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Stable Carbon and Nitrogen Isotope Ratios of Bone Collagen: Variations Within Individuals, Between Sexes, and Within Populations Raised on Monotonous Diets

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The stable carbon and nitrogen isotope ratios of collagen of seven bones from each of three rabbits raised on a monotonous diet, and of two bones from each of eight female and seven male mink raised on another monotonous diet, were determined. The ranges of $\delta^{13}C$ values and $\delta^{15}N$ values were 0·5‰ and 0·6‰ for the rabbit bones and 1·0‰ and 1·4‰ for the mink bones. Uncertainties in the $\delta^{13}C$ and $\delta^{15}N$ values for prehistoric human diets estimated from the isotopic composition of collagen from the small numbers of bones which are typically available for analysis, and thus likely to be of the order of ±1‰.

Keywords: DIETARY RECONSTRUCTION, BONE COLLAGEN CARBON-13/CARBON-12 AND NITROGEN-15/NITROGEN-14 RATIOS, NATURAL VARIABILITY, MASS SPECTROMETRY.

Introduction

The stable carbon and nitrogen isotope ratios of bone collagen reflect the corresponding isotopic composition in the diet (van der Merwe & Vogel, 1977; Burleigh & Brothwell, 1978; DeNiro & Epstein, 1978, 1981; Bender *et al.*, 1981). It is thus possible to reconstruct aspects of the diets of recent as well as prehistoric humans when their potential food sources had different $^{13}C/^{12}C$ and/or $^{15}N/^{14}N$ ratios.

Archaeologists interested in applying the isotopic method of dietary analysis to human skeletal material typically are attempting to reconstruct the subsistence patterns of whole populations (e.g. van der Merwe & Vogel, 1977; Bender *et al.*, 1981; DeNiro & Epstein, 1981; Tauber, 1981; Chisolm *et al.*, 1982). Their efforts are complicated by three types of sampling problems that are inherent to many archaeological situations. First, the number of bones available for analysis is often small. Second, the same bone is usually not available for each skeleton that was excavated. Third, bones from both sexes are usually excavated. In order to use bone collagen isotope ratios to reconstruct the diet of a population under these circumstances, it is necessary to assume, (1) that the isotopic composition of bone collagen from an individual does not differ significantly from the

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mean value that would be obtained if bones from many individuals in the population could be sampled, (2) that the isotopic ratios of different bones from an individual are the same, and (3) that the isotopic ratios of bone collagen from males and females feeding on the same diet are identical. The validity of these assumptions cannot be determined from previous work on the isotopic composition of bone collagen from individuals raised on monotonous diets (DeNiro & Epstein, 1978, 1981; Bender *et al.*, 1981) because these studies involved analysis of collagen samples prepared from aggregates of several or many bones of only a few individuals.

We present here the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of collagen extracted from the humeri and femora of eight female and seven male mink raised on a monotonous diet and from seven bones of each of three rabbits who also ate a monotonous diet. We use the results to estimate the variability of collagen isotope ratios from different bones of an individual or from the same bone of different individuals who ate the same diet. Finally, we use our estimate of the variability of bone collagen isotope ratios within and between different individuals of a population, to assess the uncertainty of reconstructions of the diets of prehistoric human populations based on the isotopic composition of collagen from small numbers of bones.

Materials and Methods

Mink were fed Purina Mink Chow and chicken parts; the rabbits were fed Purina Rabbit Chow. Strontium concentrations in these bones have been reported previously (Schoeninger, 1981).

Collagen was prepared from bone powdered to less than 0·71 mm as described previously (DeNiro & Epstein, 1981). Collagen concentrations are given as % dry weight of the bone powder.

Collagen samples were combusted using a modified version of the Stump and Frazer (1973) method (Northfelt *et al.*, 1981). The resulting CO_2 and N_2 were separated and purified in a vacuum system by cryogenic distillation. The volumes of CO_2 and N_2 gas samples were determined manometrically prior to determination of their isotope ratios by mass spectrometry. The atomic carbon to nitrogen ratios of the collagen samples are given as C/N values. The collagen isotope ratios are reported in the δ notation, where

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

$$\delta^{15}N = \left[\frac{(^{15}N/^{14}N)_{sample}}{(^{15}N/^{14}N)_{standard}} - 1 \right] \times 1000‰$$

The standard for $\delta^{13}C$ measurements is the Peedee belemnite (PDB) carbonate, while that for $\delta^{15}N$ measurements is atmospheric (AIR) nitrogen.

The means and standard deviations (1 S.D. values) for 27 analyses of a thiourea standard were $-23{\cdot}1 \pm 0{\cdot}3‰$ for $\delta^{13}C$ values, $-1{\cdot}1 \pm 0{\cdot}2‰$ for $\delta^{15}N$ values, and $0{\cdot}5 \pm 0{\cdot}0$ for atomic C/N ratios (theoretical C/N ratio is 0·5). Collagen samples prepared from two aliquots of bone powder from each of twelve samples from this and another study (Schoeninger *et al.*, 1983) were analysed. The means and standard deviations (1 S.D. values) of the differences between the twelve pairs of analyses were $1{\cdot}3 \pm 1{\cdot}6\%$ for

collagen concentrations, 0·2±0·2 for atomic C/N ratios, 0·1±0·2‰ for $\delta^{13}C$ values, and 0·2±0·3‰ for $\delta^{15}N$ values.

Results

The results of analysis of individual bones from mink and rabbit are given in Figure 1. Statistical treatment of the data is presented in Table 1.

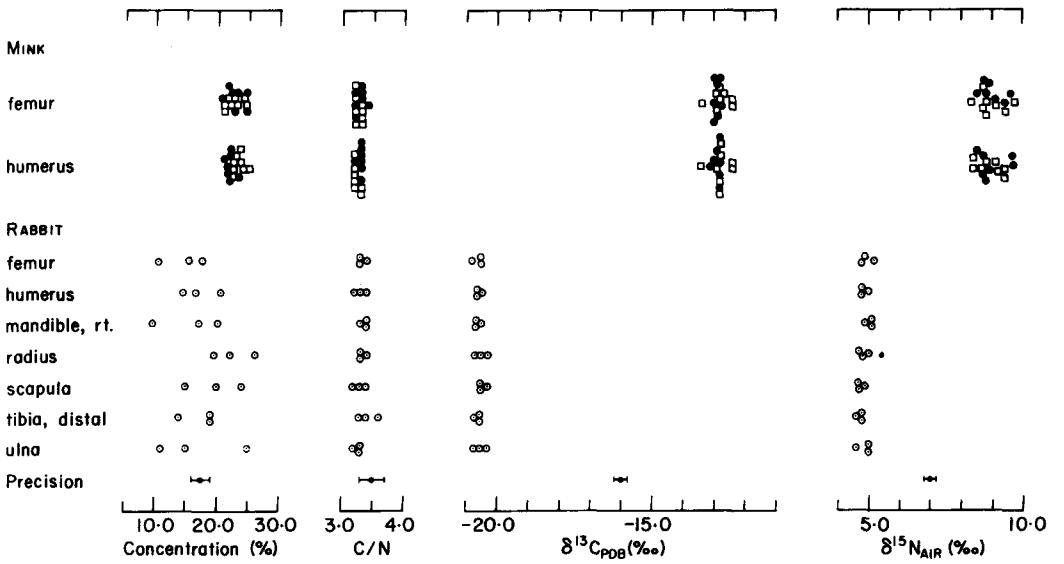

Figure 1. Concentrations, atomic carbon to nitrogen ratios, $\delta^{13}C$ values, and $\delta^{15}N$ values of collagen extracted from the humerus and the femur of each of 15 mink raised on a monotonous diet and from the seven indicated bones of each of three rabbits raised on another monotonous diet. □, Female mink; ●, male mink. The sexes of the three rabbits were not determined.

Analysis of the thirty bones from fifteen mink indicates that the variation in the isotopic composition of bone collagen from different individuals who ate the same diet is small. The largest difference between the mean value of the thirty analyses and the value for a single sample was 0·6‰ for $\delta^{13}C$ values and 0·7‰ for $\delta^{15}N$ values. The mean isotopic composition of collagen extracted from the bones of male and female mink did not differ significantly from one another for either carbon or nitrogen (Student's *t*-tests

Table 1. Means and standard deviations (1 S.D. values) of concentrations, atomic carbon to nitrogen ratios, $\delta^{13}C$ values, and $\delta^{15}N$ values for collagen extracted from the indicated bones of mink and rabbit raised on different monotonous diets

Sample	n	Concentration (%)	C/N	$\delta^{13}C_{PDB}$(‰)	$\delta^{15}N_{AIR}$(‰)
All mink bones	30	22·6±1·2	3·3±·01	−12·8±0·2	+9·0±0·4
Female mink bones	16	22·8±1·2	3·2±0·1	−12·8±0·3	+8·9±0·4
Male mink bones	14	22·3±1·2	3·3±0·1	−12·9±0·1	+9·0±0·4
Mink humeri	15	22·6±1·0	3·3±0·1	−12·8±0·2	+9·0±0·4
Mink femora	15	22·5±1·4	3·3±0·1	−12·8±0·2	+9·0±0·4
All rabbit bones	21	17·7±4·6	3·3±0·1	−20·5±0·1	+4·9±0·2

at $P=0{\cdot}5$ level), suggesting that there is no difference in the isotopic relationship between collagen and diet for the two sexes. The mean isotopic composition of collagen extracted from mink humeri and femora did not differ significantly from one another for either carbon or nitrogen (Student's t-tests at $P=0{\cdot}05$ level), suggesting that differences in the isotopic composition of collagen extracted from different bones of an individual are small. The isotopic ratios obtained for collagen from different bones in rabbits were also consistent with this conclusion. The largest difference between the mean isotopic composition of collagen from all the rabbit samples and that of collagen from a single bone was 0·3‰ for $\delta^{13}C$ values and 0·3‰ for $\delta^{15}N$ values.

Discussion

The results of this study indicate that there are only small variations in the $\delta^{13}C$ and $\delta^{15}N$ values of collagen from different bones of an individual or of different individuals who ate the same diet, and that the differences between the isotope ratios of bone collagen from males and females that ate the same diet are negligible. The variations we observed here, on the order of 1‰ or less, are small when compared to the variations in the isotopic composition of bone collagen that result from eating different amounts of C_3 and C_4 plants, legumes and non-legumes, or terrestrial and aquatic organisms. For example, C_3 and C_4 plants differ by 10–15‰ in $\delta^{13}C$ values, while the $\delta^{13}C$ values of aquatic and terrestrial food sources differ by 7–10‰ (van der Merwe & Vogel, 1977; DeNiro & Epstein, 1978; Tauber, 1981; Chisolm *et al.*, 1982; Schoeninger *et al.*, 1983). Similarly, legumes and non-legumes differ in $\delta^{15}N$ values by 6–10‰, while the $\delta^{15}N$ values of aquatic and terrestrial food sources differ by 5–15‰ (DeNiro & Epstein, 1981; Schoeninger *et al.*, 1983). Small sample sizes and incomplete sampling of bones from prehistoric human populations, problems which are inherent to archaeological samples, are thus not likely to contribute significantly to the uncertainty of dietary reconstruction based on isotope ratios of bone collagen in cases in which all members of the population consumed a similar diet. The data presented here indicate that the $\delta^{13}C$ and $\delta^{15}N$ values of collagen from a single bone will lie within about 1‰ of the isotopic ratios that would be obtained from the analysis of a larger sample of bones either from the individual or from other individuals of the same population who ate the same diet.

Archaeological evidence suggests that individuals who lived in the Tehuacan Valley of Mexico during the Venta Salada phase all had the same diet (MacNeish, 1967). The $\delta^{13}C$ and $\delta^{15}N$ values of bone collagen from eight individuals from this period fall within 1‰ ranges (DeNiro & Epstein, 1981). Ranges in the $\delta^{13}C$ values of human bone collagen from individuals of the same provenance and social status have generally been of this magnitude (van der Merwe & Vogel, 1977; Bender *et al.*, 1981; Tauber, 1981; Chisolm *et al.*, 1982). The previously published data are thus consistent with the conclusions of this study.

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