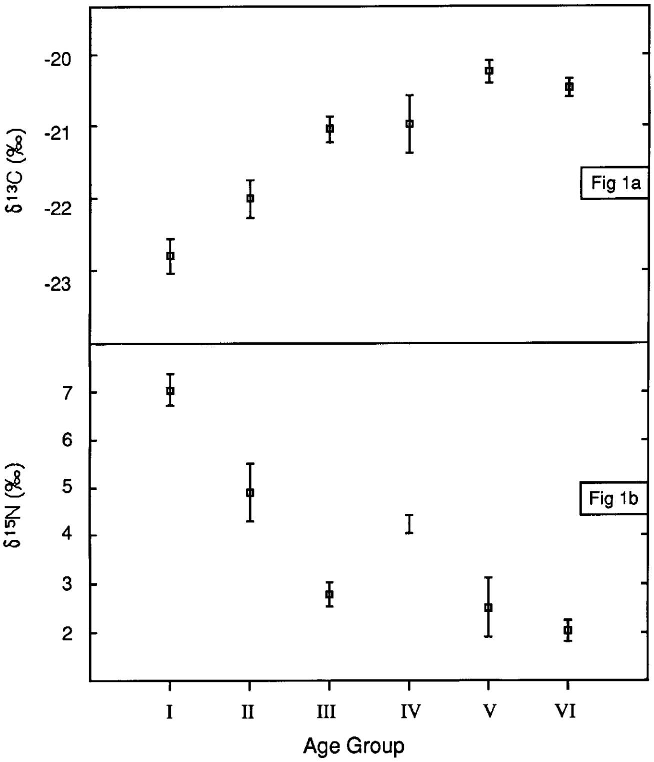

Fig. 1 The average **a** carbon and **b** nitrogen isotope ratios for the different age classes of the bears. The bar on each point gives the measured standard error for that class, with the exception of the nitrogen result for age-class IV, which gives the range for the two measures made

1976). To do this, they have metabolic mechanisms to use recycled protein and their supply of stored fat to form amino acids for maintenance-level protein synthesis. Since gestation and birth take place during winter hibernation when the mother is not eating, the bone protein of the forming fetus must also incorporate carbon from this fat and from the recycled protein.

While these hibernating metabolic processes have not been isotopically studied in modern bears, they do provide a convincing explanation for the observed isotopically light values for the young cave bears. Fat has a δ^{13}C signature about 6‰ more negative than that of collagen (Tieszen and Boutton 1989) and protein recycling may also involve fractionation towards more negative values. Thus, foetal collagen derived from a hibernating mother's stored fat and from her protein recycling will be more isotopically negative than her own collagen. This is unlike the situation for the foetal collagen of the humans and foxes discussed above, in which the carbon isotope ratios are the same as those of the mother and which reflect her diet.

This hypothesis could be further tested. Since modern bears do not urinate while hibernating, they must have a mechanism for avoiding toxic concentrations of the urea which results from protein catabolism. This is done by totally recycling the nitrogen (Nelson et al. 1975) and so we might expect this unusual process to be reflected in the stable nitrogen isotopic (δ^{15}N) values of any collagen produced during hibernation.

Nitrogen processing in non-hibernating animals results in a δ^{15}N value for bone protein approximately +3‰ higher than that of the food consumed (Wada et al. 1980; Minagawa and Wada 1984; Schoeninger and DeNiro 1984; Ambrose 1993), an isotopic "trophic level effect". These fractionating processes also affect other proteins. For example, the δ^{15}N values of the finger-nails of a human mother and her new-born child are the same, but the child's finger-nail becomes about 3‰ more positive as the child is breast-fed, and then returns to the adult value after weaning (Fogel et al. 1989). If these fractionating processes operate in non-hibernating species, we might expect them to be especially apparent in the tissue of new-born bears, whose hibernating mothers metabolically recycle their urea while the fetus is forming.

The δ^{15}N values (Table 1; Fig. 1b) subsequently determined for the cave bear samples fully support this prediction, both in the sense and the magnitude of the effect. Unlike humans, the cave bear is not born with the same δ^{15}N value as that of its mother, but with a value 5‰ higher (about two "trophic levels"). This must be due to metabolic recycling of the mother's proteinaceous tissue. The value falls to one "trophic level" as the cub ages, reflecting the isotopic differences between the mother's diet and her milk and eventually, it reaches adult value some time after the cub is weaned. As with the δ^{13}C data, this conclusion is in accord with the δ^{15}N results for the adult bear teeth (Bocherens et al. 1994).

The rate of isotopic change must reflect both the gradual change of the cub's diet after birth and its growth rate. Since there is no detailed information on the weaning age or growth of this extinct animal, we cannot quantitatively compare the observed isotopic change with that predicted from a food-mixing curve. In fact, if our overall interpretation is correct, the isotopic data may in themselves provide an accurate measure of the bear's maturation process. For example, the δ^{15}N-value for sample B2:173-3 seems out of rank for the estimated age of the animal. While we do not have sufficient bone-size data for this suite of samples to enable a detailed quantitative comparison with the isotopic results, there was sufficient remaining material to compare the smallest shaft diameters of the tibia of groups 1 and 2. We found that sample B2:173-3 was in fact small for group 2, and on this criterion it should perhaps originally have been placed in the "neonate" group as the isotopic data suggest.

Conclusions and suggestions for further research

The data obtained for both carbon and nitrogen isotopes show that new-born cave bears began life with anomalous isotopic ratios, as compared to the other species for which we have data, and that these ratios gradually changed to those expected for animals feeding on terrestrial diets. These anomalous values are consistent with present understanding of the metabolism of existing hibernating bear species, and so we conclude that this extinct species was also a hibernator with a similar metabolism.

This conclusion immediately suggests further studies in a number of areas. First, we predict that existing hibernating bears will have similar isotopic signatures. If this is found to be true, it will substantially confirm our conclusion for the cave bears. Next, we suggest that the isotopic signature may be an accurate measure of maturity for hibernating bear species, both present and extinct.

Further, if these conclusions are correct, the isotopic method should prove useful in testing the hibernating metabolisms of other species and in the chemical study of these strange metabolic processes. The possibility extends beyond bone collagen to other proteins and tissues (e.g. teeth, bone carbonate) which have different formation mechanisms, maturation times and turn-over times. As well, the different amino acids in protein have different isotopic signatures, as do the different carbon atoms in the amino acids (Hare et al. 1991; Abelson and Hoering 1961; Keeling 1996). Can we examine metabolic details by the isotopic signatures of these compounds?

Such studies may have applications beyond the metabolism of hibernation. For example, starvation apparently induces metabolic responses in non-hibernating animals (e.g. anorexic humans) that are similar to a bear's hibernating metabolism (Nelson 1989; Nedergaard and Cannon 1990). Can these isotopic measures provide useful information in studies of starvation, both for present and for past populations? The isotopic consequences of starvation should be distinguishable from those of hibernation, as starving individuals have no stored, isotopically light fat on which to draw, but does starvation induce fractionation from protein recycling? There is much to be explored.

Last, the conclusion that the cave bears were hibernators with metabolisms similar to modern bears provides very useful information to biologists studying the behaviour of these extinct animals and to archaeologists interested in their interactions with prehistoric mankind. It provides an explanation for the large numbers of bones of bears of all ages found in the cave sites, as it is known that modern bears sometimes fail to achieve proper hibernating mode and then die of starvation (Lundberg et al. 1976). Finally, we speculate that any winter-time interactions of cave-dwelling humans with cave bears must have been delicate, as modern hibernating bears are easily aroused, quickly capable of self-defense, and likely to be "grouchy as a bear" if rudely awoken.

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