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Carbon isotope ratios of apatite from fossil bone cannot be used to reconstruct diets of animals

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The reconstruction of animals' diets from measurements of stable isotope levels in fossils relies on the fact that the $^{13}C/^{12}C$ ratio of animal carbon reflects the $^{13}C/^{12}C$ ratio of dietary carbon[1,2]. Two phases in fresh bone, collagen and the carbonate occurring in apatite, the predominant bone mineral, have isotopic ratios that are related to the $^{13}C/^{12}C$ ratio of the diet[1]. The isotopic ratios of both phases have been used to study the diets of extant animals[3,4]. Reconstruction of the diets of fossil animals using the isotopic method has been limited to analysis of collagen preserved in bone[5-8]. It has not been possible to use the $^{13}C/^{12}C$ ratios of carbon in the inorganic phase of fossil bone for dietary reconstruction because most fossil bones contain significant amounts of calcium carbonate, deposited after the animal's death, that contribute to the CO_2 evolved from the bone during acid hydrolysis[3,4]. However, Sullivan and Krueger[9] recently presented data which led them to conclude that the $^{13}C/^{12}C$ ratio of CO_2 extracted by acid hydrolysis from the apatite phase of fossil bone records information about an animal's diet. We have now determined the $^{13}C/^{12}C$ ratios of both the apatite phase and the collagen of 24 fossil animal and human bones, and our results indicate that the $^{13}C/^{12}C$ ratio of fossil bone apatite cannot be used for dietary reconstruction.

Sullivan and Krueger[9] treated fossil bone with acetic acid to remove any secondary calcium carbonate that might have been present. They found that the $^{13}C/^{12}C$ ratios of the apatite carbonate fractions, which resisted acetic acid dissolution, were linearly correlated with $^{13}C/^{12}C$ ratios of the collagen for fossil as well as fresh bones. Because the collagen $^{13}C/^{12}C$ ratio is related to the isotopic composition of an animal's diet[1], they concluded that the $^{13}C/^{12}C$ ratios of apatite from fossil bone can be used to reconstruct aspects of the diets of animals that lived in the past.

There is a serious complication with the proposal[9] that the $^{13}C/^{12}C$ ratios of apatite in fossil bone can be used to reconstruct diet. Comparisons of the ^{14}C content of apatite with the ^{14}C content of collagen from the same bone or of other carbon-containing materials (such as charcoal) excavated in conjunction with the bone in question indicate that the apatite carbon can undergo exchange with carbon encountered in the post-mortem environment, either in groundwater or in the atmosphere (see, for example, refs 10–13). These diagenetic isotopic exchange processes can also affect the $^{13}C/^{12}C$ ratios of apatite[4]. To illustrate the magnitude of the stable isotopic shifts that can occur during diagenesis, we present the results of analysis of several suites of fossil bones.

We determined the $^{13}C/^{12}C$ ratios of the apatite and collagen of 6 modern and 24 fossil bones. These data, along with the ages and collection sites of the bones, are given in Table 1. Collagen was extracted from powdered bone as described previously[5] and its $^{13}C/^{12}C$ ratios determined following combustion by a modification of the Stump and Frazer method[14,15]. The $^{13}C/^{12}C$ ratios of the CO_2 liberated from apatite by reaction with anhydrous phosphoric acid were determined on separate aliquots of powdered bone that had been soaked in 50:50 (v:v) glacial acetic acid:water for two days before oxidation of the organic matter using sodium hypochlorite[1]. X-ray diffraction analysis demonstrated that the secondary calcium carbonate present in the fossil bones was removed by the acetic acid treatment.

We present a plot of the apatite $\delta^{13}C$ values against the collagen $\delta^{13}C$ values in Fig. 1. The line in Fig. 1 represents the relationship between collagen and apatite $\delta^{13}C$ values given by Sullivan and Krueger (see Fig. 1 legend). The data points for the six modern bones all lie close to this line. On the other hand, the differences between the observed apatite $\delta^{13}C$ value and that expected from the collagen $\delta^{13}C$ value for the fossil bones, based on the Sullivan-Krueger relationship, range from +4.6‰ to −12.1‰. This observation indicates that exchange processes have shifted the apatite $\delta^{13}C$ values from their original values, towards either more positive or more negative values, by as much as 12‰. Such a shift is significant when one considers that the average difference between the $\delta^{13}C$ values of C_3 and C_4 plants is about 14‰[16], while that between aquatic and most terrestrial food sources is about 7‰[1,8]. Determination of the

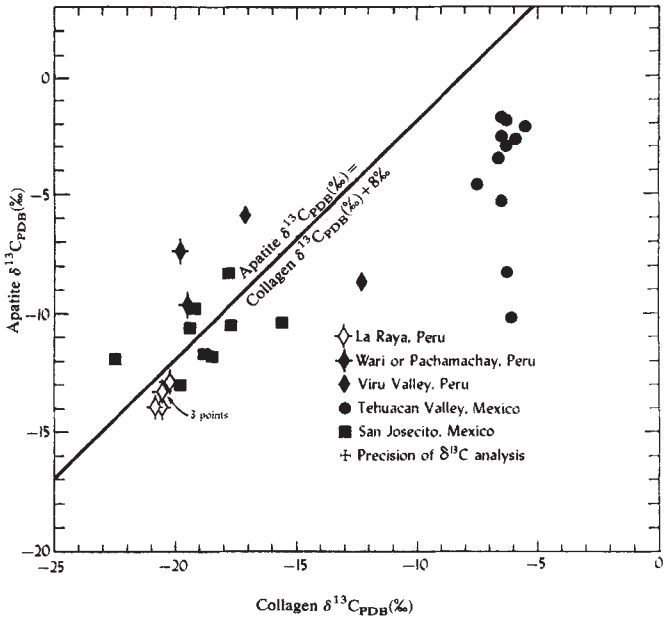

Fig. 1 The relationship between the $\delta^{13}C$ values of the apatite and collagen fractions of modern (open symbols) and fossil (closed symbols) bones analysed for this study. The line indicates the relationship given by Sullivan and Krueger[9]. Linear regression analysis of the data for fossil bones presented here produced the line: apatite $\delta^{13}C = 0.47$ collagen $\delta^{13}C - 1.29‰$ ($r = 0.80$). Sullivan and Krueger[9] indicated that apatite $\delta^{13}C$ = collagen $\delta^{13}C$ + 8‰. Linear regression analysis of their data yielded the line: apatite $\delta^{13}C = 1.07$ collagen $\delta^{13}C + 8.9‰$ ($r = 0.99$). The relationship given by Sullivan and Krueger is used in the discussion in the text. The values for $\delta^{13}C$ were calculated using the PDB belemnite carbonate as standard from the relationship:

$$\delta^{13}C = \left[\frac{(^{13}C/^{12}C)_{Sample}}{(^{13}C/^{12}C)_{Standard}} - 1\right] \times 1{,}000‰$$

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Table 1 Collection location, taxonomic identity, age and $\delta^{13}C_{PDB}$ values for the apatite and collagen fractions of the modern and fossil bones analysed for this study

Location	Species	Age (yr BP)	Apatite $\delta^{13}C_{PDB}$ (‰)	Collagen $\delta^{13}C_{PDB}$ (‰)
La Raya, Peru	*Lama pacos*	0	−13.3	−20.5
	Lama pacos	0	−12.9	−20.2
	Lama pacos	0	−14.0	−20.6
	Lama pacos	0	−14.0	−20.8
	Lama pacos	0	−13.3	−20.5
	Lama pacos	0	−13.3	−20.5
Pachamachay, Peru	*Lama glama*	4,500–3,750	−9.7	−19.5
San Josecito, Mexico	*Canis diris*	~20,000	−10.4	−15.6
	Cervus sp.	~20,000	−10.6	−19.4
	Cervus sp.	~20,000	−9.9	−19.2
	Equus sp.	~20,000	−11.9	−22.7
	Equus sp.	~20,000	−13.0	−19.8
	Felis concolor	~20,000	−11.8	−18.5
	Felis concolor	~20,000	−11.7	−18.8
	Ursus arctos	~20,000	−10.5	−17.7
	Ursus arctos	~20,000	−8.3	−17.8
Tehuacan Valley, Mexico	*Homo sapiens*	1,300–500	−8.3	−6.3
	Homo sapiens	1,300–500	−2.2	−5.5
	Homo sapiens	1,300–500	−2.7	−5.9
	Homo sapiens	1,300–500	−1.9	−6.3
	Homo sapiens	1,300–500	−3.5	−6.6
	Homo sapiens	1,300–500	−3.0	−6.3
	Homo sapiens	1,300–500	−10.2	−6.1
	Homo sapiens	1,300–500	−5.3	−6.5
	Homo sapiens	2,800–2,150	−1.8	−6.5
	Homo sapiens	2,800–2,150	−4.6	−7.5
	Homo sapiens	7,000–5,400	−2.6	−6.5
Viru Valley, Peru	*Homo sapiens*	2,200–1,500	−8.7	−12.3
	Homo sapiens	2,200–1,500	−5.9	−17.1
Wari, Peru	*Lama glama*	1,350–1,160	−7.4	−19.8

The ages given for the bones are generally based on dates obtained for other materials excavated with them and hence provide minimum and maximum limits on the actual ages.

relative amounts of C_3 versus C_4 plants or of aquatic versus terrestrial foods in the diet are the two applications of the isotopic method of dietary analysis that have been made to date[5–8]. Uncertainties in the estimates of diet $\delta^{13}C$ values on the order of 5–10‰, resulting from diagenetic exchange processes, would thus make dietary reconstruction based on fossil bone apatite $\delta^{13}C$ values completely unreliable.

Our treatment of the data reported here, as well as that used by Sullivan and Krueger[9], is based on the assumption that the fossil bone collagen $\delta^{13}C$ values have not been altered by diagenetic processes. This may not be a valid assumption[3–5]. However, some of the apatite $\delta^{13}C$ values presented in Table 1 can be interpreted without resorting to comparisons with the corresponding collagen $\delta^{13}C$ values. Eight of the 11 samples from the Tehuacan Valley come from a single period of the occupation of the site[5]. There is no evidence to suggest differences in status among these individuals[17,18]. Accordingly, it is reasonable to expect that their diets, and consequently the bone isotope ratios, would be similar. The $\delta^{13}C$ values of collagen from these samples agree to within 1‰, which is consistent with this expectation. The $\delta^{15}N$ values of collagen, which have also been shown to be determined by diet, for these same eight samples have a range of only 1‰[5]. Note, however, that the apatite $\delta^{13}C$ values for these samples range from −1.9‰ to −10.2‰. These observations indicate that the apatite carbon in these bones has undergone post-mortem exchange. The large range of $\delta^{13}C$ values suggests that individual bones have exchanged with carbon sources of different $^{13}C/^{12}C$ ratios and/or have undergone different amounts of exchange.

The results presented here demonstrate that bone apatite carbonate undergoes exchange with carbon encountered after the death of the animal and that these diagenetic exchange processes can shift the apatite $\delta^{13}C$ values by at least as much as 12‰. Uncertainties of this magnitude in estimates of diet $\delta^{13}C$ values cannot be tolerated, since differences in the $\delta^{13}C$ values of different foodstuffs whose relative consumption is of interest are only 7–14‰. The apatite carbon in some fossil bones may not have undergone post-mortem exchange. However, until a method is developed to identify such isotopically unaltered apatite, we conclude that $\delta^{13}C$ values of fossil bone apatite cannot be used to reconstruct ancient diets.

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