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Effect of Heating on the Stable Carbon and Nitrogen Isotope Ratios of Bone Collagen

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The effects of heat on the stable carbon and nitrogen isotopic ratios of collagen in bone were studied. Boiling or roasting did not change the $\delta^{13}C$ and $\delta^{15}N$ values by more than 1‰. More extreme heating, such as might occur if bones were burned or a body cremated, shifted $\delta^{13}C$ and $\delta^{15}N$ values by as much as 5 and 4‰ respectively. These large shifts were accompanied by changes in the atomic carbon-to-nitrogen ratios of the collagen. These results indicate that collagen samples extracted from prehistoric bones which display anomalous atomic carbon-to-nitrogen ratios may have been subjected to heating extreme enough to have altered their $^{13}C/^{12}C$ and/or $^{15}N/^{14}N$ ratios and therefore should not be used for dietary reconstruction.

Keywords: DIETARY RECONSTRUCTION, HEATING EFFECTS, COOKING, CREMATION, BONE BURNING, BONE COLLAGEN, CARBON-13/CARBON-12 AND NITROGEN-15/NITROGEN-14 RATIOS, MASS SPECTROMETRY

Introduction

The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of bone collagen reflect the corresponding isotope ratios of the diet (van der Merwe & Vogel, 1977; Burleigh & Brothwell, 1978; DeNiro & Epstein, 1978, 1981; Bender *et al.*, 1981; van der Merwe, 1982). It is thus possible to reconstruct the relative amounts of different types of food an animal ate if the foods had characteristically different $^{13}C/^{12}C$ and/or $^{15}N/^{14}N$ ratios. Archaeologists have used the isotopic method of dietary analysis to reconstruct the relative amounts of maize, legumes, or marine foods eaten by prehistoric human and domesticated animal populations (e.g. van der Merwe & Vogel, 1977; Burleigh & Brothwell, 1978; DeNiro & Epstein, 1981; Tauber, 1981; Chisholm *et al.*, 1982; Schoeninger *et al.*, 1983).

To date, none of the studies in which the isotopic method of dietary reconstruction was employed has involved analysis of collagen from bones that were known to have

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been heated. However, in many situations, archaeologists recover faunal or human bones that have been heated during cooking, cremation or under other circumstances (such as burning of midden areas). In order to reconstruct diet from $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of collagen from such bones, it is necessary to understand the effect of heat on bone collagen isotope ratios. It is possible that heating causes these ratios to change. For example, boiling a bone imbedded in a piece of meat might solubilize a fraction of collagen whose isotope ratios differ from that of collagen in the fresh bone. Similarly, cremation could result in combustion of a fraction of bone collagen whose isotope ratios differ from those of collagen in fresh bone. There is no published information that would permit estimation of the magnitude of isotopic changes which might occur during heating of bone.

We present here the results of studies in which $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of collagen from bones that were heated by various methods were compared with those of collagen from control (unheated) bones. Our results indicate that changes in the $\delta^{13}C$ or $\delta^{15}N$ values of bone collagen caused by mild forms of heating, such as boiling or roasting, will not produce large errors in dietary reconstruction, but that more extreme forms of heating, such as might occur during burning of discarded bones or cremation, can shift these isotope ratios by amounts large enough to render them useless for dietary reconstruction. Fortunately, such large isotopic shifts are accompanied by changes in the elemental composition of the collagen, so that it should be possible to identify collagen from prehistoric bone whose isotopic composition should not be used to estimate prehistoric diet.

Materials and Methods

We performed three experiments in which the carbon and nitrogen isotope ratios of collagen from heated and control (unheated) bones were compared. The bones we used were obtained from freshly killed animals. Most of the attached flesh was stripped from the bones by dermestid beetles. The remnants of flesh were removed mechanically.

In the first experiment, single bones from two cows, *Bos taurus*, and from one deer, *Odocoileus virginianus*, one sheep, *Ovis aries*, and one pig, *Sus scrofa*, were divided into three portions. Ribs from three llamas, *Lama pacos*, raised on the same ranch and three vertebrae from a single turkey, *Melagris gallopavo*, were also used. For each of the seven sets of bones, one piece served as the control. A second piece was boiled in distilled water for 1 h. The third piece was wrapped in aluminium foil and inserted into a piece of cow, *Bos taurus*, flesh, which was then roasted over charcoal for 2 h. The foil served to prevent possible exchange between the cow flesh and the bones of the various animals. Because of the length of the heating period, the foil did not prevent the bones from reaching the same temperatures as bones unprotected by foil would have reached. In the second experiment, whole tali from goats, *Capra* sp., were heated at different temperatures for 3 h in a muffle furnace. In the third experiment, several pig, *Sus scrofa*, femora were powdered to less than 0·71 mm and homogenized by mixing; samples of the powder were then heated at 200°C in a muffle furnace for different periods of time. The temperatures and periods of heating in the second and third experiments were chosen to simulate a range of conditions that bones might have experienced during burning or cremation by prehistoric humans (Shipman *et al.*, in press).

Intact bones from the first and second experiments were powdered to less than 0·71 mm. Collagen was prepared from bone powder, combusted, and the amounts and isotopic composition of the resulting CO_2 and N_2 determined as described elsewhere (DeNiro & Epstein, 1981; Schoeninger & DeNiro, 1984).

Collagen concentrations are reported as percentages of dry bone weight. Collagen elemental compositions are given as atomic carbon-to-nitrogen ratios. Carbon and nitrogen isotope ratios are expressed as δ values:

$$\delta = \left[\frac{R_{\text{Sample}}}{R_{\text{Standard}}} - 1 \right] \times 1000‰$$

where $R = {}^{13}C/{}^{12}C$ and the standard is the Peedee belemnite (PDB) carbonate for $\delta^{13}C$ values and $R = {}^{15}N/{}^{14}N$ and the standard is atmospheric (AIR) nitrogen for $\delta^{15}N$ values.

The mean and S.D. (1σ 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 prepared from 12 pairs of samples from this and related studies (DeNiro & Schoeninger, 1983; Schoeninger *et al.*, 1983; Schoeninger & DeNiro, 1984) was analyzed. The means and S.D. (1σ values) of the differences between the 12 pairs of analyses were $1{\cdot}3 \pm 1{\cdot}6\%$ for collagen concentrations, $0{\cdot}2 \pm 0{\cdot}2$ for atomic C/N ratios, $0{\cdot}1 \pm 0{\cdot}2‰$ for $\delta^{13}C$ values, and $0{\cdot}2 \pm 0{\cdot}3‰$ for $\delta^{15}N$ values.

Results

The effects of boiling and roasting on collagen concentration and on concentration and isotopic composition of carbon and nitrogen in collagen for bones from six species are shown in Figure 1. Neither of these heating processes reduced the collagen concentrations or changed the atomic C/N ratios of the collagen. The differences in the $\delta^{13}C$ values between boiled and control samples and between the roasted and control samples averaged $-0{\cdot}1 \pm 0{\cdot}5‰$ and $-0{\cdot}4 \pm 0{\cdot}3‰$ (mean $\pm 1\sigma$ value) respectively. The corresponding differences in the $\delta^{15}N$ values were $-0{\cdot}5 \pm 0{\cdot}7‰$ for boiled samples and $-0{\cdot}4 \pm 0{\cdot}6‰$ for roasted samples. The $\delta^{13}C$ and $\delta^{15}N$ values of all boiled or roasted samples were within about 1‰ of those of the control samples except for the llama bones, for which the $\delta^{15}N$ values of the boiled and roasted samples were 1·7‰ and 1·5‰ more negative than the value of the control.

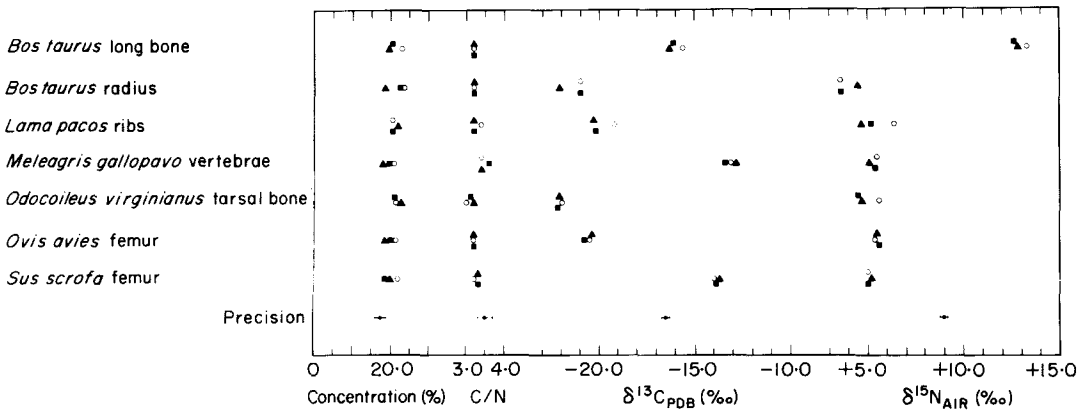

Figure 1. Effect of heating on bone collagen concentration, atomic carbon-to-nitrogen ratio, and carbon and nitrogen isotopic composition. Intact bones or bone fragments from the indicated species were boiled for 1 h or roasted inside pieces of meat for 2 h. ○, Control; ▲, boiled; ■, roasted.

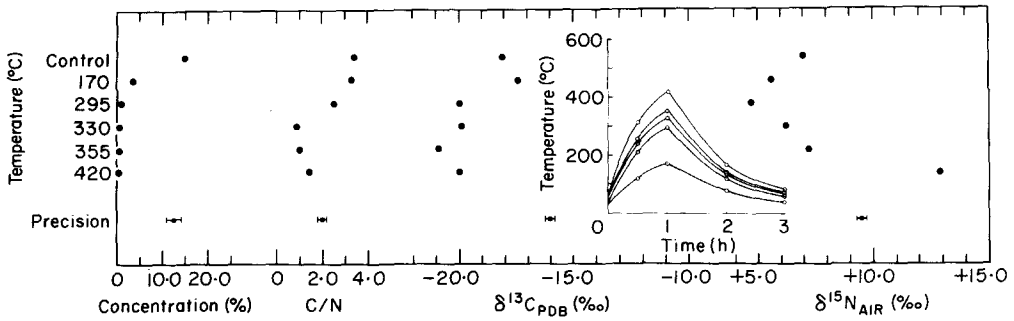

Figure 2. Effect of heating on bone collagen concentration, atomic carbon-to-nitrogen ratio, and carbon and nitrogen isotopic composition. Individual intact tali from goats selected from one herd were heated in a muffle furnace for 3 h at different temperatures, with the maximum temperature attained indicated. The actual heating curves for the individual tali are shown in the inset.

The effects of heating more extreme than boiling or roasting, such as might occur if a bone were discarded in a fire or burned during cremation, were studied by heating intact goat tali to temperatures up to 420°C in a muffle furnace. The results of this experiment are shown in Figure 2. The collagen concentrations of all the heated samples are less than those of the unheated sample. The atomic C/N ratio of the sample heated to the lowest temperature (170°C) is not different from that of the unheated sample; its $\delta^{13}C$ and $\delta^{15}N$ values are within about 1·5‰ of those of the unheated sample. In contrast, the atomic C/N ratios and some of the isotopic ratios of collagen from bones heated to higher temperatures (295°C and higher) show large deviations from the values obtained for the unheated control sample. However, since each of the collagen samples analyzed in this experiment was prepared from the talus of a different goat, some of the variation we observed may have been due to differences in bone collagen isotope ratios among different individuals (DeNiro & Schoeninger, 1983).

In order to circumvent this complication, we powdered a number of pig femora, homogenized the powder by mixing, and did another heating experiment using the muffle furnace. The results are shown in Figure 3. Heating at 200°C for increased periods of time led to decreased collagen concentrations, although the concentrations eventually reached a constant value of about 1 weight per cent. The atomic C/N ratios of the collagen sample were unchanged by heating of up to 4 h, then showed increased values for samples heated for 6 or more hours. Collagen samples with C/N ratios that were not different from that of collagen prepared from the unheated bone had $\delta^{13}C$ and $\delta^{15}N$ values that were within 1·2‰ of the values of the control sample. In contrast, the $\delta^{13}C$ and $\delta^{15}N$ values of collagen whose C/N ratios were higher than that prepared from the unheated bone differed from the isotope ratios of the control collagen by as much as 5 and 4‰ respectively.

Discussion

The results of this study indicate that the $\delta^{13}C$ and $\delta^{15}N$ values of bone collagen can be shifted when bones are heated. The magnitude of the shifts depend on the type and duration of the heating.

Boiling or roasting caused shifts of no more than about 1‰ in bone collagen $\delta^{13}C$ and $\delta^{15}N$ values, except in the case of the llama ribs. The llama ribs came from

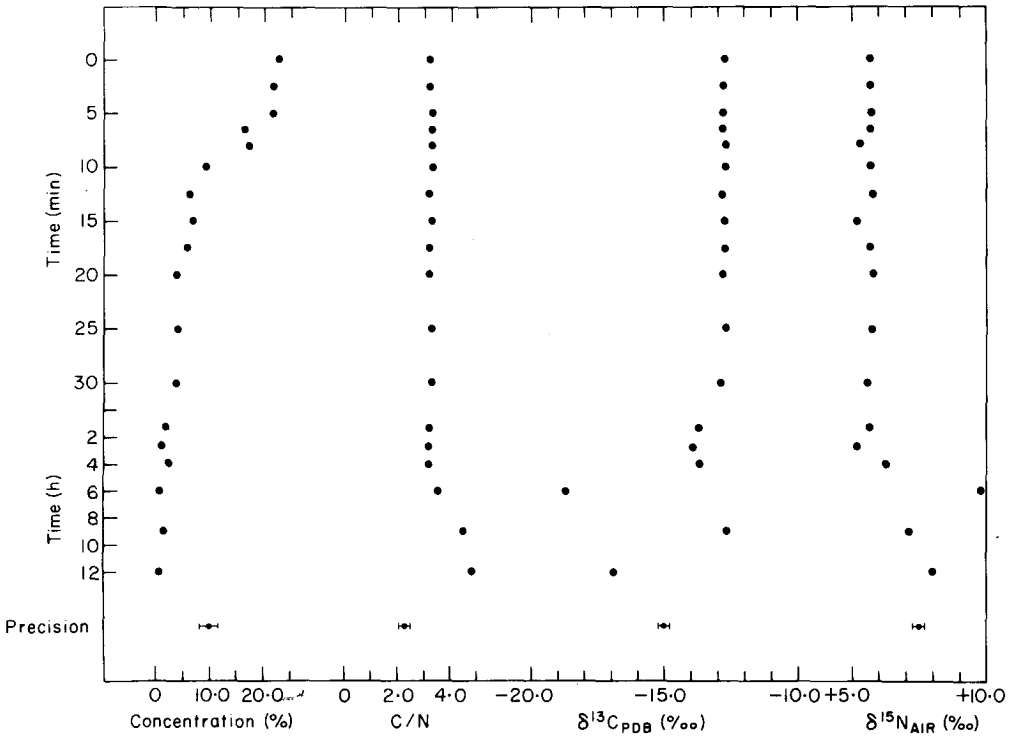

Figure 3. Effect of heating on bone collagen concentration, atomic carbon-to-nitrogen ratio, and carbon and nitrogen isotopic composition. Powdered bone from pig femora was homogenized, then heated in a muffle furnace at 200°C for the indicated periods.

different individuals. We have analyzed ribs from five other llamas (Schoeninger & DeNiro, 1984) that were raised along with the llamas whose bones were analysed in this study. The ranges in the isotope ratios of bone collagen for the six unheated ribs in the two studies, −20·6 to −19·2‰ for $\delta^{13}C$ values and +4·1 to −6·6‰ for $\delta^{15}N$ values, span the values we observed for the control, roasted, and boiled llama bones in this study. We have observed about this much variability in collagen $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of bones from different mink and rabbit raised on the isotopically monotonous diets (DeNiro & Schoeninger, 1983). Thus, the observation that boiling and roasting apparently caused larger isotopic shifts with the llama bones than with the bones from the other species we examined is probably due to the fact that only in this case did we use bones from different individuals. We conclude that the maximum shift in bone collagen $\delta^{13}C$ and $\delta^{15}N$ values caused by boiling or roasting meat is no more than about 1‰.

More extreme heating of bones in a muffle furnace, which we used to simulate the heating that would occur during cremation or burning of bones, caused shifts in the $\delta^{13}C$ and $\delta^{15}N$ values of as much as 5 and 4‰ respectively, although exposure of bone either at lower temperatures (Figure 2) or for shorter periods (Figure 3) produced smaller shifts.

The magnitude of the isotopic shifts we observed in this study must be viewed in the context of their significance for dietary reconstruction based on bone collagen isotope

ratios. In other words, if heating a bone changes the $\delta^{13}C$ or $\delta^{15}N$ value of its collagen, how much error will this isotopic shift produce in a dietary reconstruction based on the shifted isotopic ratios? The range for bone collagen $\delta^{13}C$ values is about 15‰, which covers the span from individuals eating only C_3 plants to those eating only C_4 plants (van der Merwe & Vogel, 1977; DeNiro & Epstein, 1978; van der Merwe, 1982). Similarly, the range for bone collagen $\delta^{15}N$ values is also about 15‰, which covers the span of individuals eating only marine foods to those eating only terrestrial ones (Schoeninger *et al.*, 1983; Schoeninger & DeNiro, 1984). In these cases, shifts in bone collagen $\delta^{13}C$ and $\delta^{15}N$ values of 1‰ produced by cooking would cause errors in diet reconstruction of about 7%, whereas isotopic shifts of 5‰, produced by more extreme forms of heating, would cause the diet estimates to be in error by about 33%.

Bone collagen isotope ratios have also been used to reconstruct aspects of diet in which the two extremes of feeding behavior produce smaller differences between the bone collagen $\delta^{13}C$ or $\delta^{15}N$ values. For example, the difference between the bone collagen $\delta^{13}C$ values of agriculturalists subsisting only on C_3 plants and fisher-gatherers subsisting only on marine food sources is about 7‰ (Tauber, 1981; Chisholm *et al.*, 1982; Schoeninger *et al.*, 1983). Similarly, the difference between the bone collagen $\delta^{15}N$ values of agriculturalists eating only legumes and those eating only non-legumes is expected to be about 7‰ (DeNiro & Epstein, 1981). In cases in which these aspects of diet were being reconstructed from bone collagen $\delta^{13}C$ or $\delta^{15}N$ values, the errors caused by isotopic shifts occurring during heating of bones would be about twice as large as those occurring in the cases discussed in the preceding paragraph.

We conclude from the results of this study and the foregoing discussion that possible shifts in bone collagen $\delta^{13}C$ and $\delta^{15}N$ values caused by cooking are not likely to cause large errors in the reconstruction of diet estimated from the bone collagen isotope ratios. On the other hand, more extreme forms of heating, such as would occur during cremation or burning of bone, can shift the bone collagen isotope ratios sufficiently to lead to large errors in the reconstruction of diet based on the shifted isotope values.

In light of this conclusion, the prehistorian attempting to use bone collagen $\delta^{13}C$ and $\delta^{15}N$ values for dietary analysis must avoid analyzing collagen prepared from bones which have undergone severe heating. In this regard, we observed that two other properties of collagen, namely dry weight concentration and atomic C/N ratio, changed along with the $\delta^{13}C$ and $\delta^{15}N$ values when the bones were subjected to extreme heat (Figures 2 and 3). Thus, changes in either of these parameters might be used to identify collagen from bones that had undergone extreme heating. However, collagen concentrations often decrease after bones are buried (e.g. DeNiro & Epstein, 1981) and thus low collagen concentrations do not uniquely characterize prehistoric bones that had been heated before or after burial. On the other hand, atomic C/N ratios of collagen fall within a narrow range both for recent bones and for prehistoric bones for which there is no evidence of heating. The atomic C/N ratios of collagen we have isolated from more than 150 fresh, unheated bones representing 70 extant species, from 39 recent human bones, and from 40 prehistoric human bones for which there is no archaeological evidence of heating, average $3{\cdot}1 \pm 0{\cdot}1$ (1σ value). All but one of these collagen samples had atomic C/N ratios that fell in the range between 2·9 and 3·4 (DeNiro & Schoeninger, 1983; Schoeninger *et al.*, 1983; Schoeninger & DeNiro, 1984; this study). Thus, we propose that if the atomic carbon-to-nitrogen ratio of collagen isolated from a bone is outside the range 2·9–3·4, the $\delta^{13}C$ and $\delta^{15}N$ values of the collagen should not be used to reconstruct diet because it is possible that the isotope ratios were shifted by heating. This restriction would eliminate all the bones

in this study whose collagen* $\delta^{13}C$ and $\delta^{15}N$ values were shifted by more than 1‰ during heating.

The results reported here indicate that radiocarbon dates on collagen from bones that have been heated may be in error unless corrections for heat-induced changes in ^{14}C content, based on measurements of $\delta^{13}C$ values, are applied.

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*A comment on the use of the term collagen in this context is in order. Throughout this study we used an operational definition of collagen, namely that fraction which is solubilized by treatment with 0·001 N HCl at 90°C for 10 h after bone power is treated with 1 N HCl for 20 min, washed to neutrality, treated with 0·125 N NaOH for 20 h, and washed to neutrality (DeNiro & Epstein, 1981). Clearly the material we isolated from some of the bones that were heated to high temperatures or for long periods in the muffle furnace, having C/N ratios above 3·4 or below 2·9, is not collagen in the true sense but some modified material produced by the thermal alteration of collagen. For convenience sake, we refer to this material as collagen, in spite of the fact that it is not.