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Extending the limits of paleodietary studies of humans with compound specific carbon isotope analysis of amino acids

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Abstract

Stable carbon isotopes in the bone collagen of prehistoric humans are valuable tools for determining human diet. We studied carbon isotopes in individual amino acids (IAA) in plants and collagen from herbivores and humans from North American prehistoric sites in order to determine whether more specific dietary information about Indians could be predicted. The $\delta^{13}C$ of plant amino acids ranged extensively, whereas $\delta^{13}C$ values of each amino acid from the C_3 ($n=3$) and C_4 ($n=3$) plant species were linearly related with a slope of 0.8. Essential amino acids from herbivores had $\delta^{13}C$ values that were completely different from those measured in either C_3 or C_4 plants, suggesting metabolic resynthesis in the gut by microflora. The $\delta^{13}C$ of essential amino acids from prehistoric North Americans, who had diets ranging from primarily maize-based (C_4) to hunter-gathers (C_3) subsistence, were highly correlated with $\delta^{13}C$ values of herbivore essential amino acids. There was no significant correlation of $\delta^{13}C$ in IAA from humans with those of plants. The $\delta^{13}C$ of nonessential amino acids in human bone collagen can distinguish the presence of maize in the diet, whereas the $\delta^{13}C$ of essential amino acids were transparent to a maize-derived carbon signal. Compound specific isotopic data on IAA distinguish between total carbon intake versus total protein intake and are useful for discerning the extent and nature of omnivory.

Keywords: Paleodiet; Humans; Stable carbon isotopes; Plants

1. Introduction

A major contribution to paleodietary studies of animals and humans has been the stable isotopic analysis of collagen and apatite in subfossil bones and teeth (e.g. Refs. [16,25]). For example, empirical relationships between the isotopic compositions of carbon in bulk collagen of an organism and that in the diet of an individual are used to determine sources of carbon and protein in the diet. Presence of maize, a C_4 plant ($\delta^{13}C = -12$ to $-13‰$), in human diet has been identified with carbon isotope tracers of the total bone collagen from prehistoric North and Central American Indians (e.g. Refs. [6,13,20,21]). In the simple case where the introduction of one isotopically distinct food source to an otherwise isotopically invariant diet has occurred, isotopic shifts in consumer tissues can be a faithful record of dietary change. More complicated isotopic mixtures in many human diets have been more difficult to deconvolute from bulk isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of bone collagen, because of the input of C_4 carbon from foods other than maize. For example, C_4 grasses [10], meat from animals with a C_4-based diet [2], cane sugar, and estuarine or marine fish, and invertebrates (for example, Ref. [14]) are all isotopically enriched relative to C_3 plants and common in human diets.

Some of the limitations of dietary interpretation based on bulk isotopic analyses can be overcome by analyzing more specific molecules, such as the individual amino acids (IAA) from purified collagen. Carbon in the proteins of an organism enters from the diet, but the consumption of a meat source in the diet should have a stronger influence on the isotopic composition of collagen. Animals require certain amino acids, essential amino acids, from their diet for balanced growth,

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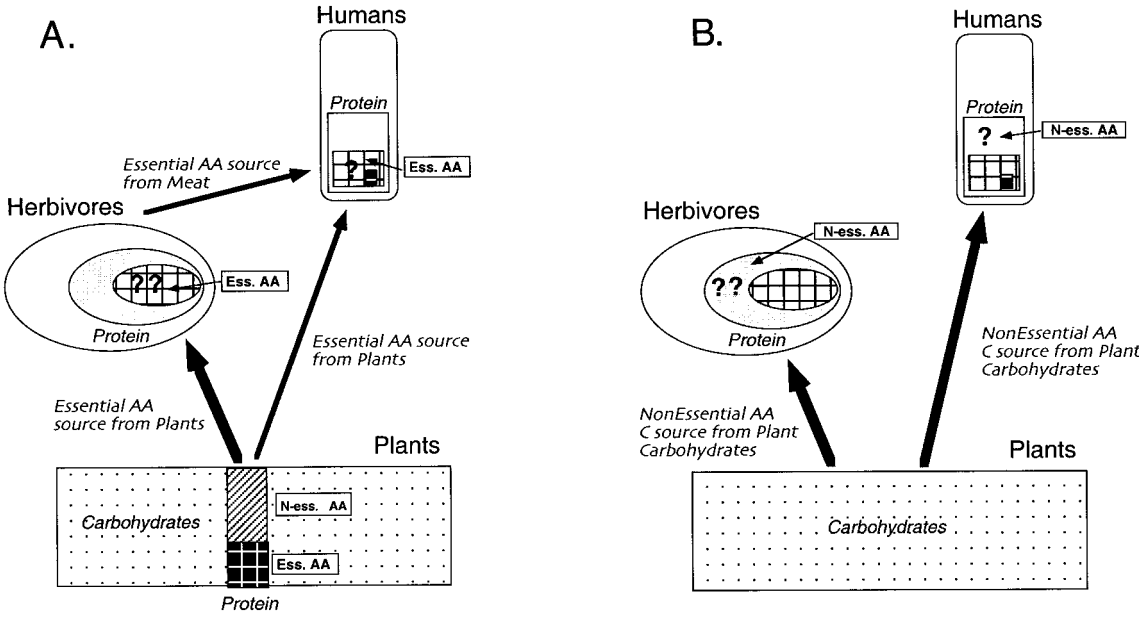

Fig. 1. Conceptual model of isotopic labeling of essential (A) and nonessential (B) amino acids in herbivore and human bone collagen. Analyses of the amino acids in plants and herbivores were performed to deconvolute the sources of carbon in the amino acids of humans.

protein synthesis, and nutrition. The carbon backbones of essential amino acids must originate from dietary protein. In the case of omnivores whose diets contain a percentage of animal products, it is likely that the essential amino acids disproportionately derive from these sources. The carbon isotopic compositions of amino acids in living organisms [1,9,11,12,18] have a very broad range because of isotopic fractionations that occur during biosynthesis. Enzymes discriminate against isotopically heavy carbon isotopes, and amino acids that are synthesized through multiple enzymatic steps are isotopically depleted, i.e. have lower $\delta^{13}C$ values. In plants, certain of the essential amino acids for animals, such as phenylalanine, leucine, and valine, are synthesized by several enzymatic steps from the molecules generated by the tricarboxylic acid (TCA) cycle. These amino acids are often the most isotopically depleted in terms of ^{13}C [9,11,18]. Amino acids that can be synthesized in vivo or incorporated directly from the diet into animal protein are called 'nonessential'. The dietary origin of the carbon in nonessential amino acids has multiple sources: protein, carbohydrates, and/or fats. Nonessential amino acids, glutamate, aspartate, proline, and hydroxyproline, can be directly produced from the TCA cycle and were grouped together in this study, because their isotopic compositions are typically uniform [9]. Thus, stable isotope tracers of both essential and nonessential groups of amino acids should provide higher dietary resolution for inferring human diet, and in particular omnivory.

In this paper, we have analyzed the $\delta^{13}C$ of IAA of plants and animals that are components of North American Indian diets. We present a model for determining the biochemical sources of total dietary carbon and protein in humans based on the isotopic composition of IAA in the essential and nonessential groups (Fig. 1). Bone collagens from humans from North America with the following subsistence strategies were analyzed: (1) hunter-gatherers (C_3), (2) maize-based diet (C_4), and (3) a maize and C_4 influenced meat dietary mixture.

Tieszen and Fagre [28] conducted experiments with mice reared on diets in which various biochemical fractions of a C_3 diet were systematically replaced with C_4 components. Dietary proteins and starch from C_4 sources resulted in significant shifts in the isotopic composition of collagen from -21.9 for an all C_3 diet to -11.8 for a $2\times C_4$ protein diet. A 92% C_4 diet resulted in a $\delta^{13}C$ value of $-9.8‰$ in the collagen fraction. On the basis of these data, we predict that the $\delta^{13}C$ values of IAA in bone collagen would have isotopic compositions similar to those in the protein fraction of their diet. Secondly, we predict that humans with a maize-based diet should show enrichment in those amino acids synthesized via the TCA cycle in human tissues. In a second series of rodent studies, Ambrose and Norr [2] concluded that dietary proteins were selectively routed into collagen, but dietary carbohydrates and lipids were not important carbon sources for collagen synthesis. Based on this model, we should find identical patterns in the $\delta^{13}C$ values of IAA in bone collagen regardless of whether humans had access to dietary maize.

In invertebrates, Fantle et al. [5] and O'Brien et al. [22] determined that the essential amino acids in animal protein were derived directly from those in their diet. Between 50 and 90% of the nonessential amino acids in

Table 1
Identification and location of samples for stable isotope analysis

Sample	Age	Location	Feeding/photosynthetic strategy	$\delta^{13}C$ bulk tissue
Plants				
Spartina	Modern	Delaware	C_4 grass	−12.5
Zea mays	Modern	Maryland	C_4 plant	−13.0
Botrichloa	Modern	Australia	C_4 grass	−12.2
Nuphar	Modern	Maryland	C_3 plant	−27.0
Acacia	Modern	Australia	C_3 shrub	−25.5
Zizannia	Modern	Maryland	C_3 grass	−28.2
Animals				
Bison	1600 AD	South Dakota	Herbivore	−16
Sylvilagus	1100 AD	Florida	Herbivore	−15
Odocoileus	2000 BC	Tennessee	Herbivore	−22.2
Humans				
	No dietary corn			
74/14	2000 BC	Ledbetter, TN	Hunter-gatherer	−21.0
25/40	2000 BC	Ledbetter, TN	Hunter-gatherer	−21.7
WO-400-2	8000 BP	Windover, FL	Hunter-gatherer	−16.0
	Dietary corn			
Sully	1600 AD	South Dakota	Corn/bison	−14.1
Chemo 31	1100 AD	Chemochobee, FL	Corn	−9.0
Panama B0	1000 AD	Panama	Corn/seafood	−11.3

butterflies and moths, however, were synthesized from dietary carbohydrates [22]. In blue crabs raised in the laboratory, nonessential amino acids raised on a high quality protein diet (i.e. zooplankton) had $\delta^{13}C$ values of IAA almost identical to nonessential amino acids in the diet. Depending on which animal model is studied, amino acids in animal proteins can be derived from carbon in all biochemical dietary sources [28] or principally from carbon in dietary proteins [2].

In our model for carbon isotopic fractionation in IAA of human bone collagen, we examined the routing of carbon by comparing $\delta^{13}C$ values of IAA in plants, herbivores, and humans (Fig. 1). The hypothesis that essential amino acids in herbivores should originate from plants in their diet was tested (Fig. 1A). Our simple model for herbivores proposes that essential amino acids from plants would be transferred without fractionation up the food chain. If essential amino acids were directly routed into bone collagen of the herbivores then three major points of potential isotopic fractionation in animals, such as microbial recycling in the gut, metabolic differences between and among animals, and starvation or illness, can be ignored. Nonessential amino acids, conversely, can originate either directly from the diet or can be synthesized by the animal during metabolism (Fig. 1B). The isotopic compositions of averaged nonessential amino acids should reflect the carbon isotopic composition of the total diet; however, individual $\delta^{13}C$ values will show variation related to enzymatic biosynthesis.

We tested whether the isotopic composition of essential amino acids in humans is more similar to those in plants at the base of the food web or in animal products. In addition, we tested the prediction that the $\delta^{13}C$ of nonessential amino acids in humans should be most strongly influenced by total dietary carbon, including carbohydrates and fats. Although one of the principal uses of $\delta^{13}C$ in humans has been to detect the presence and extent of maize in the diet, simple assessment of the influence of maize is impossible to distinguish from that of other C_4-like dietary carbon sources. We compared the $\delta^{13}C$ in IAA from humans ($n=3$) who had maize in their diet to those ($n=3$) who did not.

2. Materials and methods

2.1. Modern and archaeological samples

Location and ages of samples analyzed in this paper are listed in Table 1. The human bones from Ledbetter and Sully are described elsewhere [9,17,23,29]. Samples from Chemochobee, FL, were obtained from Bruce Smith, Smithsonian Institution, National Museum of Natural History. Modern human bone collagens were sampled from patients who were undergoing hip replacement surgery in the Washington, DC, Metropolitan area. Bone tissue was frozen in liquid nitrogen at the time of sampling.

2.2. Methods

The plants, *Zea mays* and *Spartina alterniflora*, were freeze-dried prior to hydrolysis. For all the plants, leaf tissues were selected for analysis. Bones were demineralized in EDTA, extracted with 0.1 N NaOH to remove

humic materials, then washed extensively with distilled water. One to three milligrams of tissue or demineralized bone was hydrolyzed in 1 ml of 6.0 N HCl (Pierce) at 11 °C for 20 h. After drying under a stream of N_2, the sample was derivatized with acidified iso-propanol followed by esterification with trifluoroacetic acid anhydride (TFAA), following the method of Silfer et al. [26]. The resulting sample of amino acids was diluted in about 300 µl of dichloromethane. Amino acids (1 µl) were injected on a split–splitless injector (1:10 split) at 220 °C and separated on a 25 m HP Ultra-1 column in a Varian 3400 Gas Chromatograph. The separated amino acid peaks were combusted in a Finnegan GC continuous flow interface at 940 °C, then measured as CO_2 on a Finnegan MAT 252 isotope ratio mass spectrometer. Samples were analyzed in triplicate along with standards of known isotopic composition. The $\delta^{13}C$ values of unknowns were calculated from measurements of these known standards (see also Ref. [8]).

Analytical error in measuring the derivatized amino acids was typically ±0.4‰. Errors in determining the isotopic composition of the carbon used for derivatization were approximately ±0.3‰. Standard deviations of corrected $\delta^{13}C$ values for all variance in sample and standard preparation and the correction for the addition of C from the derivative was 1.5±0.9‰. For interpretation, amino acids with $\delta^{13}C$ values within 1.5‰ of each other have statistically similar values.

3. Results and discussion

3.1. IAA from plants

Plants synthesize all of the common proteins containing amino acids [4]. The essential amino acids in animals and humans originate in plants and could be transferred up the food chain from plant to herbivore to omnivore or carnivore (Fig. 1A). Understanding the patterns of $\delta^{13}C$ in the IAA of plants is critical for the interpretation of the variability of $\delta^{13}C$ in amino acids at higher trophic levels. In Table 2, the $\delta^{13}C$ of the IAA from three C_3 and three C_4 plants are compared and span a wide range from +1.0‰ in glycine from C_4 plants to −36‰ in valine from C_3 plants.

Certain amino acids have lower standard deviations among the plant species and might be regarded as reliable dietary tracers. In the essential amino acid group, leucine, isoleucine, and phenylalanine have very consistent isotopic ratios in the three C_4 plant species that were measured. In the C_3 plant samples, leucine, phenylalanine, and lysine have low variability. For dietary analysis of protein sources, leucine and phenylalanine should be the most promising amino acids that can be used to trace the C_3 and C_4 protein components. For the nonessential amino acids, proline and glutamic acid have the least variation in both C_3 and C_4 plant samples. Moreover, the $\delta^{13}C$ of glutamic acid is almost identical to the $\delta^{13}C$ of the bulk plant (−12.5 for C_4 plants and −26.5 for C_3 plants).

High standard deviations in the remaining amino acids in plants may indicate variability in the branching points of the biosynthetic reactions forming these compounds in plants. For example, glycine (standard deviation 4.0–6.0) is involved in photorespiration and in the transfer of simple single carbon molecules (i.e. C-1) in metabolic pathways of plants. Depending on the physiological state of the plant, the C isotopic composition of glycine (two carbons) and serine (three carbons) may shift substantially, in part because the transfer of one carbon atom will have a more substantial influence on the $\delta^{13}C$. For the essential amino acids, valine has the highest standard deviations in $\delta^{13}C$. Serine and threonine also have high standard deviations, possibly because of analytical concerns. During hydrolysis of complex tissues, especially high carbohydrate materials like plants, the hydroxlyated amino acids can be destroyed with attendant isotopic alteration.

The relationship in $\delta^{13}C$ of IAA between C_3 and C_4 plants is displayed in Fig. 2. The relationship is linear with a r^2 value of 0.686, indicating that for the most part the biosynthetic pathways of C_3 and C_4 plants are similar. The slope, however, is not 1.0, which would be expected if the two groups of plants had identical physiological controls on carbon isotope compositions. Isotopic differences between the average $\delta^{13}C$ IAA values of C_4 and C_3 plants were variable (range=11–21‰). Serine, proline, and leucine, for example, have the smallest differences between the two plant groups, while alanine, threonine, and valine display the greatest differences. The data for serine suggest substantial isotopic enrichment of ^{13}C in the photorespiratory pathway of C_3 plants, which are reflected in the more positive $\delta^{13}C$ of glycine and serine. A similar pattern of enrichment in glycine and serine is seen in C_4 plants as well, suggesting that the glycine decarboxylating enzymes may be the source of isotopic fractionation.

For the purposes of this study, amino acid data are grouped in terms of essential (to animals) and nonessential amino acids in addition to glycine and serine, the major amino acids in collagen (Fig. 3). Although there is substantial variation among plants, useful patterns emerge. As a group, essential amino acids tend to be more depleted in ^{13}C compared with bulk isotopic values, while nonessential ones are slightly enriched in ^{13}C relative to bulk $\delta^{13}C$. Glycine and serine are the most enriched in ^{13}C. This relationship is important for understanding the carbon isotopic compositions of amino acids from animal bone collagen. The large spread in $\delta^{13}C$ of IAA can be inherited directly from the plant diet into animal protein. The biosynthetic imprint on isotopic fractionation in amino acids originates in plants.

Table 2
$\delta^{13}C$ IAA in plant tissue

Non-essential for animals					Essential for animals				
C_4 plants Amino acids	*Spartina*	Grass[a]	*Z. mays*	Average ± standard deviation	Amino acids	*Spartina*	Grass	*Z. mays*	Average ± standard deviation
Ala	−8.5	−4.7	−12.7	−8.6 ± 4.0	Thr	1.6	−0.3	−12.9	−3.8 ± 7.9
Gly	1.9	6.3	−5.1	1.0 ± 5.7	Val	−18.1	−6.70	−22.2	−15.7 ± 8.0
Ser	0.8	−14.3	n.d.	−6.8 ± 10.6	Leu	−21.0	−17.3	−20.6	−19.6 ± 2.0
Pro	−8.8	−10.8	−12.4	−10.6 ± 1.8	Ileu	−12.0	−10.8	−11.8	−11.5 ± 0.7
Asp	−7.9	−2.1	−6.3	−5.4 ± 3.0	Phe	−13.0	−12.6	−13.5	−13.0 ± 0.5
Glu	−13.7	−12.0	−12.8	−12.8 ± 0.9	Lys	−9.4	−12.0	−17.1	−12.8 ± 3.9
TCA[2]	−10.1	−8.3	−10.5	−9.6 ± 3.8					
C_3 plants Amino acids	*Acacia*	*Nuphar*	Wild rice	Average ± standard deviation	Amino acids	*Acacia*	*Nuphar*	Wild rice	Average ± standard deviation
Ala	−31.2	−27.4	−25.2	−28.0 ± 3.0	Thr	−15.2	−34.9	n.d.	−25.0 ± 13.9
Gly	−21.7	−13.8	−16.6	−17.4 ± 4.0	Val	−31.0	−38.6	−38.9	−36.2 ± 4.4
Ser	−19.2	n.d.	n.d.	−19.2	Leu	−33.1	−32.9	−33.2	−33.1 ± 0.2
Pro	−22.2	n.d.	−22.0	−22.1 ± 0.2	Ileu	−27.0	−24.7	−37.7	−29.8 ± 7.0
Asp	−29.3	−24.3	−19.1	−24.2 ± 5.1	Phe	−27.4	−27.7	−29.9	−28.4 ± 1.3
Glu	−25.2	−30.1	−26.2	−27.2 ± 2.6	Lys	−27.5	−30.8	n.d.	−29.1 ± 2.4
TCA	−25.6	−27.2	−22.4	−24.5 ± 2.5					

n.d.=Not determined.
[a] *Botrichloa* sp.
[b] TCA=Glu, Asp, and Pro.

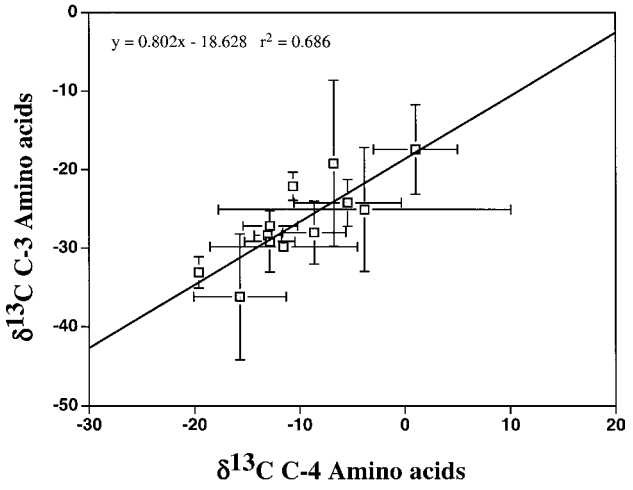

Fig. 2. The $\delta^{13}C$ of means and standard deviations of IAA from C_3 and C_4 plants taken from Table 2. This relationship is significant at the level of $P=0.0009$.

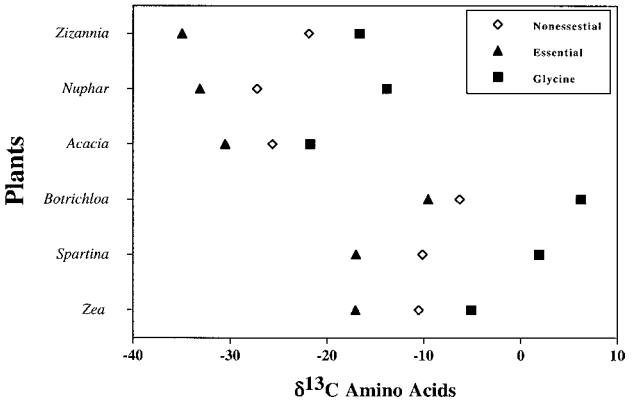

Fig. 3. The $\delta^{13}C$ of amino acid classes from individual plant species.

3.2. Carbon isotopic compositions of prehistoric herbivores

The isotopic composition of IAA in the collagen of herbivore bone was analyzed, as these animals are common food sources for North American Indians (Table 3). In Fig. 4, the $\delta^{13}C$ of animal amino acids from collagen are plotted versus those of the amino acids from the major plant source. The linear regressions for both the deer and rabbit data have a slope of approximately 1 with r^2 values from 0.55 to 0.62 and are statistically significant ($P<0.0015$). About 60% of the variation in the $\delta^{13}C$ of the amino acids in herbivore bone can be explained by variations in the isotopic compositions of amino acids in their diets.

Grouped isotopic compositions of the amino acids from these herbivores show that the nonessential amino acid data are essentially parallel for the different animals, whereas the essential amino acids vary among and between the deer, rabbit, and bison (Fig. 5). The basic observation that nonessential amino acids are enriched in ^{13}C relative to essential ones applies in herbivores as well as plants (Table 4). On the basis of the individual amino acid data, it appears that the $\delta^{13}C$ values of the nonessential amino acids may be a total dietary proxy, but the $\delta^{13}C$ of the essential amino acids in herbivores were altered. All true herbivores have alimentary tracts that are adapted to promote bacterial digestion of the fibrous compounds in plants. Microbial proteins account for as much as 90% of the amino acids in the small intestines of ruminants (for a recent review see Ref. [24]). Differences in anatomy, enzymology, and types of gut flora may impose significant unknown isotopic variability on essential amino acids in these animals.

A herbivore must obtain all of its de novo carbon from plants. Previous researchers have used the carbon isotopic compositions of bulk collagen in bone to determine the relative percentages of C_3 and C_4 carbon in animal diet (for reviews see Refs. [15,16,27]). Empirical data from field collected animals are commonly used to calculate the $\delta^{13}C$ of the original diet, and by consensus most researchers use a 5‰ as the fractionation between diet and bone collagen (e.g. Refs. [2,20]). This simple isotopic fractionation model can be taken one step further by considering the proportion of biochemical components in the diet, for example, carbohydrate, protein, and fats, to try to model carbon input into bone collagen. Ambrose and Norr [2], for example, conducted an extensive series of whole animal growth experiments with rats. Their results documented that rats fed on high protein diets had a greater proportion of their collagen synthesized via direct incorporation of amino acids from dietary protein. The isotopic composition of collagen in rats grown on diets with low protein levels gave indications that digestible, dietary carbohydrates influenced the carbon isotopic composition of bulk collagen.

3.3. Carbon isotopic composition of humans

At first glance, the range in the $\delta^{13}C$ values in IAA of human collagen was very similar to the range and distributions in $\delta^{13}C$ of IAA from plants and herbivores. This similarity is influenced by two main factors: (1) incorporation of dietary protein directly into collagen and (2) similarity in the major biosynthetic pathways that produce the nonessential amino acids in plants and animals.

The $\delta^{13}C$ of nonessential amino acids in humans should be most strongly influenced by total dietary carbon, including carbohydrates and fats. Linear relationships (not shown) between the isotopic compositions of plant amino acids had slopes very nearly 0 with r^2 values of 0.2 or less. This lack of a relationship to the primary plant signal is expected because humans, and other animals, synthesize a portion of the amino acids in collagen from other dietary sources, such as carbohydrates and fats. The relationships between the $\delta^{13}C$ of

Table 3
$\delta^{13}C$ of amino acids from prehistoric herbivore bones

Amino acids	$\delta^{13}C$ amino acids of bones		
	Deer	Rabbit	Bison
	Bone	Bone	Bone
Essentials			
Thr	−16.7	−6.7	−8.4
Val	−20.4	−21.0	−23.0
Leu	−32.4	−27.4	−24.2
Ileu	n.d.	−15.6	−21.2
Phe	−29.3	−27.5	−23.5
Lys	−19.4	−18.0	−7.7
Nonessentials			
Ala	−26.3	−15.4	−19.1
Gly	−17.1	−5.3	−12.8
Ser	−9.6	−1.7	−3.9
Pro	−21.5	n.d.	−16.5
Hyp	−17.7	−9.9	−15.0
Asp	−25.4	−11.7	−16.2
Glu	−19.9	−9.0	−15.4
Ave. TCA	−21.1	−10.2	−15.8
Ave. nonessentials	−19.6	−8.8	−14.1
Ave. essentials	−23.6	−19.4	−18
$\delta^{13}C$ bulk collagen	−22.2	−15.0	−16.0
Diet from bulk $\delta^{13}C$	−27.2[a]	−20.0	−21.0

n.d.=not determined.
[a] From of $\delta^{13}C$ collagen – 5‰.

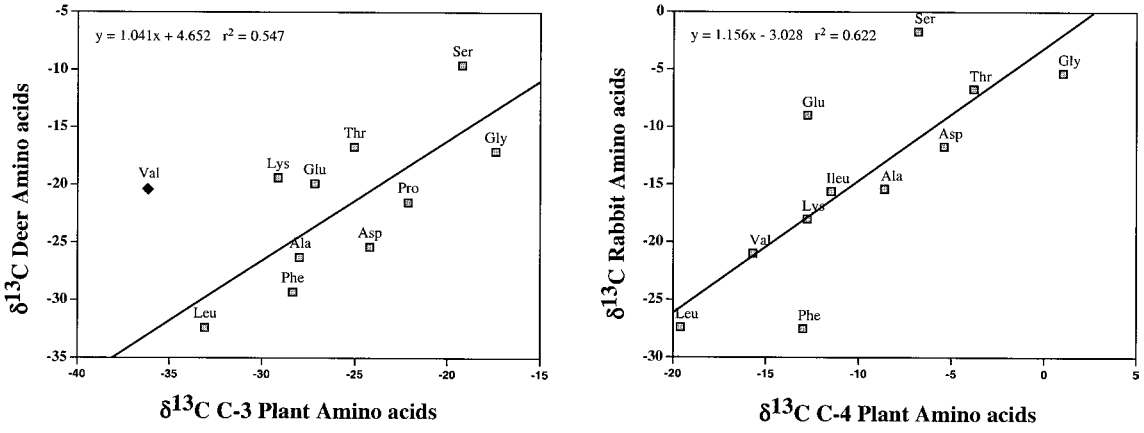

Fig. 4. The relationship between the $\delta^{13}C$ in herbivore bone collagen amino acids and corresponding values in plants. The relationship for deer was not statistically significant (P=0.0145), but that for rabbit was (P=0.0072).

IAA in meat relative to human collagen amino acids are highly significant (P=0.0026–0.0000) with r^2 values between 0.56 and 0.915 (Fig. 6). Variations in the r^2 values are not unexpected as human diets include many different types of food. The isotopic composition of bone collagen in humans, and presumably other omnivores, is strongly affected by their consumption of meat and other high protein foods (Figs. 6 and 7). Relationships of both nonessential and essential groups of amino acids from pooled, averaged individual amino acid $\delta^{13}C$ values in human collagen were correlated significantly and were strongly related to those from herbivore protein (Fig. 7).

The influence of maize in the diet was investigated by comparing essential and nonessential amino acid $\delta^{13}C$ values in humans with and without maize in their diets (Fig. 8). The slope of the line describing the relationship of essential amino acids in humans with and without dietary maize is almost 1, suggesting that the addition of the dietary staple, maize, does not contribute

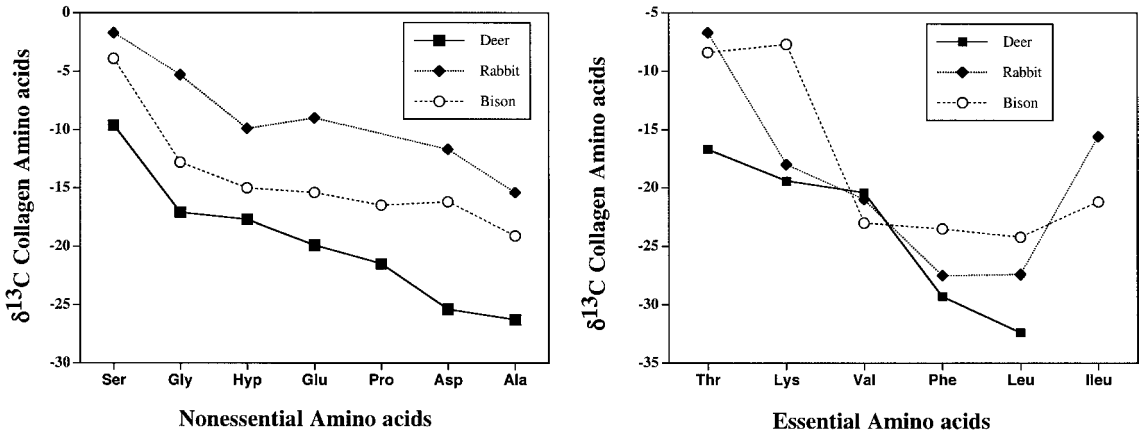

Fig. 5. Isotopic compositions of amino acids in herbivores. Parallel trends in the nonessential amino acid data indicate similar biosynthetic pathways but different carbon sources. Isotopic compositions of the essential amino acids intersect and have no distinct pattern.

Table 4
Stable carbon isotopic compositions of amino acids and total collagen from fossil human bone collagen

	Dietary corn			No corn		
Amino acids	Chemo.	Sully	Panama	Ledbetter	Ledbetter	Windover
$\delta^{13}C$						
Nonessential						
Ala	−6.5	−11.3	−10.3	−22.6	−17.0	−18.5
Gly	−0.8	−11.3	−5.6	−15.3	−15.0	−6.7
Ser	8.0	n.d.	−1.0	n.d.	n.d.	−1.4
Pro	−0.2	−14.5	−10.1	−22.4	−19.4	−15.9
Hyp	−5.6	−11.5	−8.9	−22.9	−18.4	−12.3
Asp	−5.8	−14.6	−12.2	−26.3	−21.7	−16.7
Glu	−1.1	−9.3	−9.3	−24.9	−21.0	−14.2
Ave TCA	−3.2	−12.5	−10.1	−24.1	−20.1	−14.8
Essential						
Thr	−2.2	n.d.	−5.0	n.d.	n.d.	−9.1
Val	−15.6	−25.0	−17.9	−26.1	−28.2	−14.8
Leu	−18.1	−21.8	−18.2	−31.6	−30.2	−21.0
Ileu	−14.4	n.d.	−10.6	−20.3	−19.8	−15.7
Phe	−19.2	−21.1	−23.8	−29.0	−27.0	−27.2
Lys	−17.6	−13.2	−14.2	−22.9	−19.2	−17.7
Total collagen	−9	−14.1	−11.3	−21.7	−21.0	−16.0

substantially to this amino acid pool. The analogous slope of the line for nonessential amino acids is 0.6 and is statistically highly significant, suggesting that this is the pool of amino acids disproportionally affected by maize ingestion. Ingestion of maize by prehistoric people resulted in ^{13}C enrichment in the $\delta^{13}C$ of nonessential amino acids by several ‰. Starch is the primary constituent of maize, and the $\delta^{13}C$ of the glucose in maize starch is enriched in ^{13}C by about 2‰ from bulk maize [3].

3.4. Reproducibility and variations

Measurement and analysis of compound specific isotope data from plants and bone collagen require specialized instrumentation and analytical skills. Therefore, the utility of compound specific analyses for archeological or anthropological applications may not always be appropriate. Even if larger sample sizes were analyzed, the complexity and the amount of data that are generated often give more information than is readily converted to valuable archeological interpretations. Applicability to archaeological questions must be decided in each case. How reproducible are $\delta^{13}C$ values in different individuals in a population fed a monotonous diet? When can differences in $\delta^{13}C$ of IAA be judged as 'different' versus the 'same'? How do the $\delta^{13}C$ of amino acids in plants and other organisms relate to specific metabolic, physiological, or ecological conditions? Is it always important to determine multiple sources of C_4

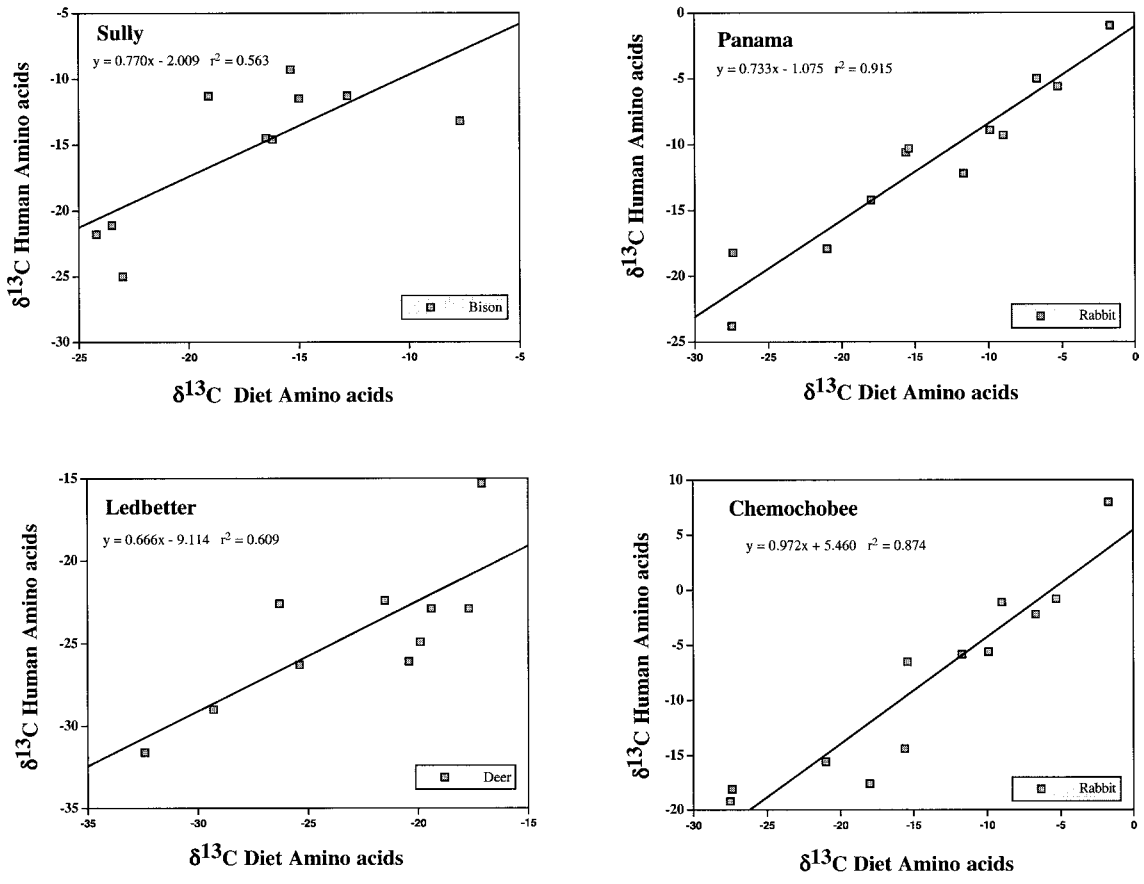

Fig. 6. Specific examples of the relationships between the $\delta^{13}C$ of IAA in human bone collagen as they relate to the $\delta^{13}C$ in amino acids of primary meat sources.

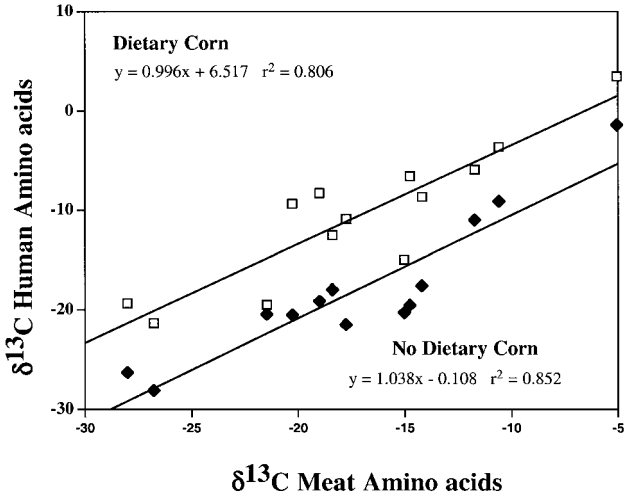

Fig. 7. Linear relationship of pooled, average values for individuals with and without maize in the diet versus $\delta^{13}C$ of IAA in meat.

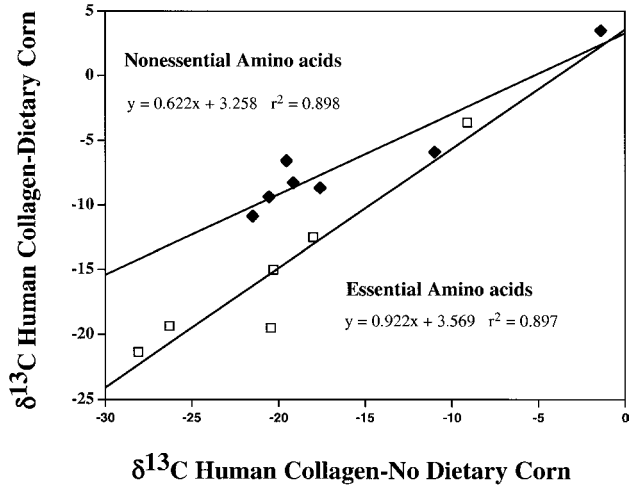

Fig. 8. The $\delta^{13}C$ of essential and nonessential IAA in humans with and without dietary maize input.

dietary input for paleodietary reconstruction? This paper is a humble start to delineate some general principles that can be tested and applied for each specifically designed study.

In answer to the first question on reproducibility, the data from contemporary American humans ($n=6$) provide some insight (Fig. 9). From these six individuals, the $\delta^{13}C$ of a majority of the amino acids had standard deviations less than 2.0‰, a variation that is comparable

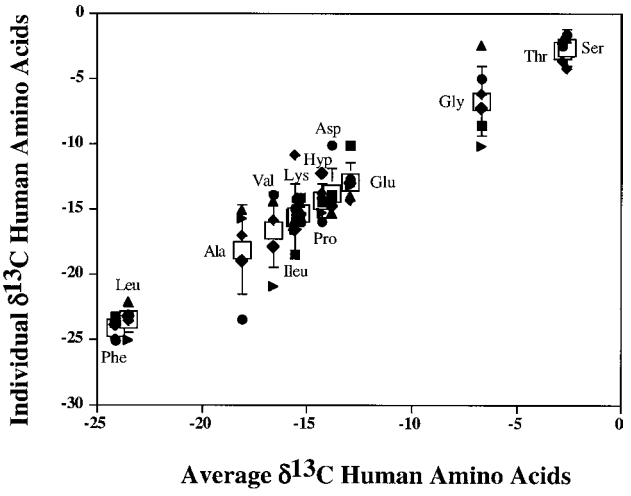

Fig. 9. Pooled values of contemporary North Americans. The open squares with error bars are the average values for all six individuals. The other data point indicates individuals plotted against the average value.

to errors in analysis and calculation (1.5 ± 0.9‰). In comparing isotopic compositions, differences in $\delta^{13}C$ greater than 2.4‰ can be interpreted as having distinct isotopic compositions. Those with greater variability may be amino acids with greater analytical error or more variable in terms of metabolic position, i.e. alanine (standard deviation=3.4), glycine (standard deviation=2.7), and valine (standard deviation=2.9).

The range in $\delta^{13}C$ of IAA within each human, herbivore, and plant of approximately 20‰ is the result of biochemical, metabolic, dietary, and physiological processes. Specific experimentation with animals or humans will lead to exact interpretations of individual data points. Abundant nutritional literature describes the relationships of dietary proteins in humans to amino acid levels in plasma (e.g. Refs. [7,19,30]), which are influenced by the complexity of metabolism. Maher et al. [19] found that isoleucine, leucine, phenylalanine, tyrosine, valine, threonine, serine, and proline plasma concentrations were correlated with the amount of these amino acids in dietary proteins. We would expect, then, a $\delta^{13}C$ relationship between the amino acids in this group (basically the essential amino acids) of animal bone collagen and those in the diet. Glycine and alanine concentrations increased after protein-free meals, and aspartate and glutamate varied inversely with their concentration in the diet. Clearly, glycine, alanine, glutamate, and aspartate are synthesized in vivo in response to dietary protein contents. Perhaps the increased variation in the $\delta^{13}C$ of alanine and glycine in all organisms is the result of their central location in nutrition and metabolism.

In addressing the last question concerning stable isotopic tracers of multiple C_4 sources in human diet, a second isotopic tracer will be needed [9,11]. Nitrogen isotopes in IAA should be able to distinguish, for example, the presence of seafood in the diet, whereas the carbon isotopic distinctions between marine or estuarine food sources versus maize are not as great. We have shown that dietary maize is disproportionately represented in the nonessential amino acids in bone collagen (Fig. 8), and nitrogen isotopic compositions of IAA should be useful for distinguishing meat from higher trophic levels as well.

4. Summary

The $\delta^{13}C$ of IAA in likely food items of North American Indians and in plants have been analyzed in order to create a baseline for interpreting the $\delta^{13}C$ of amino acids from prehistoric human collagen. The $\delta^{13}C$ of IAA from plants, even those essential to herbivores, are almost completely reset during digestion in the mammalian, herbivore gut. The correlation with herbivores and human essential amino acids is strong, however, and provides a useful analytical window to compared dietary versus metabolic influences on isotopic partitioning. The nonessential amino acids in humans who had a high percentage of maize in their diet had a different relationship to those who relied on hunter-gather subsistence. The distinction in isotopic labeling patterns between the essential and nonessential amino acids provides a window of observation on an omnivorous diet. Our data support both models proposed by Tieszen and Fagre [28] and Ambrose and Norr [2] to differing degrees depending on the specific amino acid pool and the chemical composition of the diet. With larger data sets and refinement, IAA $\delta^{13}C$ analysis holds great promise for understanding the dietary resources of humans in the archaeological record. Deconvoluting the direct influence of the diet versus human biosynthesis of amino acids should be investigated in future work. Our data on human bone collagen set boundaries for what will be observed in populations with vastly different diets.

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