

A Simple Carbon Isotope Model for Reconstructing Prehistoric Human Diet

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ABSTRACT A compilation of experimental animal data shows that neither $\delta^{13}\text{C}_{\text{collagen}}$ nor $\delta^{13}\text{C}_{\text{apatite}}$ nor $\Delta^{13}\text{C}_{\text{CO-AP}}$ indicate diagnostic reconstructions of diet, diet energy and diet protein. In contrast, plots of $\delta^{13}\text{C}_{\text{collagen}}$ against $\delta^{13}\text{C}_{\text{apatite}}$ provide a model of three regression lines (C_3 , C_4 , and marine diet protein) where position on each line indicates the energy source (C_3 , C_4 , or mixed). Neither body size nor trophic position appears to affect these relationships. Modern free-ranging, terrestrial fauna do not fit the model perhaps because they, unlike the experimental fauna, mainly use fermentation rather than digestion during energy metabolism. Archaeological humans fall as expected based on associated floral and faunal evidence. Foraging people plot at positions expected from associated C_3 fauna and plants. Those from Cahokia plot, as expected, from associated deer, nuts,

and maize whereas people from nearby smaller sites plot in positions consistent with eating more fish. Agriculturists from Ontario and Grasshopper Pueblo plot consistent with dependence on fish by the former and on turkeys by the latter. In Tierra del Fuego, people from interior regions ate more terrestrial fauna, as suggested by ethnohistoric reports, than did people from the coast. In the Southwestern Cape in South Africa individuals late in the sequence have pure C_3 diets whereas ones early in the sequence ate marine protein as suggested by independent archaeological evidence. People on San Nicolas Island depended on C_4 plants in contrast to other islands off California's coast. This simple model provides more detailed and precise dietary information than do individual isotopic measures. *Am J Phys Anthropol* 133:1112–1127, 2007. © 2007 Wiley-Liss, Inc.

The use of stable carbon isotope data from ancient human bones for diet reconstruction has multiplied in physical anthropology within the last few years (Richards et al., 2003; e.g., Lillie et al., 2003; Le Huray and Schutkowski, 2005) thanks, at least in part, to the development of automated analytical technologies. Such applications, however, often invoke assumptions about the predictive power of the isotope data that may not be firmly established. Our goal, in this manuscript, is to clarify that predictive power in order to facilitate more precise reconstructions of ancient human diet.

Nearly 30 years ago, DeNiro and Epstein (1978) demonstrated experimentally that the $\delta^{13}\text{C}$ values in the chitinous exoskeletons, muscle tissue, bone collagen, and bone apatite of animals from insects to mice showed a positive correlation with the $\delta^{13}\text{C}$ values in their diets ($\delta^{13}\text{C}_{\text{diet}}$). Shortly afterwards, Krueger and Sullivan (1984) proposed a model describing these relationships in mammalian bone, based on published data from wild fauna and archaeological humans. They reasoned that the $\delta^{13}\text{C}$ values in bone collagen ($\delta^{13}\text{C}_{\text{collagen}}$) should mostly reflect dietary protein values ($\delta^{13}\text{C}_{\text{diet protein}}$) rather than $\delta^{13}\text{C}_{\text{diet}}$ values because the majority of carbon atoms in collagen should come from dietary protein. Additionally, they reasoned that bone apatite $\delta^{13}\text{C}$ values ($\delta^{13}\text{C}_{\text{apatite}}$) should preferentially reflect the values in the energy portion of diet (i.e., diet carbohydrate, lipid, and protein not used in the animal's own protein synthesis) because bone apatite forms in equilibrium with blood carbonate that is itself a product of energy metabolism. In other words, the $\delta^{13}\text{C}_{\text{collagen}}$ values should most strongly reflect the values in dietary protein, while $\delta^{13}\text{C}_{\text{apatite}}$ values should reflect most strongly the values in dietary energy ($\delta^{13}\text{C}_{\text{diet energy}}$).

The model's publication stimulated several experimental studies designed to both test and extend its predictive power (Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Howland et al., 2003; Jim et al., 2004). Each study, to varying degrees, controlled the $\delta^{13}\text{C}$ values of dietary macronutrients, and varied the proportion of dietary protein, carbohydrates, and lipids. All concluded that the $\delta^{13}\text{C}_{\text{collagen}}$ values vary with $\delta^{13}\text{C}_{\text{diet protein}}$; but contra the original model, they concluded that $\delta^{13}\text{C}_{\text{apatite}}$ values track the $\delta^{13}\text{C}_{\text{diet}}$ better than they track $\delta^{13}\text{C}_{\text{diet energy}}$. They proposed that a linear mixing model best describes the relationship between $\delta^{13}\text{C}_{\text{apatite}}$ values and $\delta^{13}\text{C}_{\text{diet}}$ values, and that protein routing best explains $\delta^{13}\text{C}_{\text{collagen}}$ values. On theoretical grounds, Schwarcz proposed a modification to this model, "in natural human populations living at low protein intake levels...the isotopic composition of bone collagen comes closer to the linear mixing model." (Schwarcz, 2000:208). He reasoned that under conditions of low protein intake, people would be actively synthesizing the carbon backbones of their nonessential amino acids from all avail-

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TABLE 1. Experimental animal data^a

Animal	Diet: protein/energy	Protein: casein (C), plant (P), fish (F)	$\delta^{13}\text{C}$ ‰ diet	$\delta^{13}\text{C}$ ‰ protein	$\delta^{13}\text{C}$ ‰ energy ^b	$\delta^{13}\text{C}$ ‰ apatite	$\delta^{13}\text{C}$ ‰ collagen	Diet no. ^a
Monoisotopic diets								
Pig	C ₃ /C ₃	C	-25.7	-25.5	nr	-13.6	-19.6	8 (Howland et al., 2003)
Mouse	C ₃ /C ₃	P	-25.6	-24.7	nr	-16.8	-21.9	1 (Tieszen and Fagre, 1993)
Rat	C ₃ /C ₃	C	-25.2	-26.3	-25.1	-15.7	-21.4	A (Ambrose and Norr, 1993)
Rat	C ₃ /C ₃	C	-24.9	-24.5	-25.1	-14.5	-19.9	d2a4* (Jim et al., 2004)
Rat	C ₄ /C ₄	C	-12.2	-14.6	-11.7	-3.1	-7.9	d4h** (Jim et al., 2004)
Mouse	C ₄ /C ₄	P	-11.8	-13.6	nr	-3.1	-9.8	8 (Tieszen and Fagre, 1993)
Diets with different $\delta^{13}\text{C}$ values for protein and energy								
Rat	C ₃ /C ₄	P	-14.6	-25.3	-11.4	-5.2	-16.0	F (Ambrose and Norr, 1993)
Rat	C ₃ /C ₄	C	-14.7	-26.3	-11.2	-5.6	-16.9	G (Ambrose and Norr, 1993)
Rat ^c	C ₃ /C ₄	C	-12.1	-26.3	-11.3	-2.9	-13.7	C (Ambrose and Norr, 1993)
Rat ^d	C ₃ /C ₄	C	-22.5	-26.3	-12.5	-13.5	-20.7	E (Ambrose and Norr, 1993)
Rat	C ₄ /C ₃	C	-22.3	-14.6	-25.1	-13.0	-12.3	d5i (Jim et al., 2004)
Rat ^c	C ₄ /C ₃	C	-24.3	-14.3	-25.1	-13.4	-14.7	B (Ambrose and Norr, 1993)
Rat ^d	C ₄ /C ₃	C	-17.1	-14.2	-25.7	-7.7	-9.7	D (Ambrose and Norr, 1993)
Rat	Marine/C ₃	F	-23.3	-17.8	-25.1	-13.4	-14.5	d6j2* (Jim et al., 2004)
Rat	Marine/C ₄	F	-12.9	-17.8	-11.6	-3.5	-9.7	d7k2* (Jim et al., 2004)
Diets with a mixture of C ₃ and C ₄ energy								
Pig	C ₃ /C ₃ and C ₄	C	-24.7	-25.5	nr	-14.2	-20.6	3 (Howland et al., 2003)
Pig	C ₃ /C ₃ and C ₄	C	-20.5	-25.5	nr	-11.8	-18.7	4 (Howland et al., 2003)
Pig	C ₃ /C ₃ and C ₄	C	-18.0	-25.5	nr	-9.0	-16.9	5 (Howland et al., 2003)
Pig	C ₃ /C ₃ and C ₄	C	-15.9	-25.5	nr	-6.3	-15.4	6 (Howland et al., 2003)
Pig	Marine/C ₃ and C ₄	F	-15.5	-18.7	nr	-4.3	-11.5	10 (Howland et al., 2003)
Rat	Marine/C ₃ and C ₄	F	-18.3	-17.8	-18.2	-8.6	-12.2	d8l2** (Jim et al., 2004)

^a Data from: Howland et al. (2003); Tieszen and Fagre (1993) (diet 8 is 92% corn, and, following Tieszen and Fagre (1993:126), we considered the diet to have C₄ energy); Ambrose and Norr (1993); Jim et al. (2004) (* 2 points; ** 3 points).

^b Energy includes carbohydrate and lipid; "nr" refers to diets where the value for total energy was not reported (see text).

^c LP, low protein (5%).

^d HP, high protein (70%).

able dietary carbon sources (carbohydrate, protein and lipid) rather than from protein alone.

In addition to these basic relationships, dietary information is also assumed to reside in the *offset* between $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{apatite}}$ (i.e. $\Delta^{13}\text{C}_{\text{CO-AP}}$). Data from a large study of wild South African fauna appeared to support Kruger and Sullivan's 1984 model that the offset varies according to whether an animal is herbivorous, omnivorous or carnivorous (Lee-Thorp et al., 1989). The authors noted that the regression lines describing the relationships within the groups were not parallel to each other so the offset in carnivores overlapped that of herbivores at the C₃ end, which they suggested was an environmental effect. Yet, in estimating the amount of omnivory in prehistoric human populations there is little consideration of that overlap. Using archaeological data, Harrison and Katzenberg (2003) found that maize agriculturists who ate fauna with a C₃ signature had larger $\Delta^{13}\text{C}_{\text{CO-AP}}$ than did human foragers who ate C₃ plants along with marine fish even though both groups were clearly omnivorous.

Given the wealth of bone isotope data now available, it is time to reassess the original model (Krueger and Sullivan, 1984), the subsequent revision based on experimental studies (Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Howland et al., 2003; Jim et al., 2004), the proposed theoretical modification (Schwarcz, 2000), and the information recorded in $\Delta^{13}\text{C}_{\text{CO-AP}}$. To this end, we compiled $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{apatite}}$ values reported in four feeding studies of experimental pigs and rodents (Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Howland et al., 2003; Jim et al., 2004). Our compilation

suggests that most single measures fall short of expectations for diet reconstruction. It also suggests that the *absolute* value of $\Delta^{13}\text{C}_{\text{CO-AP}}$ is not specific to any particular combination of protein or whole diet, which minimizes its predictive power for diet reconstruction (see also Ambrose and Norr, 1993). Even so, the pattern of relationships between $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{apatite}}$ is consistent across pigs, rats and mice providing a simple model for diet reconstructions of greater accuracy than the other, more traditionally used, single measures.

To test the model, we plotted $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{apatite}}$ data from free-ranging faunal species (Sullivan and Krueger, 1981; Schoeninger and DeNiro, 1982; Nelson et al., 1986; Lee-Thorp et al., 1989; this study), and archaeological humans (Lee-Thorp et al., 1989; Hedman et al., 2002; Ambrose et al., 2003; Yesner et al., 2003) where the diets were independently well-characterized. The results of such testing indicate that the model permits reconstructions of ancient human diet that are more precise than those possible when using only $\delta^{13}\text{C}_{\text{collagen}}$, $\delta^{13}\text{C}_{\text{apatite}}$, or the absolute value of $\Delta^{13}\text{C}_{\text{CO-AP}}$.

MATERIALS AND METHODS

Experimental fauna

We limited the experimental animal data (Table 1) to those where the $\delta^{13}\text{C}$ values of both the animal tissues and their diets were analyzed rather than assumed. Further, we chose only feeding studies that reported $\delta^{13}\text{C}_{\text{diet}}$ protein so that the routing (or nonrouting) of dietary protein could be assessed, which eliminated the classic

TABLE 2. Free-ranging animal data

Animal	Diet: protein/energy	Number of individuals (<i>n</i>)	Mean $\delta^{13}\text{C}_{\text{apatite}}$ (PDB) \pm SD	Mean $\delta^{13}\text{C}_{\text{collagen}}$ (PDB) \pm SD	$\Delta^{13}\text{C}_{\text{CO-AP}}$ (PDB)	Study
Reindeer	C ₃ /C ₃	8	-11.9 \pm 0.9	-20.4 \pm 0.2	8.5	Nelson et al. (1986)
White-tailed deer	C ₃ /C ₃	1	-12.1	-18.9	6.8	Sullivan and Krueger (1981)
Giraffe	C ₃ /C ₃	4	-13.5 \pm 0.2	-20.4 \pm 0.5	6.9	This study; Sullivan and Krueger (1981)
Zebra	C ₄ /C ₄	2	-0.7 \pm 0.2	-9.6 \pm 1.3	10.3	This study; Sullivan and Krueger (1981)
Hippo	C ₄ /C ₄	4	-2.7 \pm 1.4	-9.3 \pm 0.8	6.6	This study; Sullivan and Krueger (1981)
Topi	C ₄ /C ₄	1	1.9	-8.4	10.3	This study
Hartebeest	C ₄ /C ₄	1	0.6	-7.8	8.4	Sullivan and Krueger (1981)
Harp seal	Marine/marine	4	-11.6 \pm 0.7	-15.1 \pm 0.6	3.5	Nelson et al. (1986)
Harbor seal	Marine/marine	4	-12.5 \pm 0.8	-14.9 \pm 0.4	2.4	Nelson et al. (1986)
Cape fur seal	Marine/marine	2	-9.8 \pm 0.5	-11.9 \pm 0.3	2.1	Lee-Thorp et al. (1989)

study by DeNiro and Epstein (1978). Meeting this restriction were most of the values reported by Ambrose and Norr (1993) and Jim et al. (2004) who fed rats, and some of those reported by Tieszen and Fagre (1993) who fed mice and Howland et al. (2003) who fed adult pigs. Pigs, which have large body size, provide a control to assess whether the small mammals used in the other three studies show patterns applicable to humans. We chose only diets that had C₃, C₄, or marine protein rather than a mixture of protein types in order to test whether these three types of protein had different effects on $\delta^{13}\text{C}_{\text{collagen}}$ or $\delta^{13}\text{C}_{\text{apatite}}$. We included two high (70%) and two low (5%) protein diets (HP and LP in Table 1) to test if protein level differentially affected either $\delta^{13}\text{C}_{\text{collagen}}$ or $\delta^{13}\text{C}_{\text{apatite}}$. The remaining diets show a range of protein from 18–20% of total diet, which is considered normal for growth and development. In cases where more than one animal in a single study was fed the same diet, we averaged their $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{apatite}}$ values.

In plotting the data, we follow the protocol of the experimental studies by combining the carbohydrate and lipid portions of the diet into the single category, energy. Protein not used for tissue synthesis also provides energy (Schwarcz, 2000:191); but while the minor contribution of such protein to overall energy probably adds noise to the system, it should not mask patterns except in the two cases where protein comprises 70% of the diet.

We calculated Pearson correlation coefficients between $\delta^{13}\text{C}_{\text{collagen}}$ or $\delta^{13}\text{C}_{\text{apatite}}$, on the one hand, and the $\delta^{13}\text{C}_{\text{diet}}$, $\delta^{13}\text{C}_{\text{diet protein}}$, or $\delta^{13}\text{C}_{\text{diet energy}}$, on the other to assess statistical significance between each pair of variables. We also plotted $\delta^{13}\text{C}_{\text{diet}}$, $\delta^{13}\text{C}_{\text{diet protein}}$, or $\delta^{13}\text{C}_{\text{diet energy}}$ against $\delta^{13}\text{C}_{\text{collagen}}$ or $\delta^{13}\text{C}_{\text{apatite}}$ and calculated the regression equations between pairs of variables to determine goodness of fit. Finally, we plotted $\delta^{13}\text{C}_{\text{apatite}}$ against $\delta^{13}\text{C}_{\text{collagen}}$ to test whether trophic level (milk protein diets versus plant protein diets) or type of protein (C₃ vs. marine versus C₃ proteins) differentially affected $\Delta^{13}\text{C}_{\text{CO-AP}}$ or the position of the data on the plot.

Free-ranging fauna

In selecting the free-ranging fauna (Table 2), by which we refer to nondomesticated and nonprovisioned fauna, we excluded any animal studies that do not report $\delta^{13}\text{C}$ values of bone apatite and collagen (Cerling et al., 2003),

or that do not identify specific animals (Krueger and Sullivan, 1984 and many in Lee-Thorp et al., 1989). Domesticated animals were not included because we could not control for the type of protein since animal feeds can vary significantly. Of the terrestrial animals, we excluded mixed feeders because we could not identify the isotopic signal of protein in such feeders. We included species of browsers (Sullivan and Krueger, 1981; Nelson et al., 1986; this study) and grazers (Sullivan and Krueger 1981; this study) from arid, tropical environments where most grasses utilize the C₄ pathway (Tieszen et al., 1979). Although variation in the $\delta^{13}\text{C}$ values of enamel ($\delta^{13}\text{C}_{\text{enamel}}$) indicates that some grazers are more selective feeders than others (Cerling and Harris, 1999; Cerling et al., 2003), we combined all of them for the purposes of this study. We also plot data from seals (Nelson et al., 1986; Lee-Thorp et al., 1989) even though they are not directly comparable to the experimental animals because the seals consume 100% marine foods. Where more than one individual from a species was available, we plotted the average $\delta^{13}\text{C}$ value for the species.

Archaeological human populations

We applied three criteria in our choice of archaeological populations (Table 3). First, there had to be extensive archaeological evidence (e.g., floral and faunal data) indicating the most likely sources of energy and protein. Second, the archaeological evidence had to indicate that the type of protein was either C₃, C₄, or marine, not a combination. These criteria eliminated several studies where the archaeological record was unclear regarding the type of protein (e.g., Ambrose et al., 1997; Prowse et al., 2004; White et al., 2001). Third, the studies had to report individual data on $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{apatite}}$ in adults. Many excellent isotope studies of archaeological populations were excluded from this study as they report only $\delta^{13}\text{C}_{\text{collagen}}$ values or report average rather than individual values (Walker and DeNiro, 1986; Chisholm and Matson, 1994; Reed, 1994).

Ten sets of archaeological populations meet these criteria. Seven sets have diets in which animal protein is expected to have a C₃ isotope signal from fauna like white tailed deer and squirrels (Olsen, 1982; Katzenberg, 1989), or rabbits (Ezzo, 1993) that feed on herbaceous (C₃) vegetation. These include one from Late Woodland Georgia (Tucker, 2002), three along the Mississippi and Illinois Rivers (Hedman et al., 2002; Ambrose et al.,

TABLE 3. Archaeological human data^a

Population set	$\delta^{13}\text{C}$ ‰ bone apatite (PDB)	$\delta^{13}\text{C}$ ‰ bone collagen (PDB)	Site name	Time period
C₃ Protein/C₃ Energy				
<i>Fisher-hunter-gatherers</i>				
Ontario ^b	-15.1	-20.7	Morrison's Island	2300 BC
Ontario ^b	-11.0	-21.0	Donaldson 1	555 BC
Ontario ^b	-13.4	-21.4	Donaldson 2	AD 5
Ontario ^b	-13.8	-20.2	Donaldson 2	AD 5
Ontario ^b	-16.7	-22.5	Levesconte	AD 175
Ontario ^b	-16.9	-21.9	Levesconte	AD 175
Ontario ^b	-16.6	-23.0	Levesconte	AD 175
Ontario ^b	-16.3	-21.9	Levesconte	AD 175
Ontario ^b	-16.6	-23.7	Levesconte	AD 175
Ontario ^b	-10.6	-18.1	Serpent Mounds	AD 415
Ontario ^b	-15.9	-22.6	Serpent Mounds	AD 400
Ontario ^b	-16.4	-22.1	Serpent Mounds	AD 400
Ontario ^b	-15.7	-22.1	Serpent Mounds	AD 400
Ontario ^b	-16.4	-22.3	Serpent Mounds	AD 400
Ontario ^b	-16.3	-21.7	Serpent Mounds	AD 400
Ontario ^b	-14.0	-19.8	Surma	AD 700
Ontario ^b	-13.6	-19.9	Surma	AD 700
Ontario ^b	-12.9	-20.0	Surma	AD 700
Ontario ^b	-12.8	-20.7	Varden	AD 918
Ontario ^b	-12.0	-21.0	Varden	AD 918
Ontario ^b	-14.4	-20.5	Varden	AD 918
Ontario ^b	-12.5	-21.0	Varden	AD 918
Ontario ^b	-15.9	-21.1	Varden	AD 918
Late Woodland Georgia ^c	-16.3	-21.5	Cannon	AD 1220
Late Woodland Georgia ^c	-15.7	-19.8	Cannon	AD 1220
Late Woodland Georgia ^c	-16.6	-21.2	Cannon	AD 1220
Late Woodland Georgia ^c	-17.1	-21.0	Cannon	AD 1220
C₃ Protein/C₄ Energy				
<i>Mississippi River valley: Floodplain sites</i>				
American Bottom ^d	-13.4	-7.6	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-12.6	-7.3	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-14.8	-7.6	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-11.7	-6.4	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-13.0	-6.6	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-12.8	-5.3	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-13.1	-6.8	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-12.9	-7.4	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-12.9	-6.8	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-13.6	-6.1	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-11.8	-7.1	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-12.5	-6.5	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-14.1	-8.0	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-12.6	-7.3	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-10.9	-6.3	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-10.0	-5.2	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-12.6	-7.4	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-11.5	-6.6	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-11.7	-6.3	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-11.8	-5.6	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-11.3	-5.8	East St. Louis Stone Quarry	AD 1250-1275
American Bottom ^d	-6.9	-14.6	Florence St	AD 1250-1275
American Bottom ^d	-7.2	-12.6	Florence St	AD 1250-1275
American Bottom ^d	-7.0	-12.0	Florence St	AD 1250-1275
American Bottom ^d	-7.5	-13.9	Florence St	AD 1250-1275
American Bottom ^d	-5.7	-11.6	Florence St	AD 1250-1275
American Bottom ^d	-7.0	-12.8	Florence St	AD 1250-1275
American Bottom ^d	-6.2	-12.4	Florence St	AD 1250-1275
American Bottom ^d	-6.9	-12.7	Florence St	AD 1250-1275
American Bottom ^d	-11.8	-5.4	Range	AD 1250-1275
American Bottom ^d	-12.6	-6.6	Range	AD 1250-1275
American Bottom ^d	-12.5	-6.0	Range	AD 1250-1275
American Bottom ^d	-11.8	-5.5	Range	AD 1250-1275
American Bottom ^d	-12.7	-6.6	Range	AD 1250-1275
American Bottom ^d	-15.8	-6.6	Range	AD 1250-1275

(continued)

TABLE 3. (Continued)

Population set	$\delta^{13}\text{C}$ ‰ bone apatite (PDB)	$\delta^{13}\text{C}$ ‰ bone collagen (PDB)	Site name	Time period
<i>Mississippi River valley: Upland Sites</i>				
American Bottom ^d	-16.5	-7.0	Corbin Mounds	AD 1250-1275
American Bottom ^d	-16.4	-9.8	Corbin Mounds	AD 1250-1275
American Bottom ^d	-14.9	-4.4	Corbin Mounds	AD 1250-1275
American Bottom ^d	-13.9	-4.8	Corbin Mounds	AD 1250-1275
American Bottom ^d	-13.7	-5.8	Corbin Mounds	AD 1250-1275
American Bottom ^d	-13.6	-8.5	Corbin Mounds	AD 1250-1275
American Bottom ^d	-13.5	-7.1	Corbin Mounds	AD 1250-1275
American Bottom ^d	-13.3	-6.5	Corbin Mounds	AD 1250-1275
American Bottom ^d	-13.1	-4.9	Corbin Mounds	AD 1250-1275
American Bottom ^d	-12.5	-5.4	Corbin Mounds	AD 1250-1275
American Bottom ^d	-11.7	-5.0	Corbin Mounds	AD 1250-1275
American Bottom ^d	-11.4	-4.6	Corbin Mounds	AD 1250-1275
American Bottom ^d	-12.5	-7.6	Hill Prairie	AD 1250-1275
American Bottom ^d	-11.7	-3.8	Hill Prairie	AD 1250-1275
American Bottom ^d	-14.0	-9.3	Hill Prairie	AD 1250-1275
American Bottom ^d	-19.2	-13.3	Hill Prairie	AD 1250-1275
American Bottom ^d	-21.2	-15.5	Hill Prairie	AD 1250-1275
Illinois River Valley ^d	-16.8	-10.8	Schild A	AD 1200
Illinois River Valley ^d	-16.3	-6.5	Schild A	AD 1200
Illinois River Valley ^d	-14.7	-8.2	Schild A	AD 1200
Illinois River Valley ^d	-14.2	-7.7	Schild A	AD 1200
Illinois River Valley ^d	-13.8	-6.9	Schild A	AD 1200
Illinois River Valley ^d	-11.9	-5.9	Schild A	AD 1200
Illinois River Valley ^d	-21.9	-10.2	Schild A	AD 1200
Illinois River Valley ^d	-16.8	-10.7	Schild A	AD 1200
Illinois River Valley ^d	-14.3	-7.6	Schild A	AD 1200
<i>Mississippi River valley: Cahokia</i>				
Cahokia ^e	-10.5	-20.3	Mound 72 (High Status)	AD 1050-1150
Cahokia ^e	-8.2	-15.8	Mound 72 (High Status)	AD 1050-1150
Cahokia ^e	-11.5	-19.3	Mound 72 (High Status)	AD 1050-1150
Cahokia ^e	-11.5	-19.9	Mound 72 (High Status)	AD 1050-1150
Cahokia ^e	-3.1	-17.5	Mound 72 (Low Status)	AD 1050-1150
Cahokia ^e	-5.9	-18.1	Mound 72 (Low Status)	AD 1050-1150
Cahokia ^e	-6.1	-19.9	Mound 72 (Low Status)	AD 1050-1150
Cahokia ^e	-5.3	-18.7	Mound 72 (Low Status)	AD 1050-1150
Cahokia ^e	-8.4	-14.8	Mound 72 (Low Status)	AD 1050-1150
<i>Grasshopper Pueblo</i>				
Southwest Pueblo ^f	-13	-8	Grasshopper Pueblo*	AD 1275-1400
Southwest Pueblo ^f	-12	-7.5	Grasshopper Pueblo	AD 1275-1400
Southwest Pueblo ^f	-11.5	-5.5	Grasshopper Pueblo	AD 1275-1400
Southwest Pueblo ^f	-11	-7.6	Grasshopper Pueblo	AD 1275-1400
Southwest Pueblo ^f	-10.5	-6	Grasshopper Pueblo	AD 1275-1400
Southwest Pueblo ^f	-9.7	-5.4	Grasshopper Pueblo	AD 1275-1400
Southwest Pueblo ^f	-13.2	-7.5	Grasshopper Pueblo	AD 1275-1400
Southwest Pueblo ^f	-12.5	-7	Grasshopper Pueblo	AD 1275-1400
Southwest Pueblo ^f	-11	-5.5	Grasshopper Pueblo	AD 1275-1400
Southwest Pueblo ^f	-11.2	-5.9	Grasshopper Pueblo	AD 1275-1400
Southwest Pueblo ^f	-10.5	-4.5	Grasshopper Pueblo	AD 1275-1400
<i>Ontario</i>				
Ontario ^b	-8.1	-14.8	Miller	AD 1152
Ontario ^b	-5.9	-13.2	Force	AD 1235
Ontario ^b	-7.3	-14.3	Force	AD 1235
Ontario ^b	-6.1	-13.6	Force	AD 1235
Ontario ^b	-7.3	-14.6	Force	AD 1235
Ontario ^b	-5.4	-11.6	Fairty Ossuary	AD 1350
Ontario ^b	-6.2	-13.7	Fairty Ossuary	AD 1350
Ontario ^b	-5.6	-11.6	Uxbridge	AD 1490
Ontario ^b	-6.5	-12.8	Uxbridge	AD 1490
Ontario ^b	-6.6	-12.7	Uxbridge	AD 1490
Ontario ^b	-6.8	-12.5	Uxbridge	AD 1490
Ontario ^b	-5.8	-11.7	Uxbridge	AD 1490
Ontario ^b	-6.9	-12.3	Uxbridge	AD 1490
Ontario ^b	-6.0	-12.7	Uxbridge	AD 1490
Ontario ^b	-6.3	-11.8	Uxbridge	AD 1490
Ontario ^b	-6.7	-12.8	Uxbridge	AD 1490
Ontario ^b	-6.9	-13.2	Kleinberg Ossuary	AD 1600
Ontario ^b	-6.9	-13.7	Kleinberg Ossuary	AD 1600

(continued)

TABLE 3. (Continued)

Population set	$\delta^{13}\text{C}$ ‰ bone apatite (PDB)	$\delta^{13}\text{C}$ ‰ bone collagen (PDB)	Site name	Time period
Ontario ^b	-7.0	-13.7	Kleinberg Ossuary	AD 1600
Ontario ^b	-6.4	-13.6	Ossossane Ossuary	AD 1636
Ontario ^b	-7.4	-12.6	Ossossane Ossuary	AD 1636
Ontario ^b	-6.9	-13.0	Ossossane Ossuary	AD 1636
Ontario ^b	-6.6	-13.3	Ossossane Ossuary	AD 1636
Ontario ^b	-5.6	-12.5	Ossossane Ossuary	AD 1636
Marine Protein/C₃ Energy				
Tierra del Fuego ^g	-17.2	-22.6	North Tierra del Fuego	prehistoric
Tierra del Fuego ^g	-17.4	-21.8	Rio Grande	prehistoric
Tierra del Fuego ^g	-16.3	-20.1	Punta Maria	post 1500 BP
Tierra del Fuego ^g	-9.6	-10.6	Maria Luisa	post 1000 BP
Tierra del Fuego ^g	-11.2	-13.3	Caleta Falsa	850 BP
Tierra del Fuego ^g	-12.1	-14.8	Caleta Falsa	850 BP
Tierra del Fuego ^g	-9.4	-13.1	Policarpo	post 1500 BP
Tierra del Fuego ^g	-12.1	-14.1	Ushuaia	post 1500 BP
Tierra del Fuego ^g	-12.2	-14.8	Hoste Island	post 1500 BP
Tierra del Fuego ^g	-14.9	-18.3	Hoste Island	post 1500 BP
Tierra del Fuego ^g	-15.4	-20.0	Navarino Island	post 1500 BP
Southwestern Cape ^h	-14.7	-16.6	-	440 BP
Southwestern Cape ^h	-14.6	-16.9	-	620 BP
Southwestern Cape ^h	-9.2	-12.4	-	800 BP
Southwestern Cape ^h	-14.0	-17.6	-	880 BP
Southwestern Cape ^h	-13.5	-16.1	-	955 BP
Southwestern Cape ^h	-12.1	-14.4	-	1170 BP
Southwestern Cape ^h	-14.1	-16.7	-	1180 BP
Southwestern Cape ^h	-13.7	-16.2	-	1220 BP
Southwestern Cape ^h	-13.7	-17.4	-	1310 BP
Southwestern Cape ^h	-12.4	-16.5	-	1330 BP
Southwestern Cape ^h	-14.3	-19.0	-	1370 BP
Southwestern Cape ^h	-13.8	-17.5	-	1390 BP
Southwestern Cape ^h	-11.9	-14.4	-	1510 BP
Southwestern Cape ^h	-13.8	-16.9	-	1775 BP
Southwestern Cape ^h	-11.9	-15.8	-	1850 BP
Southwestern Cape ^h	-12.4	-14.9	-	2000 BP
Southwestern Cape ^h	-15.0	-19.4	-	2010 BP
Southwestern Cape ^h	-12.5	-14.0	-	2100 BP
Southwestern Cape ^h	-11.7	-13.2	-	2120 BP
Southwestern Cape ^h	-12.9	-15.0	-	2130 BP
Southwestern Cape ^h	-13.1	-16.4	-	2140 BP
Southwestern Cape ^h	-11.4	-13.1	-	2150 BP
Southwestern Cape ^h	-13.4	-14.5	-	2170 BP
Southwestern Cape ^h	-13.9	-14.8	-	2210 BP
Southwestern Cape ^h	-12.7	-13.8	-	2200 BP
Southwestern Cape ^h	-13.2	-15.3	-	2420 BP
Southwestern Cape ^h	-13.1	-15.7	-	2440 BP
Southwestern Cape ^h	-10.7	-12.1	-	2530 BP
Southwestern Cape ^h	-12.4	-14.2	-	2540 BP
Southwestern Cape ^h	-12.3	-14.7	-	2660 BP
Southwestern Cape ^h	-9.5	-13.0	-	2880 BP
Southwestern Cape ^h	-10.5	-13.6	-	3220 BP
Southwestern Cape ^h	-11.3	-13.8	-	3970 BP
Southwestern Cape ^h	-13.3	-16.1	-	5680 BP
Southwestern Cape ^h	-13.0	-16.4	-	6120 BP
San Nicolas Island ^b	-8.4	-11.4	SNI 40	2030 BC
San Nicolas Island ^b	-8.2	-10.6	SNI 40	2030 BC
San Nicolas Island ^b	-10.7	-12.8	SNI 40	2030 BC
San Nicolas Island ^b	-9.7	-12.6	SNI 40	2030 BC
San Nicolas Island ^b	-8.3	-10.7	SNI 40	2030 BC
San Nicolas Island ^b	-9.6	-12.1	SNI 40	2030 BC
San Nicolas Island ^b	-8.7	-11.2	SNI 40	2030 BC
San Nicolas Island ^b	-8.5	-11.2	SNI 40	2030 BC
San Nicolas Island ^b	-8.4	-12.0	SNI 40	2030 BC
San Nicolas Island ^b	-6.9	-11.2	SNI 40	2030 BC
San Nicolas Island ^b	-7.7	-11.0	SNI 40	2030 BC
San Nicolas Island ^b	-9.0	-12.6	SNI 40	2030 BC
San Nicolas Island ^b	-8.2	-10.6	SNI 40	2030 BC
San Nicolas Island ^b	-8.4	-11.8	SNI 16	1350 BC
San Nicolas Island ^b	-10.5	-13.1	SNI 16	1350 BC
San Nicolas Island ^b	-9.9	-11.4	SNI 16	1350 BC

(continued)

TABLE 3. (Continued)

Population set ^a	$\delta^{13}\text{C}$ ‰ bone apatite (PDB)	$\delta^{13}\text{C}$ ‰ bone collagen (PDB)	Site name	Time period
San Nicolas Island ^b	-9.7	-12.7	SNI 16	1350 BC
San Nicolas Island ^b	-9.7	-13.1	SNI 16	1350 BC
San Nicolas Island ^b	-9.5	-12.4	SNI 16	1350 BC
San Nicolas Island ^b	-6.6	-10.3	SNI 16	1350 BC
San Nicolas Island ^b	-9.1	-12.6	SNI 16	1350 BC
San Nicolas Island ^b	-9.3	-12.0	SNI 16	1350 BC
San Nicolas Island ^b	-7.1	-10.0	SNI 16	1350 BC
San Nicolas Island ^b	-10.1	-11.2	SNI 16	1350 BC
San Nicolas Island ^b	-9.5	-12.0	SNI 16	1350 BC
San Nicolas Island ^b	-10.3	-12.2	SNI 16	1350 BC
San Nicolas Island ^b	-9.3	-12.1	SNI 18	AD 1650
San Nicolas Island ^b	-10.4	-12.8	SNI 18	AD 1650
San Nicolas Island ^b	-9.3	-11.7	SNI 18	AD 1650
San Nicolas Island ^b	-9.5	-12.7	SNI 18	AD 1650
San Nicolas Island ^b	-8.4	-10.3	SNI 18	AD 1650

^a Values normalized to today's $\delta^{13}\text{C}$ value of atmospheric CO_2 by subtracting 1.5‰ from the value reported in the original reports. Studies include those indicated in footnotes.

^b Harrison and Katzenberg (2003).

^c Tucker (2002).

^d Hedman et al. (2002).

^e Ambrose et al. (2003).

^f Ezzo (1993).

^g Yesner et al. (2003).

^h Lee-Thorp et al. (1989).

* Grasshopper Pueblo stable isotope values taken from Ezzo's Figure 5.37 (1993:63).

2003), and Grasshopper Pueblo in the American Southwest (Ezzo, 1993). The final two sets are from Ontario (Harrison and Katzenberg, 2003) where some of the associated middens also contain Great Lakes fish, which have $\delta^{13}\text{C}$ values $\sim 2\text{--}4\%$ higher than that of fauna that feed on C_3 vegetation (Katzenberg, 1989; Yerkes, 2005).

The plant portion of the diets in these seven populations varied more widely. Two of these groups used mainly C_3 plants, including the nonagriculturists from Ontario (Katzenberg, 1989; Harrison and Katzenberg, 2003), and those from the Late Woodland of Georgia (Milanich, 1994; Tucker, 2002). These foraging people obtained the majority of both energy and protein from foods that would have had C_3 signals.

Five of the seven sets depended on maize agriculture including the agriculturists from Ontario (Harrison and Katzenberg, 2003), two sets from the American Bottom region (Hedman et al., 2002), Cahokia (Ambrose et al., 2003), and Grasshopper Pueblo (Ezzo, 1993). Floral analyses indicate that while some C_3 plants supplemented the diet at all these sites, maize was the major plant staple (Bohrer, 1982; Katzenberg, 1989; Emerson, 2002). At Grasshopper Pueblo, the populations may also have eaten CAM succulents (Bohrer, 1982), which should exhibit $\delta^{13}\text{C}$ values close to those in C_4 plants. In these five populations, animal protein is expected to have a C_3 signal while the majority of energy came from sources with a C_4 signal.

The three final sets of prehistoric populations depended on marine animal protein. These include the hunting and gathering inhabitants of San Nicolas Island off the California coast (Harrison and Katzenberg, 2003), Tierra del Fuego at the tip of South America (Yesner et al., 2003), and the southwestern Cape of South Africa (Lee-Thorp et al., 1989). San Nicolas, an isolated island with no large indigenous terrestrial mammals, supported a fishing-foraging adaptation (Vellanoweth and Erlandson, 1999), which probably included marine mammal hunting

as reported for other Channel Islands (Cassidy et al., 2004). At sites across Tierra del Fuego, marine fauna dominate the faunal record although wild camelids are reported in faunal assemblages from the northernmost regions (Borrero, 1989) and ethnohistoric reports mention camelid hunting (Yesner et al., 2003). Marine fauna are common in the prehistoric record in the Cape region of South Africa although human dependence on these resources decreased over time (Parkington et al., 1988; Lee-Thorp et al., 1989). The plants commonly consumed by people in all three regions are C_3 (Martinez-Crovetto, 1982; Sealy, 1986) although at San Nicolas, some C_4 grasses occur (Thomas, 1995). In all three regions, animal protein should have been marine, and the majority of energy should have a C_3 signal, except later in time in the southwestern Cape of South Africa.

No archaeological population known to have consumed purely C_4 protein could be included for this analysis. Although several southwestern pueblo populations probably consumed fauna with a C_4 signal (i.e., bison and turkey), the stable isotope studies report only collagen values (Spielmann et al., 1990) and so could not be included in our study.

We compared the isotope data from the experimental fauna, the free-ranging animals, and the archaeological humans. To do so, we subtracted 1.5‰ from $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{apatite}}$ values for the archaeological samples to normalize to today's atmospheric $\delta^{13}\text{C}$ value (Marino and McElroy, 1991).

RESULTS AND A MODEL

Experimental fauna

The data compiled from the four experimental studies show interesting relationships (Fig. 1, Tables 4 and 5). There are significant correlations between $\delta^{13}\text{C}_{\text{apatite}}$ values and $\delta^{13}\text{C}_{\text{diet}}$ values, between $\delta^{13}\text{C}_{\text{collagen}}$ and

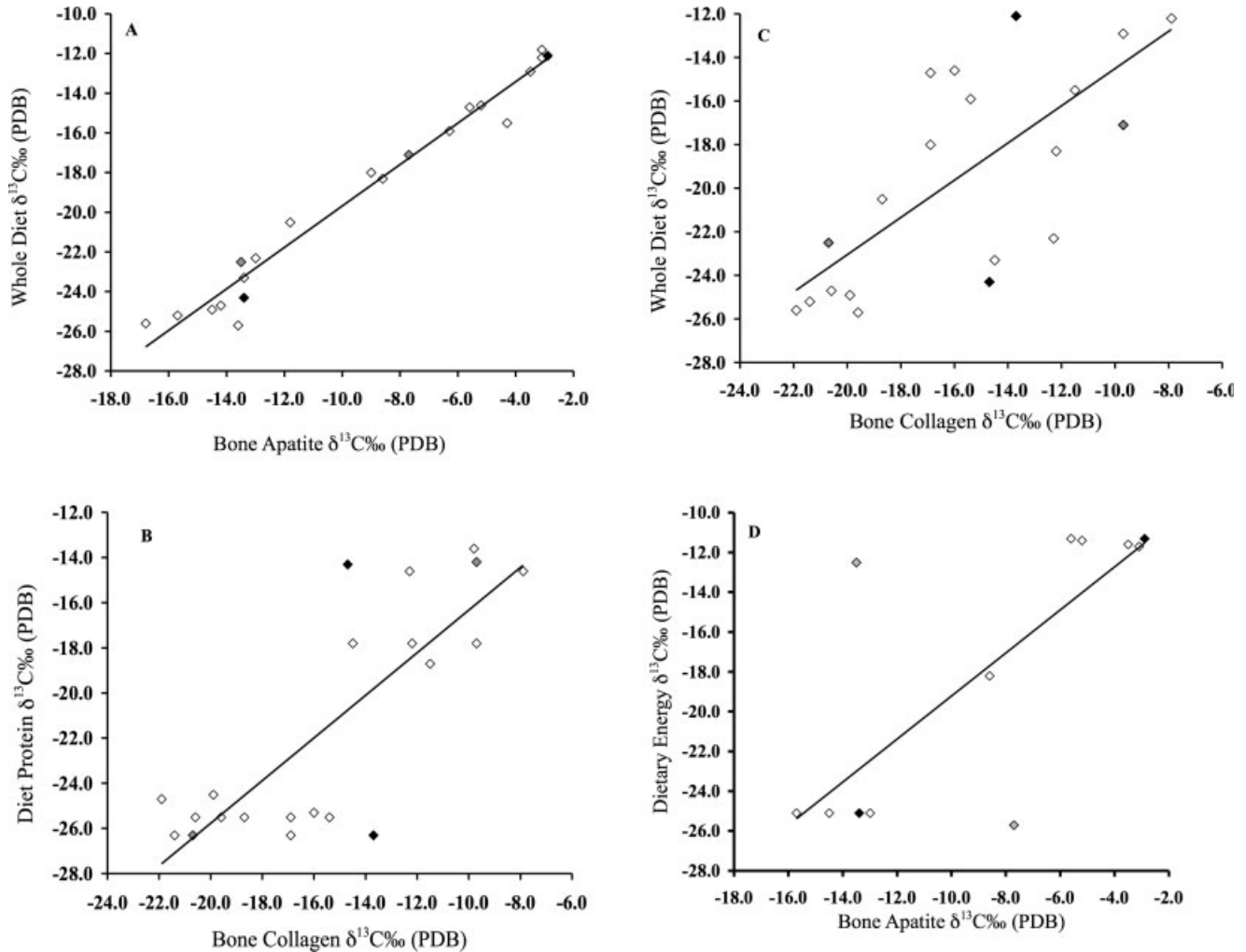


Fig. 1. Data from mice, rats and pigs fed controlled diets. Data from: Ambrose and Norr, 1993; Howland et al., 2004; Jim et al., 2003; Tieszen and Fagre, 1993, high protein diets indicated by grey symbols; low protein by black symbols. (a) $\delta^{13}\text{C}_{\text{apatite}}$ is significantly correlated with $\delta^{13}\text{C}_{\text{diet}}$ ($r^2 = 0.97$, $P < 0.001$, d.f. = 19) and unaffected by protein level or animal body size although there are examples in which a range of $\delta^{13}\text{C}_{\text{diet}}$ values associates with similar values for $\delta^{13}\text{C}_{\text{apatite}}$. (b) $\delta^{13}\text{C}_{\text{collagen}}$ is significantly correlated with $\delta^{13}\text{C}_{\text{diet protein}}$ ($r^2 = 0.65$, $P < 0.001$, d.f. = 19) although low protein diets are poorly correlated with $\delta^{13}\text{C}_{\text{collagen}}$ and there is notable scatter about the line. (c) $\delta^{13}\text{C}_{\text{collagen}}$ is significantly correlated with $\delta^{13}\text{C}_{\text{diet}}$ ($r^2 = 0.54$, $P < 0.001$, d.f. = 19). Animals on low protein diets are affected more than those on high protein diets and there is notable scatter for mixed diets. (d) $\delta^{13}\text{C}_{\text{apatite}}$ is significantly correlated with $\delta^{13}\text{C}_{\text{diet energy}}$ ($r^2 = 0.59$, $P = 0.002$, d.f. = 11); but prediction of $\delta^{13}\text{C}_{\text{diet energy}}$ from $\delta^{13}\text{C}_{\text{apatite}}$ is highly inaccurate on high protein diets.

$\delta^{13}\text{C}_{\text{diet protein}}$, between $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{diet}}$, and between $\delta^{13}\text{C}_{\text{apatite}}$ and $\delta^{13}\text{C}_{\text{diet energy}}$ as reported previously (DeNiro and Epstein, 1978; Ambrose and Norr, 1993; Tieszen and Fagre, 1993; among others). Yet, in most of the cases reviewed here, the data indicate that such reconstructions require considerations of multiple potential sources for error.

The plot of $\delta^{13}\text{C}_{\text{apatite}}$ and $\delta^{13}\text{C}_{\text{diet}}$ has the least scatter of all four graphs (Fig. 1a). $\delta^{13}\text{C}_{\text{diet}}$ is 9.7‰ more negative than $\delta^{13}\text{C}_{\text{apatite}}$, a value nearly identical to that first reported by DeNiro and Epstein (9.6‰ in 1978), and later by Ambrose and Norr (9.4‰ in 1993) in rats. The amount of protein in the diet (70% protein vs. 5% protein) has no effect on the relationship as the offsets for animals fed both high (grey symbols) and low (black symbols) protein fall close to the regression line. This

demonstrates graphically Ambrose and Norr's 1993 observation that animals fed excess protein metabolize dietary amino acids such that the carbon backbones are available for incorporation into bone's mineral fraction in a manner similar to that of dietary carbohydrates and dietary lipids.

The offset also seems unaffected by animal body size (pigs versus mice and rats, Table 5). The average offsets of the two groups are not significantly different from one another, and the ranges overlap. In marked contrast to the offset reported here for pigs (10.2‰), tooth enamel from two pigs on pure C_3 diets average 13.4‰ for $\Delta^{13}\text{C}_{\text{diet-enamel}}$ (Passey et al., 2005). Although it is not clear what impact there may be from analyzing tooth enamel rather than bone, Passey et al. (2005) suggest that the difference between their pig data and those

TABLE 4. Offsets in the experimental animal data^a

Animal	Diet: protein/energy ^b	Protein: casein (C), plant (P), fish (F)	$\Delta^{13}\text{C}$ ‰, diet-apatite	$\Delta^{13}\text{C}$ ‰, protein- collagen	$\Delta^{13}\text{C}$ ‰, diet- collagen	$\Delta^{13}\text{C}$ ‰, energy- apatite	$\Delta^{13}\text{C}$ ‰, collagen- apatite	Diet no. ^a
Monoisotopic diets								
Pig	C ₃ /C ₃	C	12.1	5.9	6.1	–	–6.0	8, Howland et al. (2003)
Mouse	C ₃ /C ₃	P	8.8	2.8	3.7	–	–5.1	1, Tieszen and Fagre (1993)
Rat	C ₃ /C ₃	C	9.5	4.9	3.8	9.4	–5.7	A, Ambrose and Norr (1993)
Rat	C ₃ /C ₃	C	10.4	4.6	5.0	10.6	–5.4	d2a4*, Jim et al. (2004)
Rat	C ₄ /C ₄	C	9.1	6.7	4.3	8.6	–4.5	d4h**, Jim et al. (2004)
Mouse	C ₄ /C ₄	P	8.7	3.8	2.0	–	–6.7	8, Tieszen and Fagre (1993)
Diets with different $\delta^{13}\text{C}$ values for protein and energy								
Rat	C ₃ /C ₄	P	9.4	9.3	–1.4	6.2	–10.8	F, Ambrose and Norr (1993)
Rat	C ₃ /C ₄	C	9.1	9.4	–2.2	5.6	–11.3	G, Ambrose and Norr (1993)
Rat ^c	C ₃ /C ₄	C	9.2	12.6	–1.6	8.4	–10.8	C, Ambrose and Norr (1993)
Rat ^d	C ₃ /C ₄	C	9.0	5.6	1.8	–1.0	–7.2	E, Ambrose and Norr (1993)
Rat	C ₄ /C ₃	C	9.4	2.4	10.1	12.1	+0.7	d5i, Jim et al. (2004)
Rat ^c	C ₄ /C ₃	C	10.9	–0.4	9.6	11.7	–1.3	B, Ambrose and Norr (1993)
Rat ^d	C ₄ /C ₃	C	9.4	4.5	9.7	18.0	–2.0	D, Ambrose and Norr (1993)
Rat	Marine/C ₃	F	9.9	3.4	8.9	11.7	–1.1	d6j2*, Jim et al. (2004)
Rat	Marine/C ₄	F	9.4	8.2	3.3	8.1	–6.2	d7k2*, Jim et al. (2004)
Diets with a mixture of C ₃ and C ₄ energy								
Pig	C ₃ /C ₃ and C ₄	C	10.5	4.9	4.1	–	–6.4	3, Howland et al. (2003)
Pig	C ₃ /C ₃ and C ₄	C	8.7	6.8	1.8	–	–6.9	4, Howland et al. (2003)
Pig	C ₃ /C ₃ and C ₄	C	9.0	8.6	1.1	–	–7.9	5, Howland et al. (2003)
Pig	C ₃ /C ₃ and C ₄	C	9.6	10.1	1.5	–	–9.1	6, Howland et al. (2003)
Pig	Marine/C ₃ and C ₄	F	11.2	7.2	4.0	–	–7.2	10, Howland et al. (2003)
Rat	Marine/C ₃ and C ₄	F	9.7	5.6	6.1	9.6	–3.6	d8l2**, Jim et al. (2004)

^a Data from: Howland et al. (2003); Tieszen and Fagre (1993); Ambrose and Norr (1993); and Jim et al. (2004) (* 2 points, ** 3 points).

^b Energy includes carbohydrate (CHO) and lipid. “–” refers to diets where the value for total CHO and lipid was not reported (see text).

^c LP, low protein (5%).

^d HP, high protein (70%).

from the Howland study (Howland et al., 2003) is due to the differential digestion of food components from the mixed diets in the Howland study. They base this partially on data from two other pigs, which inadvertently consumed a mixed diet for 1 week and had a slightly lower average $\Delta^{13}\text{C}_{\text{diet-apatite}}$ value (i.e., 12.4‰, Passey et al., 2005). Yet, one of the pigs from the Howland study displays a $\Delta^{13}\text{C}_{\text{diet-apatite}}$ value (i.e., 12.1‰, Table 4, diet 8) that approaches those from Passey et al. (2005). This suggests that differential digestion of mixed versus non-mixed diets is not the sole cause of the difference between the two studies. Additional samples are needed to clarify the impact of body size, and of enamel versus bone; but since humans commonly eat mixed diets and most reconstructions of prehistoric human diet depend on data from bone rather than tooth enamel, the Howland pigs seem to provide a reasonable comparison for those interested in prehistoric human diet.

The data concur with proposals that the type of digestive physiology rather than body size or individual species parameters is most significant in determining $\Delta^{13}\text{C}_{\text{diet-apatite}}$ values (Cerling and Harris, 1999; Hedges and van Klinken, 2000). The experimental animals have simple gastrointestinal tracts and depend mainly on endogenous enzymes to metabolize food. Humans do the same. In Passey et al. (2005), the only species that differs significantly (Mann Whitney U tests) from any other is cow. Cows, which are ruminants, depend largely on fermentation by exogenous micro-organisms in food metabolism. This contrasts with all the other fauna in their study, as well as in those considered in the present

study. All the fauna, except the cows, are monogastric animals and depend on minimal fermentation. Thus, in the Passey et al. study on tooth enamel, as in the present study of bone apatite, body size does not appear to be the most significant variable. In the present work, the data from the experimental animals appear internally consistent and relevant for reconstructing the $\delta^{13}\text{C}_{\text{diet}}$ of ancient humans.

On average, $\delta^{13}\text{C}_{\text{diet}}$ can be calculated from $\delta^{13}\text{C}_{\text{apatite}}$ with relatively high accuracy as reported previously within single studies (Ambrose and Norr, 1993):

$$\delta^{13}\text{C}_{\text{diet}} = 1.04 \times \delta^{13}\text{C}_{\text{apatite}} - 9.2 \quad (r^2 = 0.97) \quad (1)$$

Even so, a $\delta^{13}\text{C}_{\text{apatite}}$ value of -14‰ associates with a 4‰ range of values for $\delta^{13}\text{C}_{\text{diet}}$ (-26 to -22‰), and $\delta^{13}\text{C}_{\text{apatite}}$ values from -17 to -14‰ associate with a $\delta^{13}\text{C}_{\text{diet}}$ value of -26‰ . Therefore, in reconstructing $\delta^{13}\text{C}_{\text{diet}}$ from $\delta^{13}\text{C}_{\text{apatite}}$ a range of possible diet values should be considered, and checked against other lines of evidence.

The plot of $\delta^{13}\text{C}_{\text{collagen}}$ versus $\delta^{13}\text{C}_{\text{diet protein}}$ shows greater scatter (Fig. 1b). On average, $\delta^{13}\text{C}_{\text{diet protein}}$ is 6.0‰ more negative than $\delta^{13}\text{C}_{\text{collagen}}$. The highest and the lowest $\Delta^{13}\text{C}_{\text{diet protein-collagen}}$ values are from animals fed 5% protein (-0.4‰ and $+12.6\text{‰}$). These two individuals have $\delta^{13}\text{C}_{\text{collagen}}$ values that are only 1‰ different from each other even though they have drastically different $\delta^{13}\text{C}_{\text{diet protein}}$ values demonstrating graphically that a significant amount of carbon in bone collagen is coming

TABLE 5. Large versus small fauna^a

Animal	n	$\Delta^{13}\text{C}_{\text{diet-apatite}}$		Sig.	$\Delta^{13}\text{C}_{\text{diet-protein-collagen}}$		Sig.	$\Delta^{13}\text{C}_{\text{diet-collagen}}$		Sig.	$\Delta^{13}\text{C}_{\text{diet energy-apatite}}$	
		Mean \pm SD	(range)		Mean \pm SD	(range)		Mean \pm SD	(range)		Mean \pm SD	(range)
All	21	9.7 \pm 0.9	(8.7 to 12.1)		6.0 \pm 3.0	(-0.4 to 12.6)		3.6 \pm 3.7	(-2.2 to 10.1)		9.2 \pm 4.4	(-1.0 to 18.0)
Pigs	6	10.2 \pm 1.3	(8.7 to 12.1)	NS ^b	7.2 \pm 1.9	(4.9 to 10.1)	NS ^c	3.1 \pm 2.0	(1.6 to 6.4)	NS ^d	No data	
Mice and rats	15	9.5 \pm 0.6	(8.7 to 10.9)		5.6 ^e \pm 3.3	(2.4 to 10.1)		4.2 \pm 4.1	(2.2 to 10.1)		9.3 ^f \pm 2.2	(5.6 to 11.2)

^a Calculated from data in: Howland et al. (2003); Tieszen and Fagre (1993); Ambrose and Norr (1993); Jim et al. (2004).

^b Not significant, $P \leq 0.09$, $t = 1.30$, d.f. = 19.

^c Not significant, $P \leq 0.12$, $t = 1.65$, d.f. = 17.

^d Not significant, $P \leq 0.20$, $t = 1.33$, d.f. = 19.

^e Low protein values not included in the calculation for this mean, $n = 11$ (see text).

^f High protein values not included in the calculation for this mean, $n = 11$ (see text).

Sig. Significance of nonparametric Mann-Whitney U tests.

from diet energy (Ambrose and Norr, 1993). Removal of these two individuals does not change the average $\Delta^{13}\text{C}_{\text{diet-protein-collagen}}$ value although it lowers the standard deviation from 3‰ to 2.3‰ and the range from 12.2‰ to 7.7‰. The data from animals fed high protein diets fall as expected on the regression line.

Body size shows no effect on $\Delta^{13}\text{C}_{\text{diet-protein-collagen}}$ within the sample. The average value for the large mammals is not significantly different from that in the small mammals, and the ranges overlap when the low protein values are removed (Table 5). None of the pigs were fed low protein diets. The $\delta^{13}\text{C}_{\text{diet-protein}}$ can be calculated from $\delta^{13}\text{C}_{\text{collagen}}$:

$$\delta^{13}\text{C}_{\text{diet-protein}} = 0.94 \times \delta^{13}\text{C}_{\text{collagen}} - 6.9 \quad (r^2 = 0.65) \quad (2)$$

Given the scatter in the data, accuracy is about $\pm 2\%$ when other evidence indicates normal intake of protein. In cases where a low protein diet is suspected, the accuracy should be considered as $\pm 3\%$.

The plot of $\delta^{13}\text{C}_{\text{collagen}}$ versus $\delta^{13}\text{C}_{\text{diet}}$ (Fig. 1c) also shows a great deal of scatter with tighter adherence to the regression line at the ends and more scatter near the middle of the line. Within the sample, the $\delta^{13}\text{C}_{\text{diet}}$ is 3.6‰ more negative than the $\delta^{13}\text{C}_{\text{collagen}}$ (Table 5). This average value is similar to that originally reported by DeNiro and Epstein (1978) for rats on controlled diets (3.4‰), and lower than that reported by Lee-Thorp (Lee-Thorp et al., 1989) for free-ranging fauna (5.0‰). The data for the two experimental animals fed 5% protein (black diamonds) do not fall on the regression line. This appears to refute Schwarcz's expectation that low protein diets would produce an "isotopic composition of bone collagen...closer to the linear mixing model..." (Schwarcz, 2000:208) where $\delta^{13}\text{C}_{\text{collagen}}$ would accurately predict $\delta^{13}\text{C}_{\text{diet}}$. Data from animals fed high protein diets fall as expected near the regression line.

Body size probably has little effect on $\Delta^{13}\text{C}_{\text{diet-collagen}}$. The average value for pigs does not differ significantly from that of small animals, and the ranges overlap (Table 5). Additional experimental studies with larger sample sizes, expanded to include more species, could determine the actual magnitude of any body size effect; but the available data indicate that $\delta^{13}\text{C}_{\text{diet}}$ can be calculated from $\delta^{13}\text{C}_{\text{collagen}}$ within about 4‰ across body sizes.

$$\delta^{13}\text{C}_{\text{diet}} = 0.86 \times \delta^{13}\text{C}_{\text{collagen}} - 6.0 \quad (r^2 = 0.54) \quad (3)$$

The error, however, is not equal throughout the range of $\delta^{13}\text{C}_{\text{collagen}}$ values. When $\delta^{13}\text{C}_{\text{collagen}}$ is around -8% or -22 to -20% , the diets can be predicted quite accurately as nearly 100% C_4 or C_3 , respectively. In contrast, when $\delta^{13}\text{C}_{\text{collagen}}$ indicates a mix of C_4 and C_3 , ($\delta^{13}\text{C}_{\text{collagen}} = -18$ to -12%), alternative lines of evidence are needed to assess the representation of different food items in the diet.

The most scatter appears in the plot of $\delta^{13}\text{C}_{\text{apatite}}$ versus $\delta^{13}\text{C}_{\text{diet energy}}$ (Fig. 1d). On average, $\delta^{13}\text{C}_{\text{diet energy}}$ is about 9‰ more negative than $\delta^{13}\text{C}_{\text{apatite}}$. The two animals fed high protein diets (grey symbols) are clear outliers, and their positions show graphically that diet protein contributed significantly to dietary energy (Ambrose and Norr, 1993). Their removal from the calculations does not affect the average; but results in less scatter in the data. Animals fed low protein diets fall as expected on the regression line. There are no pigs for

which the specific isotope values of diet energy are known so the influence of body size on this relationship remains untested. The $\delta^{13}\text{C}_{\text{diet energy}}$ can be calculated from $\delta^{13}\text{C}_{\text{apatite}}$:

$$\delta^{13}\text{C}_{\text{diet energy}} = 1.1 \times \delta^{13}\text{C}_{\text{apatite}} - 8.4 \quad (r^2 = 0.59) \quad (4)$$

In cases of normal protein intake, the error is about $\pm 2\%$ although more samples are needed representing the middle part of the range of diet values. This level of accuracy probably reflects the fact that the majority of diet is used as energy. When protein intake is high and there is a good chance that the $\delta^{13}\text{C}_{\text{diet protein}}$ is different from $\delta^{13}\text{C}_{\text{diet energy}}$, the error increases to more than $\pm 4\%$.

Overall, the preceding considerations demonstrate several points that build on and extend the conclusions stated previously within individual studies. Yet, the amount of variation around all of the lines shown in Figure 1 indicates that using only one of these measures for reconstructing diet, diet protein, or diet energy is fraught with nondiagnostic solutions.

The model

There is a significant correlation between $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{apatite}}$, with a great deal of scatter around the line (Fig. 2a). For example, a $\delta^{13}\text{C}_{\text{apatite}}$ value $\sim -13\%$ correlates with $\delta^{13}\text{C}_{\text{collagen}}$ values of both -21% and -12% , resulting in $\Delta^{13}\text{C}_{\text{CO-AP}}$ values of about -7% and 1% . Further, there is great redundancy in absolute $\Delta^{13}\text{C}_{\text{CO-AP}}$ values (Table 4) such that approximately -6% associates with pure C_3 diets (diet 8 in pig), marine and C_4 diets (diet d7k2), mixed C_3 and C_4 diets (diet 3), and pure C_4 diets (diet 8 in mouse).

In contrast, $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{apatite}}$ show tighter distributions when the data are controlled for dietary protein (C_3 , C_4 , or marine). Three regression equations (Fig. 2b) from the same data plotted in Figure 2a, show far less scatter around each individual line. The C_3 and C_4 protein lines are parallel to one another with significantly different y-intercepts. Therefore, in contrast to using absolute $\Delta^{13}\text{C}_{\text{CO-AP}}$ values, this model of three regression lines provides a way to distinguish between diets with C_3 protein and those with C_4 protein. For both protein types, the diets with C_4 carbohydrate and lipid fall at the upper end of the line and those with C_3 carbohydrate and lipid fall at the lower end of the line. Diets with mixed C_3 and C_4 sources of carbohydrate and lipid fall in between the endpoints of the line attributed to their specific dietary protein.

The marine protein regression line, however, is not parallel to the other two. In addition, there is overlap in the C_4 and marine protein distributions. This may be the result of small sample size; but at this point, other lines of evidence are needed to distinguish marine versus C_4 protein in the diet. Even so, the model provides better precision in diet reconstruction than does the absolute value of $\Delta^{13}\text{C}_{\text{CO-AP}}$ or than $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{apatite}}$ used independently.

All three regressions have slopes around two, which means that for each 1% increase in $\delta^{13}\text{C}_{\text{collagen}}$, $\delta^{13}\text{C}_{\text{apatite}}$ increases by almost 2% . Since diet protein is held constant in each of the three lines and since both variables ($\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{apatite}}$) represent diet, the increase in $\delta^{13}\text{C}_{\text{apatite}}$ largely represents increases in the $\delta^{13}\text{C}$ values of diet energy, which consists largely of carbohy-

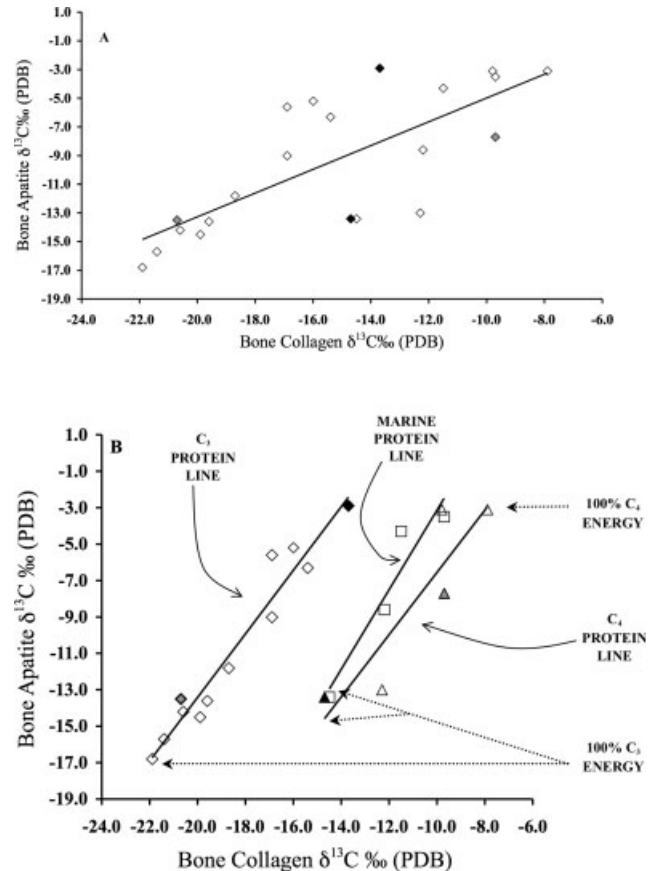


Fig. 2. $\delta^{13}\text{C}_{\text{apatite}}$ plotted against $\delta^{13}\text{C}_{\text{collagen}}$ in the same experimental animals as in Fig. 1. (a) $\delta^{13}\text{C}_{\text{apatite}}$ correlates significantly with $\delta^{13}\text{C}_{\text{collagen}}$ ($r^2 = 0.57$, $P < 0.001$, d.f. = 19, $y = 0.83x + 3.30$); but the scatter results in non-diagnostic diet reconstructions when using $\Delta^{13}\text{C}_{\text{CO-AP}}$. (b) Controlled for dietary protein, the same data sort into three regression lines (C_3 protein: $y = 1.74x + 21.4$, $r^2 = 0.95$, $P < 0.001$, d.f. = 11, marine protein: $y = 2.18 + 18.6$, $r^2 = 0.90$, $P = 0.05$, d.f. = 3, C_4 protein: $y = 1.71x + 10.6$, $r^2 = 0.80$, $P = 0.04$, d.f. = 4) that show little to no overlap. In each case, diets with C_3 energy correlate with more negative bone $\delta^{13}\text{C}_{\text{collagen}}$ values than do those with C_4 energy. The C_3 protein line is parallel to the C_4 protein line with significantly different y-intercepts ($P = 0.05$). Animals fed low and high levels of protein fall as expected.

drate and lipid except in diets with excessively high protein levels. The slope is also consistent with proposals that fewer of the carbon atoms in diet energy are going into bone collagen (except in cases where protein is excessively low) than are coming directly from diet protein. Even so, the closeness of the C_3 and C_4 protein lines indicates that the rest of the diet is also contributing, and argues against a complete protein routing model for $\delta^{13}\text{C}_{\text{collagen}}$ values. Only 6% displace the two lines from one another even though the two types of protein are 14% different from one another.

Although few in number, the bone collagen and apatite $\delta^{13}\text{C}$ values of experimental animals fed either high or low protein diets suggest that the level of dietary protein doesn't influence the position of any sample on this plot in a manner different from normal diets. The two animals fed 5% protein fall on their respective protein lines (C_3 or C_4) at the end expected from their energy

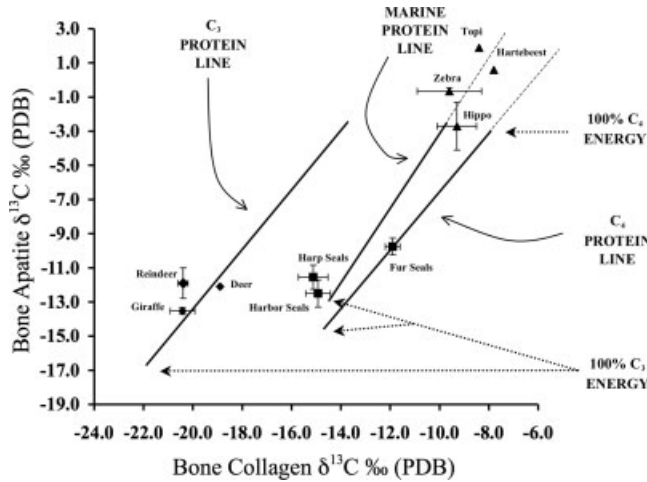


Fig. 3. Free ranging fauna with well-characterized diets plotted over the regression lines described in Fig. 2b. The C_3 browsers fall on the C_3 protein line at the C_3 energy end of that line as expected. In contrast, the C_4 grazers fall above the C_4 protein line (see text). Seals fall as expected. Data from Lee-Thorp et al. (1989), Nelson et al. (1986), Sullivan and Krueger (1981), and the present study.

source (C_3 or C_4). Those fed 70% protein diets also fall on their respective protein lines in between the endpoints as expected for diets with mixed energy sources. In these cases the mixture of energy comes from the use of excess protein as energy. Therefore, unlike some of the relationships described in Figure 1, the level of protein should not affect identification of the type of the protein, carbohydrate and energy using this model. Even so, additional samples of fauna fed high and low protein diets are needed to reach conclusions about specific effects such diets have on these values.

The type of the protein (Table 4) also seems to have no influence on the $\delta^{13}C_{\text{collagen}}$ or $\delta^{13}C_{\text{apatite}}$ values. Animals that were completely herbivorous (i.e., fed plant protein, carbohydrate and lipid) do not fall below those animals that were omnivorous (i.e., fed milk protein). Thus, this small sample provides no support for expectations that the level of omnivory within a single species can be identified by their $\Delta^{13}C_{\text{CO-AP}}$ values.

Free-ranging fauna

The free-ranging fauna (Fig. 3) show a complex pattern when plotted on the model described by the experimental animals. Animals expected to consume mostly C_3 plants (reindeer, deer, and giraffe) bracket the C_3 protein line at the C_3 energy end of the line whereas those grazing on C_4 grasses (zebra, hartebeest, topi, and hippo) fall 2–4‰ above the C_4 protein line. Plants collected in the same area as these grazers and the giraffe have $\delta^{13}C_{\text{plant}}$ values of -12.0‰ and -12.2‰ for C_4 plants, and -27‰ for C_3 plants (this study; Cerling and Harris, 1999). These values suggest that the large, terrestrial fauna reported here have $\Delta^{13}C_{\text{diet-apatite}}$ values of 14‰, which is the same offset reported between diet and tooth enamel ($\Delta^{13}C_{\text{diet-enamel}}$) in large, herbivorous mammals (Cerling and Harris, 1999). It is markedly different from the average of 9.6‰ found in the experimental fauna. In contrast, the free-ranging, terrestrial fauna show $\Delta^{13}C_{\text{diet-collagen}}$ values with a range of 2.4–6.0‰, which

brackets the average value for the experimental fauna (i.e., 3.6‰). The explanation for the higher $\Delta^{13}C_{\text{diet-apatite}}$ values is likely the different types of digestive physiology used for energy production by the experimental versus the free-ranging fauna as discussed earlier. Giraffes and the grazers reported here depend on foregut or hindgut fermentation, whereas the experimental animals and humans depend mainly on endogenous enzymes with only minor amounts of energy coming from fermentation. The larger $\Delta^{13}C_{\text{diet-apatite}}$ in the free-ranging terrestrial fauna may be due to the excretion of ^{13}C -depleted methane (Hedges and van Klinken, 2000).

Differences in metabolic breakdown of food provide an alternative explanation for the difference in offsets observed between herbivores and carnivores in Lee-Thorp and colleagues' landmark 1989 study. Although individual faunal species are not identified in that study, the herbivores are described as "grazers, mixed feeders and browsers" (Lee-Thorp et al., 1989:589), which implies that these employ fore or hindgut fermentation in food metabolism. In contrast, the carnivores include felids, jackals and bat-eared foxes (Lee-Thorp et al., 1989:589), which do little fermentation. Thus, it may be that the differences in digestive physiology between the two groups are as important as are the dietary differences in accounting for the significantly different regression lines described by the carnivores and herbivores in that study. If true, this implies that comparing human $\Delta^{13}C_{\text{CO-AP}}$ values relative to those in large bodied herbivores will not indicate the level of omnivory. In any case, the differences in metabolism combined with the results shown here suggest that animals utilizing significant amounts of fermentation during metabolic breakdown of food are not valid analogs for reconstructing diets in humans and other animals depending largely on endogenous enzymes for metabolism of food.

The three faunal species eating marine foods are all seals and have simple gastrointestinal tracts. Their data bracket the marine protein line as expected, although they fall at the C_3 energy end of the line. This position is probably due to the ingestion of ^{13}C -depleted fat (DeNiro and Epstein, 1977) in all these animals' diets. The Cape fur seal data overlap the C_4 protein line for reasons that are unclear at this time. With this exception, the marine faunal data are consistent with the pattern exhibited by the experimental fauna although more mammals eating marine foods are needed to reach a firm conclusion.

Archaeological humans

Sites where diets are expected to be C_3/C_3 . The individuals from nonagricultural sites in southern Ontario and Late Woodland Georgia largely fit the model (Fig. 4) as expected for fishing and foraging people. Some individuals from Ontario, however, have more negative $\delta^{13}C_{\text{collagen}}$ values than expected, which may reflect the ecological aspects of their forested environment (Krigbaum, 2003). Other individuals fall on the C_3 protein line at a position indicating ingestion of mixed C_3/C_4 energy, which could be accomplished by eating a small proportion of C_4 plants as energy. This suggests the presence of previously unrecognized foods with C_4 signals.

Sites where diets are expected to be C_3 protein with C_4 energy. Only a few individuals from the American Bottom sites and from Cahokia (Fig. 5a) fall where

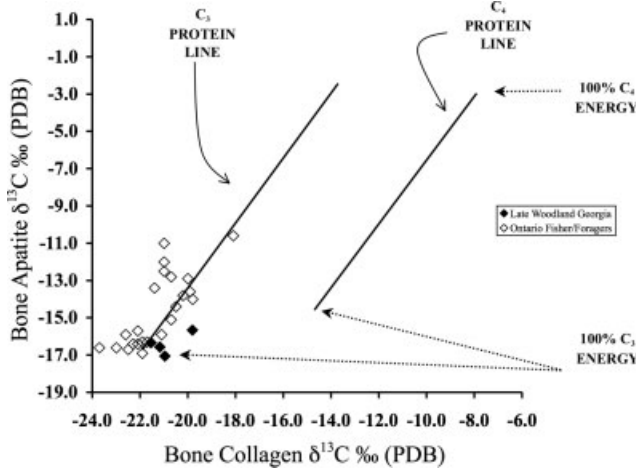


Fig. 4. Archaeological populations lacking evidence for maize agriculture plotted over regression lines in Fig. 2b. As expected, they plot in positions similar to experimental animals fed diets with C_3 protein and C_3 energy. Data from Harrison and Katzenberg (2003) and Tucker (2002).

expected near the C_3 protein regression line. These are the individuals from Cahokia and a few individuals from Upland sites who clearly obtained the majority of their protein from deer, squirrels or other foods with a C_3 signal (e.g., nuts). Many have $\delta^{13}C_{\text{apatite}}$ values that are less negative than expected (i.e., they fall above the line) reflecting the ingestion of maize as the main source of energy at Cahokia. Little carbon from the maize is being incorporated into bone collagen whereas it is being incorporated into bone apatite.

Most of the individuals from the American Bottom sites, however, exhibit $\delta^{13}C_{\text{collagen}}$ values that fall between the C_3 protein and C_4 protein lines close to the C_4 energy end of the line. These people are expected to ingest largely C_4 energy from maize (Hedman et al., 2002); but their position between the two protein lines suggests that many of these people obtained significant protein as well as energy from foods that were enriched in ^{13}C . Eating carnivorous or anadromous fish as their main source of animal protein could account for many of these individuals (Yerkes, 2005). Those individuals with the least negative $\delta^{13}C_{\text{collagen}}$ values probably obtained protein from maize in addition to fish.

The samples from American Bottom sites and Cahokia show a large range ($\sim 12\text{‰}$) in both $\delta^{13}C_{\text{collagen}}$ and $\delta^{13}C_{\text{apatite}}$ values even though all these sites are within 25 km of each other. The individuals from American Bottom sites who fall closer to the C_3 protein line probably lived a significant portion of their lives as local or foreign foragers (Hedman et al., 2002); perhaps obtaining deer for trade or tribute for Cahokia. Both high and low status individuals from Cahokia (Ambrose et al., 2003) are at the low end of the range of values for $\delta^{13}C_{\text{collagen}}$, consistent with proposals that people at Cahokia controlled hunting territories or a tribute system for acquiring meat (Kelly, 1997; Yerkes, 2005).

All of the individuals from Grasshopper Pueblo and Ontario agricultural sites (Fig. 5b) fall near the part of the model indicating that a majority of their dietary energy came from C_4 sources as expected. Both sets of

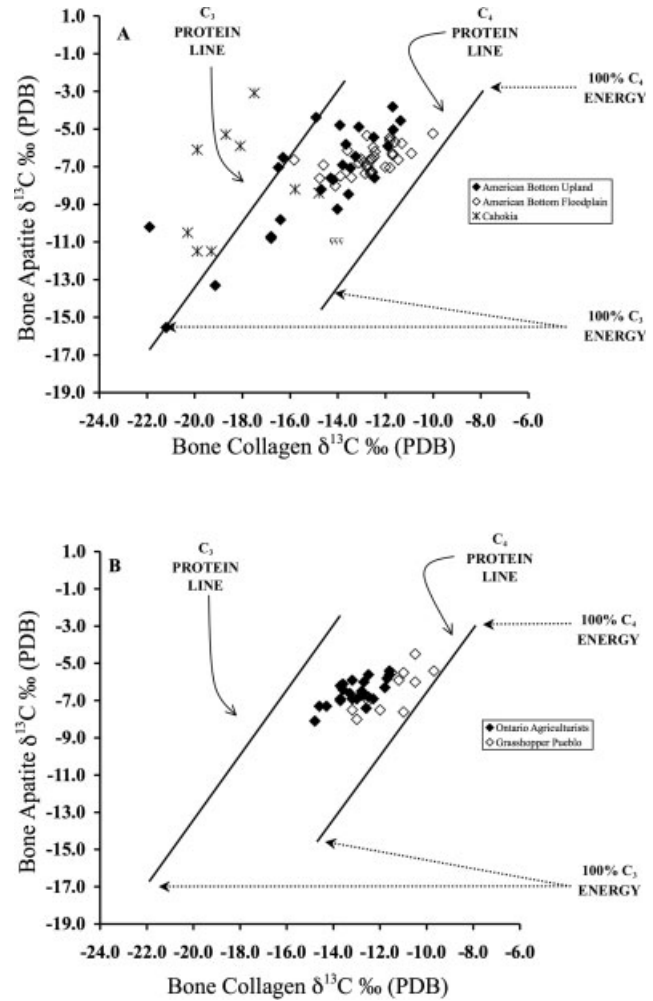


Fig. 5. Archaeological maize agriculturists expected to have eaten animal protein with a C_3 signal with fish available seasonally at Ontario (Katzenberg, 1989), Cahokia and American Bottom sites (Yerkes, 2005). Data from Ambrose et al. (2003), Ezzo (1993), and Hedman et al. (2002). (a) People at Cahokia exhibit values consistent with a diet of deer, nuts, and maize; but no fish. Those from smaller American Bottom sites have higher $\delta^{13}C_{\text{collagen}}$, consistent with eating fish (Katzenberg, 1989). (b) Data from Ontario and Grasshopper Pueblo plot between the C_3 and C_4 protein lines. In Ontario, this is due to fish intake (Katzenberg, 1989) whereas at Grasshopper Pueblo, the data suggest that maize-fed turkeys were eaten more frequently than is commonly appreciated (see text). Individuals from these sites show less variation than those from the Mississippi River sites, which suggests greater intragroup similarity in diets.

data fall between the C_3 and C_4 protein lines. The position of the Ontario populations (i.e., 2‰ to the right of the C_3 protein line) provides further support for the importance of carnivorous freshwater fish and migratory waterfowl in their diets (Katzenberg, 1989). At Grasshopper Pueblo, the main animal source of protein is supposed to be mule deer, however, the data are more consistent with significant consumption of the meat or eggs of domestic turkeys that have been fed maize. Turkeys from eastern pueblos have C_4 isotope signals (Kellner et al., nd), maize is evident in prehistoric turkey

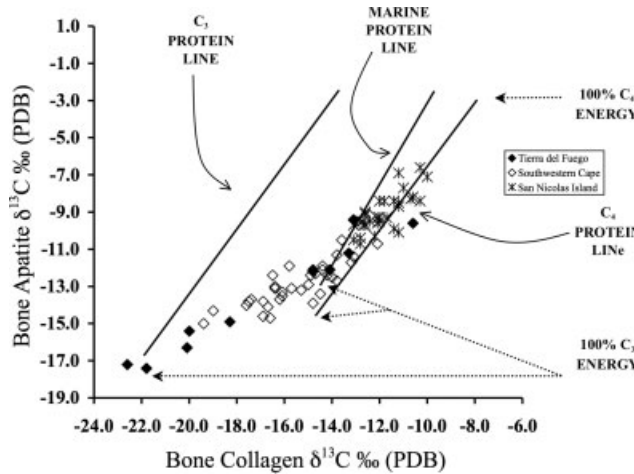


Fig. 6. Populations with diets of marine protein and C_3 energy from various wild plants. Individuals from Tierra del Fuego who plot on the marine protein regression line are from coastal sites whereas those who plot near the C_3 protein regression line are from more inland sites (Yesner et al., 2003). Individuals from the Southwestern Cape of South Africa with greater emphasis on C_3 foods come from later in the archaeological sequence as expected (Parkington et al., 1988). Individuals from San Nicolas Island off the California Coast that overlap the C_4 protein line suggest that reported C_4 grasses and CAM succulents (Thomas, 1995) were more important than commonly assumed. Data from Harrison and Katzenberg (2003), Lee-Thorp et al. (1989) and Yesner et al. (2003).

coprolites at Cedar Mesa Utah (Chisholm and Matson, 1994), and turkey bones, which make up 20% of the bird remains at Grasshopper Pueblo, show cutmarks consistent with processing for consumption and (McKusick, 1982; Olsen, 1990; Munro 2006). The isotope data are also consistent with proposals that the people exploited the protein-rich seeds of succulents in the region. Overall the data are less variable than those from the American Bottom or Cahokia, which suggests less intra-population variation in diet and may, in turn, indicate less status-related differences in food access at these sites.

Sites where diets are expected to include marine protein with C_3 energy. The individuals from Tierra del Fuego, the Southwestern Cape, and San Nicolas Island show a large range of isotope values (Fig. 6). Those from Tierra del Fuego show a bimodal distribution along both axes. Those individuals who fall near the C_3 protein regression line at the level of 100% C_3 energy are from more northern areas, and the data are consistent with ethnohistoric reports of camelid hunting and with guanaco bones in the sites (Borrero, 1989; Yesner et al., 2003). Those individuals who fall close to the marine protein regression line at the level of 100% C_3 energy, come from southern coastal areas, and their placement is consistent with consumption of significant amounts of marine foods (Yesner et al., 2003).

Individuals from the Southwestern Cape show a continuous range of variation in values, with many individuals falling as expected on the marine protein regression line at the level of 100% C_3 energy. Other individuals, however, fall between the C_3 and marine protein regression line at the level of 100% C_3 energy. Those that fall

closer to the C_3 protein line come from later in the sequence than those that fall close to the marine protein line. These positions are consistent with the archaeological evidence indicating a decline in dependence on marine foods later in the archaeological sequence (Parkington et al., 1988).

The individuals from San Nicolas Island, located far off the California coast, show less intragroup variation than do the other two groups and several individuals fall on the model in a position indicating ingestion of C_4 protein with mixed C_3 and C_4 energy. Although all of the inhabitants must have depended heavily on marine foods, some appear to have included significant amounts of C_4 protein in their diets. This suggests that the ingestion of warm season grasses and/or succulents was significant, which contrasts to other islands nearer the coast where neither isotopic nor floral data provide evidence for the ingestion of C_4 foods (Walker and DeNiro, 1986; Goldberg, 1993). It further suggests that the inhabitants of the distant island of San Nicolas did not import C_3 foods (e.g., acorns, etc.) from the mainland as did other Santa Barbara Channel Island populations. The relative isolation of San Nicolas Island from the other Channel Islands and from the California mainland, in combination with the unpredictable nature of storms in the region (Fagan, 2004), may account for the self-reliance of these people.

CONCLUSIONS

Considering the data from experimental fauna from all 4 studies revealed a level of error around each prediction of $\delta^{13}C_{\text{diet}}$, $\delta^{13}C_{\text{diet protein}}$, and $\delta^{13}C_{\text{diet energy}}$, which was not obvious within each individual study. Overall, using $\delta^{13}C_{\text{collagen}}$ or $\delta^{13}C_{\text{apatite}}$ independent of one another produces reconstructions that are not diagnostic of a particular diet, diet energy or diet. The same is true for the absolute values of $\Delta^{13}C_{\text{CO-AP}}$. In contrast, bivariate plots of $\delta^{13}C_{\text{collagen}}$ against $\delta^{13}C_{\text{apatite}}$ appear quite promising in this regard. The experimental data published over the last 10 years plot along regression lines identified as C_3 , C_4 , and marine diet protein. The animal data plot on individual lines in positions that indicate their dietary energy source (i.e., C_3 , C_4 , or mixed). Trophic position does not appear to affect these relationships as animals fed casein (milk protein) plot similarly to those fed plant protein. Modern, free-ranging fauna, when considered relative to the data for plants in the same region as the fauna, appear to have larger $\Delta^{13}C_{\text{diet-apatite}}$ values than the experimental animals. Digestive physiology for energy production in the free-ranging fauna (mainly foregut and hindgut fermentation) may affect $\Delta^{13}C_{\text{diet-apatite}}$ in a manner different from that in the experimental animals, which rely largely on endogenous enzymes in food metabolism. If supported in further investigations, this implies in turn, that these types of large bodied herbivorous mammals are not accurate analogs for humans in dietary reconstructions.

The model permitted straightforward comparison of archaeological populations for the identification of major dietary components. It revealed subtle inter- and intra-population variations in diet that were not obvious when using $\delta^{13}C_{\text{collagen}}$ or $\delta^{13}C_{\text{apatite}}$, or $\Delta^{13}C_{\text{CO-AP}}$ values independent of one another, and that elucidated social aspects within and between human groups. Most foraging people from Ontario and Late Woodland Georgia fall

at the position for diets that included C_3 protein and C_3 energy as expected although a few individuals from Ontario consumed some of their energy from foods with a C_4 signal.

Populations with archaeological evidence for cultivated maize show both inter- and intra-population variation. Many individuals from the complex site of Cahokia fall, as expected on the C_3 protein line, whereas the majority of individuals from smaller, nearby Upland and Floodplain sites probably ate freshwater fish. The striking differences in diet between Cahokia, on the one hand, and its smaller neighbors, on the other, highlight the complex social and cultural interactions that must have occurred in the region prior to European contact. Agriculturists from Ontario plot as expected for diets in which the majority of energy comes from maize; some individuals fall in positions consistent with obtaining the majority of their protein from Great Lakes fish. In sharp contrast to expectations, individuals from Grasshopper Pueblo fall on the model in positions that indicate less dependence on mule deer than on maize-fed turkeys that are often discounted as major food items (although see Kohler et al., 2005; Munro 2006).

Many of the hunter/gatherers associated with marine fauna and C_3 flora fall on the marine protein line but there are notable exceptions. People from more northern, interior regions of Tierra del Fuego fall near the C_3 protein line at the C_3 energy end of the line providing increased support for ethnohistoric reports and midden remains pointing to use of terrestrial fauna. In the Southwestern Cape region of South Africa individuals from late in the sequence show less reliance on marine fauna than those earlier in the sequence. On San Nicolas Island several individuals fall in a position that indicates the inclusion of C_4 foods in contrast to populations from other islands of the Santa Barbara Channel region, emphasizing the economic isolation of San Nicolas' inhabitants. The fit of these carefully chosen archaeological populations to the model described by the experimental fauna, supports application of the model to archaeological populations where faunal and floral evidence is inadequate for estimating the main sources of dietary protein and energy.

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