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Stable carbon isotope ratios in Asian elephant collagen: implications for dietary studies

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Summary. Stable carbon isotope ratios in bone collagen have been used in a variety of dietary studies in modern and fossil animals, including humans. Inherent in the stable isotope technique is the assumption that the isotopic signature is a reflection of the diet and is persistent in collagen because this is a relatively inert protein. Carbon isotope analyses of bones from a southern Indian population of Asian elephant (*Elephas maximus*), a long-lived mammal that alternates seasonally between a predominantly C_3 (browse) and C_4 (grass) plant diet, showed two patterns that have important implications for dietary interpretation based on isotopic studies. Relative to the quantity of the two plant types consumed on average, the $\delta^{13}C$ signal in collagen indicated that more carbon was incorporated from C_3 plants, possibly due to their higher protein contribution. There was a much greater variance in $\delta^{13}C$ values of collagen in sub-adult (range -10.5‰ to -22.7‰, variance = 14.51) compared to adult animals (range -16.0‰ to -20.3‰, variance = 1.85) pointing to high collagen turnover rates and non-persistent isotopic signatures in younger, growing animals. It thus seems important to correct for any significant relative differences in nutritive value of food types and also consider the age of an animal before drawing definite conclusions about its diet from isotope ratios.

Key words: Stable carbon isotopes – Collagen – Diet – Asian elephant – *Elephas maximus*

Stable carbon isotope ratios in bone collagen have been used to interpret various aspects of diet in modern and fossil animals, including humans (see Rundel et al. 1989 for a review). For instance, $^{13}C/^{12}C$ ratios may reflect the proportions of C_3 and C_4 plants (van der Merwe and Vogel 1978; DeNiro and Epstein 1978a) or the marine versus terrestrial components in the diet (Tauber 1981; Chisholm et al. 1982; Sealy and van der Merwe 1985; Ramsay and Hobson 1991).

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The principle behind the stable isotope technique is simple. Plants and animals can be grouped into fairly well-defined categories based on their stable carbon isotope ratios, as a consequence of variation in photosynthetic systems and their positions in the food web. Stable carbon isotope ratios are expressed as parts per mil (‰) relative to the Pee Dee belemnite (PDB) standard as follows (Craig 1957):

$$\delta^{13}C\ ‰ = \{[(^{13}C/^{12}C)\ \text{sample}/(^{13}C/^{12}C)\ \text{standard}] - 1\} \times 1000$$

Plants with the C_3 photosynthetic pathway of carbon fixation typically have $\delta^{13}C$ values in the range of -25‰ to -30‰ while those with C_4 pathway are in the range of -10‰ to -14‰ (Smith and Epstein 1971). In a terrestrial food web these widely differing $\delta^{13}C$ values of primary producers could be expected to be reflected in the $\delta^{13}C$ values of tissues of an animal feeding on these categories.

Among various biochemical fractions, collagen has been widely used for the isotopic analysis not only because it is a component of bone, thus permitting studies on fossil animals, but also because it is relatively inert compared to intra-cellular proteins (Libby et al. 1964; Stenhouse and Baxter 1977). Relative to the diet source, collagen is enriched in the heavier isotope due to metabolic fractionation by about 3‰–6‰; the exact enrichment value is believed to be species specific (DeNiro and Epstein 1978b). Dietary interpretations have to be made after applying this correction to the $\delta^{13}C$ values obtained in collagen of an animal.

Although the question of actual collagen turnover rates and the persistence of the "isotopic memory" with time has been raised earlier (Tieszen 1978; Lewin 1983; Sukumar et al. 1987), it has been generally ignored in studies of dietary reconstruction from stable isotope ratios. It has been shown experimentally in gerbils that $\delta^{13}C$ of tissues such as hair, brain, muscle and liver can change quite rapidly in response to changes in diet (Tieszen et al. 1983). If collagen too turned over sufficiently rapidly, its isotopic signature could potentially fluctuate in response to short-term changes in dietary components. This would have

important implications for the interpretation of diet from stable isotope measurements.

It is also possible that carbon from dietary sources may not be represented in an animal tissue in the same proportion as these are ingested quantitatively because of qualitative differences in nutrient content of food items.

We have thus investigated these two aspects of diet in relation to stable carbon isotope composition. In particular, we have asked the following questions: (a) How does mean $\delta^{13}C$ value in animal collagen correlate with actual dietary intake observed in the field? (b) How does the variance in $\delta^{13}C$ relate to age of an animal? What are its implications for collagen turnover? For our investigation we have chosen a long-lived mammal, the Asian elephant (*Elephas maximus*), which is known to have seasonal preferences for C_3 (browse) or C_4 plants (grasses) (Sukumar 1989).

Materials and methods

We collected bone samples primarily from mandibles of Asian elephants which died in the Nilgiri-Eastern Ghats region of southern India during 1981–1989. This region is characterized by a diversity of tropical vegetation types, including evergreen forest, moist deciduous forest, dry deciduous forest and dry thorn forest, with all but the first type featuring C_4 grasses in the undergrowth. It also holds one of the largest populations of wild Asian elephants, estimated at 4000–5000. This region and its elephant population have been the subjects of long-term ecological studies, more details of which are available elsewhere (Sukumar 1989). Elephants were aged from their dentition (Roth and Shoshani 1988) and from body measurements such as shoulder height (Sukumar et al. 1988). Our samples covered an age range from new-born to 60 years. We have categorized them into infant (0–1 year), sub-adult (1–25 years) and adults (25–60 years) for the further discussion in this paper. For convenience we have defined adult elephants as those animals in which growth in body weight has slowed down considerably and the asymptotic height is practically attained (by age 25 years, Sukumar et al. 1988), rather than sexually mature animals (above 15 years). Field observations were made during 1981–1983 and 1988–1989 on feeding habits of the elephants in this region by sampling techniques described elsewhere (Altmann 1974; Sukumar 1989).

Collagen was extracted from powdered bone as a gelatin by methods described in DeNiro and Epstein (1978b). Samples were sealed under vacuum into quartz tubes with CuO wire and combusted at 800° C to prepare CO_2 gas (Boutton et al. 1983). The CO_2 was analysed for stable carbon isotope composition in a VG Micromass 602D mass spectrometer with an overall precision of ±0.2‰ (1 SD) as evidenced by replicate measurements on samples and an (internal) glucose standard.

Results and discussion

The $\delta^{13}C$ values versus age of the sampled elephants are shown in Fig. 1 and summarized for three age classes in Table 1. There is considerable scatter in the values for infants (−9.4 to −21.5‰) and sub-adults below 25 years (−10.5 to −22.7‰), while adult elephants above this age have a much narrower range (−16.0 to −20.3‰).

The $\delta^{13}C$ values of collagen in new-born or suckling infants would reflect the diet of their mothers over a certain period of time prior to sampling, with some shift due to fractionation firstly in the production of milk by the mother (Minson et al. 1975) and later in collagen synthesis

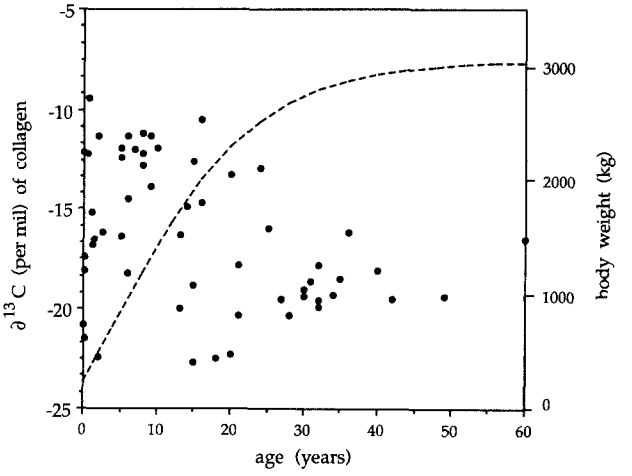

Fig. 1. Plot of $\delta^{13}C$ (‰) against age (years) in Asian elephants. The growth curve for body weight (kg) versus age (years) in female Asian elephants (*broken line*) is also shown for comparison. This is based on the function: Weight (at time t) = 3055 $(1-e^{-0.092\ (t+6.15)})^3$ kg (Sukumar et al. 1988)

Table 1. $\delta^{13}C$ values of elephant bone collagen in different age classes

$\delta^{13}C$ values	Age classes (years) 0–1 Infant	1–25 Sub-adult	25–60 Adult
Range	−9.4 to −21.5‰	−10.5 to −22.7‰	−16.0 to −20.3‰
Mean (±SD)	−16.4‰ (±4.44)	−15.3‰ (±3.81)	−18.6‰ (±1.36)
Variance	19.71	14.51	1.85
Sample size	7	33	16

The variances in $\delta^{13}C$ of sub-adult and adult age classes are significantly different (F ratio = 7.84; df = 32, 15; $P < 0.01$)

(DeNiro and Epstein 1978b). Since collagen can be expected to show a high turnover in such young animals, the isotopic signature would be transitory if the diet of the mothers had changed with season. This is also supported by our analyses of bone samples that we obtained towards the end of our study from a female elephant and its foetus (data not included in the above figures). The $\delta^{13}C$ values of mother (−13.8‰) and foetus (−17.1‰) were different, strongly indicating that in the foetus this was influenced by the recent feeding behaviour of the mother.

The difference of about 3‰ in mean $\delta^{13}C$ value between the sub-adult and adult age-classes (Table 1) could be due to a slightly lower preference for browse (C_3 type) by sub-adults compared to adults, a result also supported by direct observations on feeding habits of the elephant population (Fig. 2). Sub-adults consumed only 42% C_3 plants on average compared to 50% consumed by adults over a year.

However, the mean $\delta^{13}C$ values of collagen do not exactly reflect the quantitative intake of C_3 plants and C_4 plants by elephants. In the study area the common C_3 food plants have a mean $\delta^{13}C$ value of −27.2‰ while the C_4

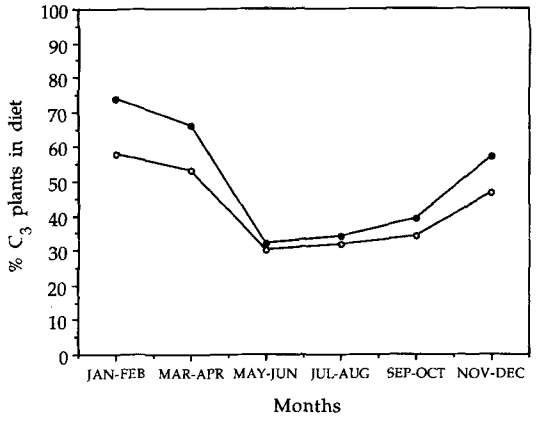

Fig. 2. Percentage of time spent in feeding on C_3 (browse) plants on average during different seasons by adult elephants (*solid circles*) and sub-adult elephants (*open circles*) in the study area. The percentage of C_4 plants in the diet is simply equal to 100 − percentage C_3 plants. The dry months in the study area are December-April and the wet months May-November

grasses have a mean value of −12.8‰ (values reported in Sukumar et al. 1987). Assuming a $\delta^{13}C$ shift of 4.5‰ in elephant collagen relative to diet (see Sukumar et al. 1987; Tieszen et al. 1989), the mean $\delta^{13}C$ of adult elephants (−18.6‰) indicates a diet of c. 70% C_3 plants and not 50% as observed in the field. This difference could arise from a higher contribution of carbon, in this case protein, from C_3 food plants (range 3–26% dry weight) compared to C_4 plants (range 1.5–10%) (see Sukumar 1989 for published values of nutrient content).

Of even greater significance is the much higher variance in $\delta^{13}C$ values of sub-adults compared to adult elephants. This could arise due to two plausible reasons. There could be greater heterogeneity in the foraging behaviour of younger elephants, with individuals having predominantly grazing (on C_4 plants), browsing (on C_3 plants) or mixed feeding preferences. The second plausible reason could be higher collagen turnover rates in younger animals, making the isotopic signature sensitive to changes in isotopic ratios of diet over relatively short-time periods.

Behavioural heterogeneity in feeding among sub-adult elephants is not likely for a number of reasons. Elephant society consists of closely-knit matriarchal family herds, in which sub-adults stay with adult females until death, and males disperse from the family only at the age of puberty (about 15 years) (Douglas-Hamilton and Douglas-Hamilton 1975; Moss 1988; Sukumar 1989). Sub-adults thus follow the adult females in their movements for foraging in different habitat types. The development of behaviour, including foraging, in elephants is a long process involving considerable amount of learning by the juveniles from the adults. From these considerations it is highly unlikely that inter-individual variation in foraging could be very marked in sub-adults but not in adult elephants. It is especially unlikely that certain sub-adults would be consuming only C_4 grasses or C_3 browse for a number of years (which one might wrongly conclude from a too simplistic interpretation of the $\delta^{13}C$ data) when they have access to a wide variety of both plant types in this region.

It is more logical to interpret the difference in variance of $\delta^{13}C$ values between sub-adults and adults as due to high collagen turnover in younger animals combined with a seasonal alternation between C_3 and C_4 plants in the diet. In actively anabolizing, growing animals it could be expected that collagen resorption and synthesis would be higher than in those which have stopped growing or show negative metabolism (catabolism). The assumptions about the inert nature of bone collagen inherent in the stable isotope method can be largely traced to studies showing low uptake of ^{14}C (a radioactive isotope), derived from nuclear weapons tests, in the collagen of adult humans aged over 70 years (Libby et al. 1964; Stenhouse and Baxter 1977). As stated by Libby et al. (1964) such an assumption would certainly not be valid for younger ages because bone growth involves both destruction of much of the original collagen fibre and synthesis of new fibre. There is in fact experimental evidence that collagen resorption could be considerable (Woessner 1968).

Elephants continue to gain in height and in weight beyond puberty, perhaps even with a slightly accelerated secondary growth similar to humans, attaining their asymptotic height/weight after 25 years (Laws et al. 1975; Sukumar et al. 1988). They also alternate between a predominantly C_4 diet during the wet season and a C_3 diet during the dry season (Fig. 2). The diet of individuals may seasonally shift to even greater dependence on C_3 or C_4 plants than that indicated in Fig. 2 which gives only average values for the populations. For some elephant herds, the diet might be up to 90% C_4 type during the wet season in tall grass habitat and 90% C_3 type during the dry season in short grass habitat (personal observations). In growing animals with high collagen turnover this feeding behaviour could shift isotope ratios sufficiently to obscure the true nature of the overall diet. Given a certain degree of inter-individual variation, animals sampled after a near-monotonous diet of one plant type (C_3 for e.g.) for a particular time period (say, a few months in this case) could show extreme $\delta^{13}C$ values (e.g. indicating C_3 diet). On the other hand, the isotopic ratio would fluctuate much less in adults which have stopped growth.

What are the implications of these results for isotope studies of diet? For determining dietary habits, stable isotope ratios have to be interpreted with caution if the species is a mixed feeder in which nutritive differences in dietary components and seasonal effects are likely to be present. This would include studies of seasonal migrations between coastal and inland food resources, determining quantitative intake of C_3/C_4 plants or leguminous/non-leguminous plants, food niche separation in an animal community and so on. An omnivore feeding on protein-rich meat and protein-poor plants for instance could show an isotopic signature heavily biased in favour of the meat source. While it can be assumed that the isotope ratio of collagen in an adult provides an integrated record of diet over a sufficiently long time period (the exact time scale depending on longevity and metabolic rate of the species), the same may not be true of younger animals. We would therefore suggest that the age of the individual animal sampled is at least approximately determined before definite conclusions are drawn about its feeding ecology. If the age cannot be determined, as may be the case with much

fossil material, a large sample size would be necessary to reduce or eliminate the chances of sampling only sub-adult individuals which may give spurious results. Our results have particular relevance to paleodietary studies of humans, a species with similar life-history characteristics (age at sexual maturity, cessation of growth and longevity) to elephants.

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