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Chapter 25

Stable Isotope Analysis of Bone Collagen, Bone Apatite, and Tooth Enamel in the Reconstruction of Human Diet

A Case Study from Cuello, Belize

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Stable isotope analysis of bone collagen is now a well-established method of studying ancient human diet. Carbon isotope values distinguish between C_3 and C_4 plants in the terrestrial food web; nitrogen values can indicate marine resource exploitation, terrestrial climate, and trophic level. Unfortunately, the relative contributions of the protein, carbohydrate, and fat portions of the diet to bone collagen and bone apatite are still not fully understood. Stable isotope data for human burials from the Preclassic Maya site of Cuello, Belize demonstrate that isotopic analysis of both tissues is necessary for proper dietary reconstruction of all but the simplest ancient food webs. Equally important are isotopic analyses of the fauna and flora available for human exploitation, and the integration of these data with archaeological evidence. At Cuello, it appears that maize-eating dogs may have been a significant dietary component, but there is no evidence that deer were tamed or loose-herded as ethnohistoric accounts suggest.

Archaeological excavations conducted at Cuello, Belize by Norman Hammond and co-workers have uncovered the earliest known Preclassic Maya site, with its Swasey ceramic phase dating to 1200 BC (*1*). The 180 human burials discovered so far have provided a wealth of information on Preclassic Maya ritual and ideology (*2, 3*), population characteristics and health (*4*), and diet (*5, 6*).

At Cuello, the presence of maize cobs of varying size (*7, 8*), along with manioc and perhaps other root crops (*9*), and large numbers of deer, dog and turtle remains (*10, 11*), raises questions about the nature of the Preclassic Maya diet. Cupule sizes suggest that early, small-cobbed maize was replaced by progressively larger cobs, inferring increased production and consumption. Stable carbon isotope ratio ($^{13}C/^{12}C$, relative to a standard) determinations for several unidentified animal

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bones from Cuello, for the purpose of radiocarbon date correction (*12*), also indicated a significant C_4 component (one of two dominant photosynthetic pathways, in which C_3 and C_4 refer to the number of carbon atoms in a molecule formed during the first stage of photosynthesis) to their diet and thus the possibility of dog-raising and/or deer-taming, both observed in historic times by Bishop Landa, Francisco Hernande, and others (*13-15*). Finally, the large number of human burials at Cuello permits the study of gender- or status-based differences in diet, including a comparison between sacrificial victims from two mass burials and those from regular, individual graves.

Stable Isotopes and Diet

Stable isotope analysis of bone collagen is now a well-established method of studying ancient human diet (*16-18*). Carbon isotope values distinguish between C_3 and C_4 plants in the terrestrial food web; nitrogen values can indicate marine resource exploitation, terrestrial climate, and trophic level. Stable isotope ratios of carbon in bone apatite and tooth enamel are also reflective of diet, provided that appropriate pretreatment procedures are employed to remove adsorbed and diagenetic carbonate (*19-23*).

Unfortunately, allocation of the protein, carbohydrate, and lipid portions of the diet to bone collagen and bone apatite are still not fully understood (*24-26*). It now appears that $\delta^{13}C$ values for collagen represent mostly dietary protein while those for apatite reflect the whole diet (*27*), rather than just the energy components (*28*). For herbivores, isotopically similar plant proteins and carbohydrates supply carbon to collagen and apatite, respectively. For omnivores and carnivores, lipids from animal foods are also important contributors to apatite. Furthermore, under conditions of nutritional stress carbohydrates and lipids may be routed to collagen growth and maintenance.

We argue that analysis of both collagen and apatite fractions should be routinely done to avoid over-estimating the importance of the protein portion of prehistoric diets.

Finally, interpretation of analytical data must take into consideration the ontogeny and turnover rates of collagen and apatite tissues in bone and teeth. Bone collagen certainly reflects long-term diet for adults, although high-protein diets may stimulate higher rates of bone turnover. Elevated metabolism and growth rates of juveniles may also result in more rapid collagen turnover, while turnover rates are much higher for trabecular than for cortical bone (*29*). Bone apatite is likely to turn over faster than bone collagen, but still probably represents diet over several years, while tooth enamel apatite is laid down rapidly and is not replaced.

Analytical Methods

Collagen was extracted from the poorly preserved Cuello bones by slow dissolution of the mineral component in dilute hydrochloric acid, neutralization of humic acids with sodium hydroxide, and separation of fatty residues with a 2:1:0.8 chloroform, methanol and water mixture. Collagen pseudomorphs were freeze-dried and percent yields calculated prior to combustion in closed quartz tubes with granular Cu and CuO wire. The resulting carbon dioxide and nitrogen gases were cryogenically purified and

isolated on a vacuum line in the Archaeometry Laboratories at Harvard University. Samples consistently produced carbon and nitrogen gases with an elemental C:N ratio between 2.9 and 3.6, carbon weight percents around 40, and nitrogen weight percents around 15, all indicative of the integrity of the original collagen sample (*30*).

Apatite was produced from cleaned bone or tooth enamel by dissolving the organic phase in Clorox and removing diagenic and adsorbed carbonate contaminants with dilute 1 M acetic acid. Carbon dioxide gas was liberated in 100% phosphoric acid at 90 °C in a sealed and evacuated reaction vessel. Weight percent yields of carbon dioxide were consistent with our in-house enamel and apatite standards.

The carbon and nitrogen isotope ratios were measured on a VG Prism 2 ratio mass spectrometer. Cryogenically distilled gas samples were introduced through the manifold, while some collagen samples were introduced directly into the Carlo Erba CHN analyzer, which combusts the organic material on-line and sends the sample gas directly to the mass spectrometer source. The latter method has the advantage of rapidly producing reliable results with sample sizes on the order of 0.3 milligrams.

Isotope ratios are reported using the delta notation in parts per thousand or per mil (‰), relative to the PDB (Peedee Formation, South Carolina, *Belemnitella americana* marine fossil limestone) standard in the case of carbon, and to AIR (atmospheric N_2) in the case of nitrogen. C_3 plants average about -26‰ for carbon, and C_4 plants about -12‰. Herbivores typically show a positive fractionation in their bone collagen of about 5‰, to about -21‰ and -7‰ respectively for pure feeders. Nitrogen isotope ratios are a function of climatic effects, trophic levels, and marine vs. terrestrial food consumption. For accurate interpretation of both carbon and nitrogen isotope ratios it is necessary to establish baseline values for the region and time period under investigation.

Isotopic Ecology

Both archaeological fauna from Cuello and modern forest-dwelling fauna from the Orange Walk, Belize area were extensively tested to establish a baseline for the interpretation of the human remains. Archaeological deer (*Mazama americana, Odocoileus virginianus*), peccary (*Tayassuidae sp.*), and mud turtle (*Kinosternon sp.*) have an average $\delta^{13}C$ of -20.8 ± 1.4‰, while modern deer, peccary, tapir (*Tapirus bairdii*), kinkajou (*Potos flavus*), paca (*Agouti paca*) and nine-banded armadillo (*Dasypus novemcinctus*) average -22.4 ± 1.1‰. The Industrial Effect on atmospheric carbon dioxide accounts for the 1.6‰ negative shift for modern organisms relative to Preclassic fauna. Additional care must be taken with the selection of modern organisms, since the modern landscape includes less forest than the Preclassic, not to mention extensive stands of sugar cane, a C_4 crop. The only wild archaeological fauna with a C_4 component to the diet is the insect-eating armadillo, with an average $\delta^{13}C$ of -16.4 ± 2.8‰. Armadillos frequently make their burrows in cornfields, and would have been easily caught there.

The isotopic values for a number of marine organisms were also determined, despite the minimal evidence at Cuello for the consumption even of riverine fish. Bone collagen values for several fish species ($n = 9$) from Caye Caulker, about 20km offshore, have average $\delta^{13}C$ values of -7.3 ± 2.0‰ and average $\delta^{15}N$ values of 6.8 ± 1.4‰, while the flesh of conch, whelks, winkles, and flat tree oysters ($n = 11$)

averages -13.3 ± 1.8 and 3.5 ± 1.3 respectively. These values are characteristic of the Caribbean marine foodweb, which is based in part on extensive stands of flowering marine grasses like *Thalassia testudinum* (turtle grass) which have enriched carbon and depleted nitrogen isotope ratios (*31-33*). The differences between Caribbean values and those for other parts of the New World (*34*) emphasize the importance of establishing baseline values for each food source in all studies of human diet.

Deer and Dogs

The collagen $\delta^{13}C$ values for all archaeological deer specimens analyzed are consistent with those of C_3 plant eaters. Five brocket deer (*Mazama americana*) average -22.0 ± 0.8‰, while five white-tailed deer (*Odocoileus virginianus*) average -20.5 ± 0.9‰. These values are consistent with those for 49 white-tailed deer from several Maya sites published by Gerry (*35*). There is thus no isotopic evidence of human maintenance of deer populations, which presumably would have involved supplying them with maize feed or at least tolerating some access to maize fields. At Dos Pilas, a cache of subadult deer limbs was excavated (*36*), with one individual exhibiting a completely healed femoral fracture. It has been considered unlikely that this individual would have survived without human intervention (*37*), while isotopic analysis did not show any enrichment in $\delta^{13}C$ (*38*). The animal apparently survived for several months, but perhaps not long enough for its bone isotopic composition to change significantly if it were fed maize. As Wright (*38*) correctly notes, evidence of maize consumption would support the semi-domestication hypothesis while the converse is not necessarily true.

In contrast, small dogs (*Canis familiaris*) averaging less than 20 pounds (*11*) usually had a substantial C_4 component to their diet, although usually not as much as their human masters. For 12 individuals, collagen $\delta^{13}C$ values average -15.6 ± 3.9‰ and $\delta^{15}N$ values 7.5 ± 2.0‰. In most cases, the variation in canine diet probably reflects a combination of household scraps and scavenging for food, rather than a stable household diet. The unimodal distribution of their age at death (about 1 year), along with the chopped-up and broken nature of their limb bones (presumably for marrow extraction) (*11*), suggests that dogs were utilized as a low-cost meat resource, as observed in the 1500's by Francisco Hernande (*14*). Two dogs have very positive $\delta^{13}C$ (-10‰) and $\delta^{15}N$ (9‰) values, indicating significantly more maize consumption than was typical of the average human diet ($\delta^{13}C$ = -13‰), and suggestive perhaps of fattening for sacrificial purposes (*13, 39*). Three others have no significant C_4 component ($\delta^{13}C$ = -20 to -23‰).

Gerry's (*35*) isotopic data (ave. $\delta^{13}C$ = -9‰, n=19) for dogs from three Classic Maya sites, on the other hand, show greater reliance on C_4 food sources than the human population there (ave. $\delta^{13}C$ = -10‰), perhaps as a result of scavenging on human fecal material in addition to eating maize.

Human Diet at Cuello

Based on the quantity of faunal remains, white-tailed deer were the most important meat source at Preclassic Cuello, followed by freshwater turtle and dogs (*10*). Peccary, armadillo, and an assortment of mammalian, reptilian and avian species

constitute only a small percentage of the faunal remains; fish and shellfish are negligible in number, but a wide variety of marine and estuarine species are represented. Maize was domesticated long before the Preclassic period, and would probably be the major plant staple consumed.

Fifty-four human individuals have been isotopically tested, most for more than one tissue type (i.e., bone collagen, bone apatite, tooth enamel). There are slight indications that the percentage of C_4-based food items in the Preclassic adult male diet at Cuello actually decreased over time, from a high in the Early Middle Preclassic of -11.2‰ (n = 2), to -12.1‰ (n = 3) in the Late Middle Preclassic, to -13.4‰ (n = 7) in the Late Preclassic (Table I). This would appear contrary to the commonly-held belief that maize consumption intensified during this period; in fact this may reflect changes in dietary protein sources rather than the maize contribution to the diet. There does not seem to be any change in female diet throughout the Preclassic (n = 11). Our small sample hints that in the Early Middle Preclassic (n = 5), males may have had greater access to C_4-based food sources, averaging about 2‰ more positive in their bone collagen than females; in the Late Preclassic a larger sample (n = 13) shows no gender-based difference.

Three sacrificed individuals from the Early Cocos Period mass burial have significantly enriched $\delta^{13}C$ collagen values (-10.7 ± 0.2‰, n=3), strongly inferring that they were not native to the Cuello area, or had substantially different diets for a significant part of their later life (Table II).

Overall, the Preclassic inhabitants of Cuello have average $\delta^{13}C$ collagen values of -12.9 ± 0.9‰ (n = 28); $\delta^{15}N$ values of 8.9 ± 1.0‰ (n = 23); $\delta^{13}C$ bone apatite values of -9.8 ± 1.0‰ (n = 16); and $\delta^{13}C$ tooth enamel values of -8.7 ± 2.3‰ (n = 33). The C_4 contribution to human tissues may be calculated by simple interpolation. Using collagen $\delta^{13}C$ end-members of -21‰ for pure C_3 diets and -7‰ for pure C_4 diets, we can estimate the C_4 contribution to human collagen carbon was approximately 58% at Preclassic Cuello. Using apatite end-members of -14‰ and 0‰, we can similarly estimate that the C_4 contribution to human bone apatite carbon was approximately 30%. This latter figure may be taken as the average C_4 content of the diet, while the consumption of $\delta^{13}C$-enriched dog meat would have enhanced the collagen C_4 carbon content, which overemphasizes the protein portion of the diet. The even more positive tooth enamel values probably reflect both a high-maize juvenile diet, as well as the pre-weaning diet where the child is a trophic level above the lactating mother.

Alternatively, one may use the experimentally-derived model produced by Ambrose and Norr (*27*) in their controlled-diet study using rats:

$$\delta^{13}C_{carbonate} = 8.3733 + 0.93957 \times \delta^{13}C_{diet}$$

$$\delta^{13}C_{diet\cdot collagen} = 4.6061 + 0.52263 \times \delta^{13}C_{diet\cdot protein}$$

The average $\delta^{13}C$ value of the whole diet, based on the Cuello human bone apatite value of -9.8‰, would then be -19.3‰; using the Cuello human bone collagen value of -12.9‰, the average $\delta^{13}C$ value of dietary protein would be -15.9‰. With C_3 plant foods averaging -26‰, maize about -12‰, wild animal (deer, turtle) flesh about -23‰ (flesh, with a high lipid component, is about 2‰ more negative than collagen) and

Table I. Stable Isotope Data for Preclassic Maya from Cuello, Belize

Phase	*Group*		$\delta^{13}C_{collagen}$	$\delta^{15}N_{collagen}$	$\delta^{13}C_{apatite}$	$\delta^{13}C_{enamel}$
Swasey?	Female		-11.8		-9.1	-7.3
Bladen	Males	ave.	-11.2	9.8	-8.7	-8.8
		s.d.	0.2	0.1	0.2	1.4
		n	2	2	2	2
Bladen	Females	ave.	-13.6	8.2	-10.0	-12.3
		s.d.	1.1	0.9	1.3	3.8
		n	3	3	2	2
Bladen	All adults	ave.	-12.6	8.8	-9.3	-10.6
		s.d.	1.4	1.0	1.1	3.4
		n	5	5	4	4
Lopez	Males	ave.	-12.1	8.4	-9.9	-6.9
		s.d.	0.4	0.4	0.6	0.0
		n	3	3	3	1
Lopez	All adults	ave.	-12.3	8.5	-9.8	-9.3
		s.d.	0.5	0.4	0.5	2.3
		n	4	4	4	2
Middle Preclassic	All adults	ave.	-13.2	8.0	-11.2	-9.3
		s.d.	0.3	0.3	1.5	1.8
		n	4	3	2	4
Cocos	Males	ave.	-13.4	8.9	-9.4	-8.5
		s.d.	0.6	1.1	0.1	2.9
		n	7	6	2	6
Cocos	Females	ave.	-13.3	9.4	-10.4	-9.5
		s.d.	0.8	0.7	0.0	1.2
		n	6	4	1	5
Cocos	Juveniles	ave.	-12.5	10.9	-10.2	-7.7
		s.d.	0.0	0.0	0.0	0.8
		n	1	1	1	9
Cocos	All	ave.	-13.3	9.3	-9.8	-8.4
		s.d.	0.7	1.1	0.5	1.9
		n	14	11	4	20

ave. = average of n individuals; s.d. = standard deviation of the mean

Table II. Stable Isotope Data for Individual and Mass Burials at Cuello, Belize

Group	*Subgroup*		$\delta^{13}C_{collagen}$	$\delta^{15}N_{collagen}$	$\delta^{13}C_{apatite}$	$\delta^{13}C_{enamel}$
Single Burials	Males	ave.	-12.8	8.8	-9.8	-8.5
		s.d.	0.9	0.9	1.3	2.5
		n	15	13	8	11
Single Burials	Females	ave.	-13.2	8.9	-9.8	-9.7
		s.d.	0.9	0.9	0.9	2.6
		n	11	8	5	9
Single Burials	Juveniles	ave.	-12.5	10.9	-9.7	-8.4
		s.d.	0.0	0.0	0.0	1.5
		n	1	1	1	11
Single Burials	All	ave.	-12.9	8.9	-9.8	-8.7
		s.d.	0.9	1.0	1.0	2.3
		n	28	23	16	33
Mass Burials	All	ave.	-10.7	9.7	-9.4	-9.6
		s.d.	0.2	0.8	0.6	2.7
		n	3	3	6	6

ave. = average of n individuals; s.d. = standard deviation of the mean

dog meat as positive as -12‰ to -16‰, it is clear that maize did not constitute an overwhelming percentage of the whole diet. Although legumes and some leafy vegetables and nuts contain over 20% protein, maize is only about 10% protein, and there is some evidence that plant protein is not assimilated in bone as well as animal protein (*27*). The average value for Cuello human dietary protein, enriched by 3.4‰ relative to the whole diet average, can thus best be explained by the regular consumption of C_4-enriched dog meat and the occasional armadillo. Small but measurable differences between adult males and females in the Preclassic may potentially be explained by greater male consumption of C_4-enriched meat sources or considerably more maize and/or maize beer.

Maya Diet

These results complement the isotopic data available from other Maya sites in Belize (*35, 40, 41*) and demonstrate overall an historic trend towards increasing reliance on maize and other $\delta^{13}C$-enriched food sources, followed by a reduction in the modern era (Figure 1). Data from Maya sites elsewhere in Mesoamerica (*35, 38, 42*) show that residents of the Peten area of Guatemala and in Honduras were considerably more dependent on maize than the Belize Maya, with those at Copan truly subsisting on a corn and beans diet (Figure 2). In contrast to Belize, the wildlife of the Copan valley were rapidly eradicated by growing populations. In the Peten, more meat was

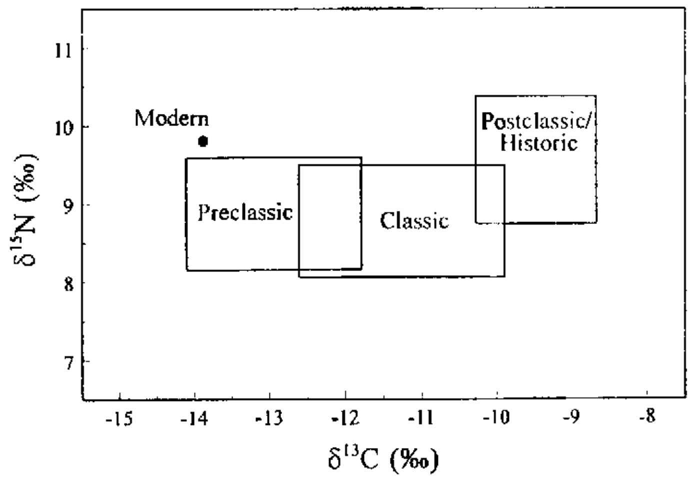

Figure 1. Chronological Comparison of Human Bone Collagen Data (5, 6, 35, 40) from Belize

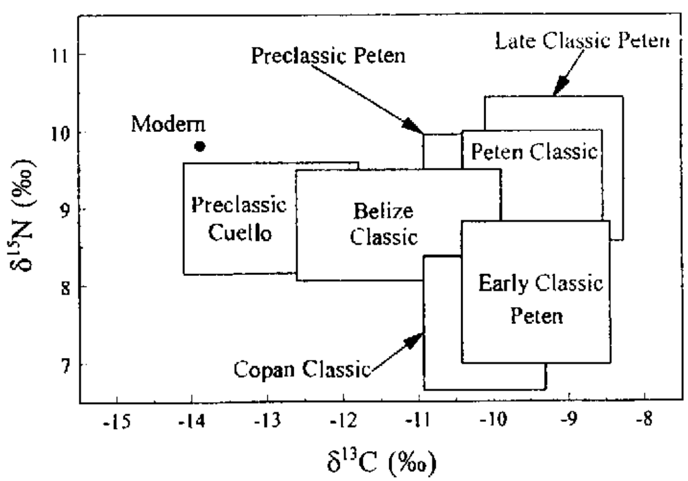

Figure 2. Comparison of Human Bone Collagen Data from Different Maya Regions (5, 6, 35, 38, 40, 42)

consumed, but the residents there were still more dependent on maize than their contemporaries in Belize who had access to a wider range of ecozones and who presumably had a lower population density. Scholars have conflicting opinions over whether dogs were an important food resource for the Maya (*11, 35, 43*), but as the only domesticated animal available in areas with diminished wild game, it is likely that they were eaten particularly when other resources were scarce or out-of-season. If their bones were crushed and boiled to extract marrow, they may be under-represented in the faunal record, or lumped together with other unidentified mammals (*10, 11*). At Cuello, the quantity of dog bones found in midden and domestic contexts with other food remains, the evidence of cut marks and bone crushing, and the average mortality age of one year, all enhance the isotopic interpretation of their regular consumption by the Preclassic Maya.

Conclusion

It would appear that geography and local ecology played the greatest role in determining the diet of the ancient Maya, with relatively minor local differences in terms of gender or status. The historic practice of raising dogs for food is now documented at Preclassic Cuello, and illustrates long-term continuity in this cultural practice. Ethnohistoric accounts of taming or loose-herding deer are not supported by the isotopic data, and reminds us to exercise caution in the use of historic documents to reconstruct ancient Maya lifeways. This study emphasizes the importance of establishing an isotopic baseline from local fauna and flora, of analyzing both bone collagen and bone apatite in humans, and integrating these data with other archaeological information to reconstruct prehistoric diet.

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