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Bone Chemistry and Ancient Diet

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Introduction

Dietary practices are a fundamental aspect of human life, from the basic caloric necessities to the socioeconomic variables which affect food production, acquisition, preparation, and consumption. Archaeologists have used many different data sources to study ancient dietary practices, including direct evidence from animal bones, macrobotanical plant remains, pollen and phytoliths in the soil, organic residues in pottery, and coprolites, as well as indirect evidence from skeletal pathology, dental wear patterns, ethnographic observations, writings, and artistic depictions. Nevertheless, for most such studies, the results are just the determination of the main menu, with animal foods the only source of semiquantitative dietary estimates.

It was only in the late 1970s that a new area of research developed – bone chemistry – which has expanded considerably our understanding of human dietary practices (Vogel & van der Merwe 1977). Biochemically, you are what you eat, and bones, teeth, and other tissues preserved in the archaeological record may be analyzed and provide direct information about the diet of individuals. This has led to research on how diets may vary based on age, sex, and socioeconomic status, as well as between different sites and time periods (here are some of the recent synthetic publications about stable isotope analyses and human diet: Tykot 2004, 2010; Pollard & Heron 2008; Lee-Thorp 2008; Price & Burton 2011).

Specifically, the combination of stable carbon and nitrogen isotopes in bone collagen and other tissues, and carbon and oxygen isotopes in bone apatite or tooth enamel, may be used to reconstruct prehistoric diet because of differential isotope fractionation between certain plant groups of atmospheric carbon dioxide during photosynthesis and trophic level increases in nitrogen isotopes. This allows us to distinguish between plants that follow different photosynthetic pathways; those which are nitrogen fixing versus absorption; and consumption of foods from different trophic levels, especially aquatic fish and mammals versus terrestrial plants and animals, but also between hunter-gatherers and agriculturalists, and even address short-term dietary change through microsampling of tooth roots and hair. Trace elements such as barium and strontium provide additional information about food sources and diet, while oxygen isotope analysis is used to study potential seasonality of shellfish gathering, and along with strontium and lead isotope analysis the mobility of the consumers.

Reliable isotope results have been obtained from samples of our early human ancestors, while the use of modern instruments which require only tiny samples and have a low per-sample cost has led to hundreds of archaeological bone chemistry studies around the world, with frequent publications in the *Journal of*

Archaeological Science, the *American Journal of Physical Anthropology*, *Archaeological and Anthropological Sciences*, and the *International Journal of Osteoarchaeology*.

Key Issues

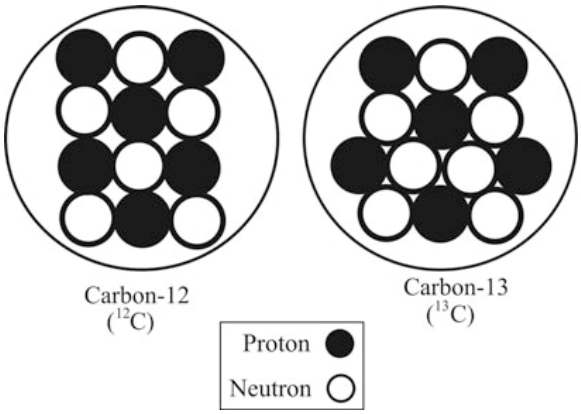
Principles of Stable Isotope Analysis

There are two stable isotopes each for carbon (^{12}C, ^{13}C) and nitrogen (^{14}N, ^{15}N), and three for oxygen (^{16}O, ^{17}O, ^{18}O) (Fig. 1). The lightest isotopes for each are the most abundant in nature (c. 99 % for each), while the small but measurable amount of variation in the heavier isotopes is measured in parts per thousand or per mil (‰). High-precision stable isotope measurements using stable isotope ratio mass spectrometers are reported using the delta notation ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$) relative to internationally recognized standards:

$$\delta^{13}\mathrm{C}\,(\text{in }‰\text{ or per mil}) = [\{(\text{sample } {}^{13}\mathrm{C}/{}^{12}\mathrm{C})/(\text{standard } {}^{13}\mathrm{C}/{}^{12}\mathrm{C})\} - 1] \times 1000$$

For carbon and oxygen, the standard is *Belemnitella americana* from the Pee Dee limestone formation in South Carolina, while for nitrogen, it is AIR.

While all terrestrial plants photosynthesize CO_2 from the atmosphere and turn it into complex carbon-based molecules, there are three different photosynthetic pathways for plants which result in differences in their carbon isotope ratios which are then passed on to their consumers (Fig. 2). Trees, shrubs, and grasses from temperate regions follow the C_3 (Calvin-Benson) pathway, and have $\delta^{13}C$ values averaging about −26.5 ‰, while grasses native to hot, arid environments follow the C_4 (Hatch-Slack) pathway and have $\delta^{13}C$ values averaging about −12.5 ‰ (although maize is more positive, c. −10 ‰). There are also some differences between particular species of plants and for the same plant grown at different latitudes (Fig. 3). Succulent plants like cactus utilize the CAM (crassulacean acid metabolism) photosynthetic pathway, with carbon isotope ratios often similar to those of C_4 plants but much more variable due to local ecological settings. In heavily forested areas, a canopy effect occurs due to incomplete atmospheric mixing and results in even more negative carbon isotope ratios. Atmospheric carbon isotope ratios have become depleted by about 1.5 ‰ since the industrial revolution, so values obtained on modern terrestrial plants and animals must be adjusted accordingly for comparison with most archaeological studies.

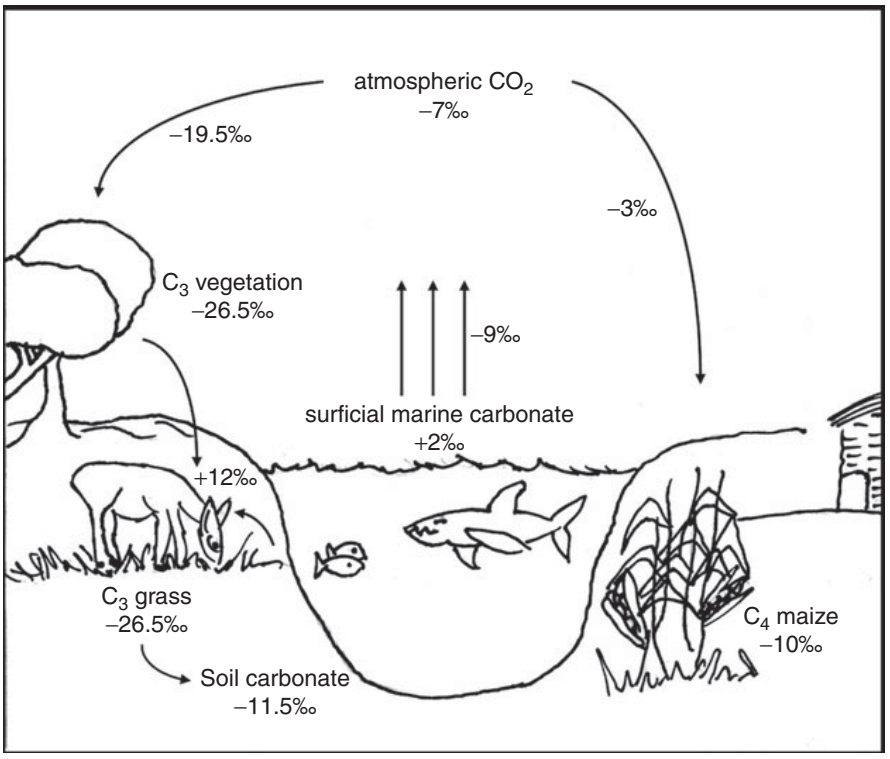


Bone Chemistry and Ancient Diet, Fig. 1 Stable isotopes with different numbers of neutrons in the nucleus

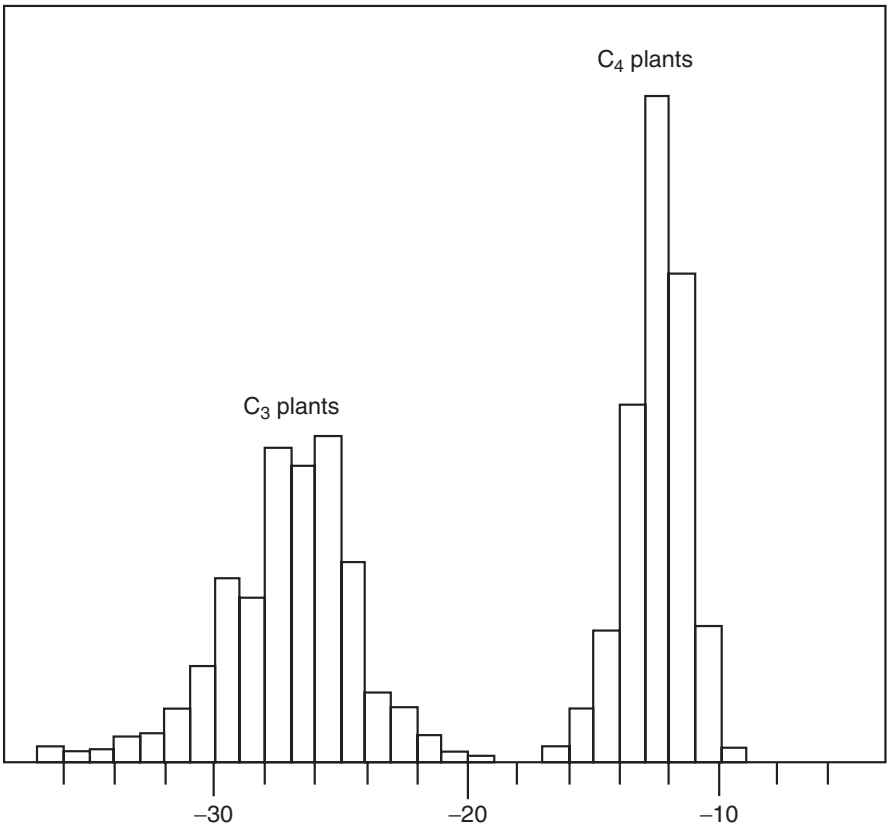
Wheat, barley, and rice are the most widely used domesticated C_3 cereal crops, while maize (corn), millet, and sorghum are the main C_4 domesticates. Stable carbon isotope analysis therefore has been widely used to address the importance of maize in the Americas and millet in Europe, Africa, and Asia.

For isotopic analysis of human remains to study diet, different tissues may be tested. Bone is made of a complex organic material called collagen (mostly a combination of essential and nonessential amino acids) and the bone mineral known as apatite (calcium hydroxyphosphate carbonate). Tooth enamel has a similar structure to bone apatite, while tooth roots have both collagen and apatite. Hair is made of keratin, a proteinaceous compound like collagen.

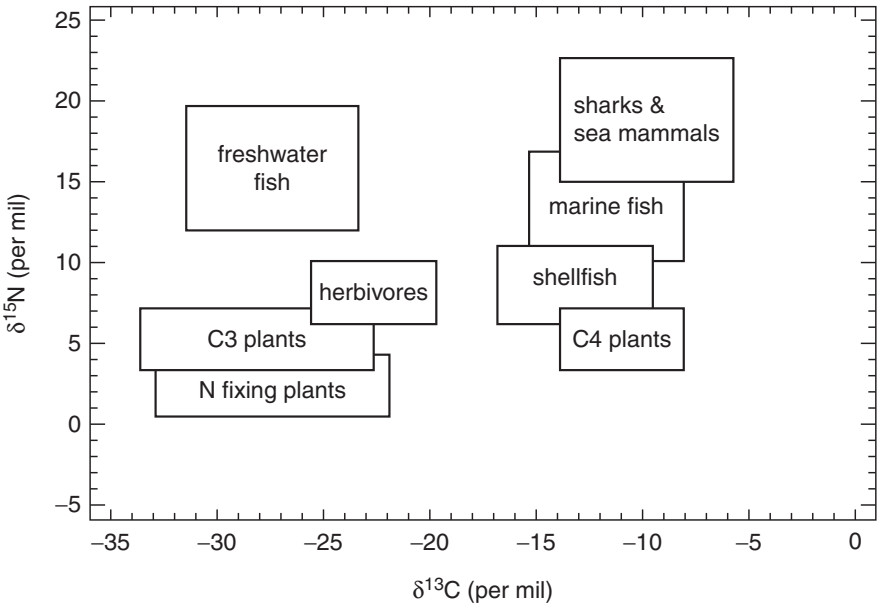
Empirical carbon isotope data for large mammals, along with data for laboratory-raised



Bone Chemistry and Ancient Diet, Fig. 2 General pattern of photosynthesis showing carbon isotope fractionation



Bone Chemistry and Ancient Diet, Fig. 3 Carbon isotope ratios for modern C_3 and C_4 plants



Bone Chemistry and Ancient Diet, Fig. 4 Carbon versus nitrogen isotope ratios for a typical food web. Isotope ranges may vary by region

rats and mice, indicate that bone collagen $\delta^{13}C$ is metabolically enriched about +5 ‰ relative to diet, although this value is affected by the proportion of protein in the total diet and any differences in $\delta^{13}C$ values between protein and energy sources. Studies have shown that bone collagen is disproportionately produced from the protein portion of the diet, while bone apatite and tooth enamel are produced from a mixture of dietary protein, carbohydrates, and fats (lipids). Experimental data on rats shows that bone apatite is enriched about +9.5 ‰ relative to the whole diet, regardless of the variety or isotopic composition of the foods consumed (Ambrose & Norr 1993; Tieszen & Fagre 1993), while data for larger herbivores and humans suggest that the diet-apatite spacing is about +12 ‰ (Tykot et al. 2009). Stable carbon isotope analysis of both bone collagen and apatite thus permits quantitative estimates of several dietary patterns.

Carbon isotope ratios for freshwater and marine foods are more variable than terrestrial plants and animals, due to differences in carbonate values in the water and trophic status, with marine (saltwater) fish having positive $\delta^{13}C$ values similar to that of maize. Fish and sea mammals, however, typically have much higher nitrogen isotope values, and their high protein content contributes much more carbon to bone collagen than does maize (c. 10 % protein) or other plants. In contrast, carbon isotope ratios for bone apatite, which equally represent dietary carbohydrates, fats, and proteins, allow for the identification of just a few percent of C_4 plants in an otherwise C_3-based diet.

Nitrogen isotope ratios for plants depend primarily on whether they obtain their nitrogen by symbiotic bacterial fixation or directly from soil nitrates, but also vary according to rainfall, altitude, and other factors. Plant values also may be elevated due to human-led fertilization practices. From their local baseline values, nitrogen isotope ratios increase about 3 ‰ for each trophic level due to metabolism, with lots of variation among marine organisms. Humans dependent on terrestrial plants and animals usually have $\delta^{15}N$ values in bone collagen of about 6–10 ‰, whereas consumers of freshwater or marine fish and sea mammals may have $\delta^{15}N$ values of 15–20 ‰. The most accurate interpretations may be made when there are isotopic data available for the animal and plant foods likely to have been consumed by a specific population, so testing is often done not just on human remains but also faunal and floral samples found at archaeological sites (Fig. 4).

Oxygen isotope analyses are also used to study ancient diet. Oxygen isotope values relate

directly to local climate, temperature, and humidity and are used for determining the seasonality of shells, climate studies, mobility, and their impacts on dietary patterns. Analyses have been done on oxygen in both the carbonate and phosphate components of bone apatite and tooth enamel. Strontium isotope ratios directly represent the geographic area of food production/acquisition and thus the mobility of dietary resources and/or their consumers. Strontium isotope analysis has been used for migration studies in many parts of the world (see Meiggs & Freiwald's entry on ▶ Human Migration: Bioarchaeological Approaches in this encyclopedia).

Bone collagen and bone apatite have resorption/replenishment rates estimated at 7–10 years or more, so that isotopic analysis of bones of adults provides the average diet for many years prior to death. Tooth tissues, however, do not turn over, so their isotope values represent diet from the time of formation, regardless of age at death. Tooth formation begins in utero for deciduous teeth and ranges from 0 to 18+ years of age for permanent teeth. The analysis of multiple teeth from the same adult individual may reveal the age of weaning (first the introduction of solid foods and later the cessation of breast feeding) since a nursing infant is effectively a carnivore and will have much higher $\delta^{15}N$. Differences between juvenile teeth and adult bone values may reflect changes in diet due to geographic movement (e.g., for marital reasons) or change in status.

Stable isotope analysis of multiple human tissues can provide a science-based dietary life history of an individual. Although collagen is rarely preserved in bones predating the Upper Paleolithic and even for recent time periods is often badly degraded in hot and moist environments, bone apatite has provided reliable results throughout the Holocene and tooth enamel for early hominins back into the Miocene. Microanalysis of tooth segments (or hair or fingernails, when preserved) may be done to address short-term isotopic dietary variability, perhaps due to seasonal mobility or harvesting practices.

Sample Preparation and Stable Isotope Analysis

For isotope analysis of archaeological bone, the first step is to remove any potential contamination, either from the soil or from added preservatives, for a sample weighing about 1 g. It is then necessary to separate the specific tissues to be analyzed. For collagen, this involves demineralization of the bone using acid and separation from any residual lipids. The procedures used in the Laboratory for Archaeological Science at the University of South Florida involve demineralization in 2 % hydrochloric acid (72 h), removal of base-soluble contaminants using 0.1 M sodium hydroxide (24 h before and after demineralization), and dissolution of residual lipids in a 2:1:0.8 mixture of methanol, chloroform, and deionized water (24 h) (Fig. 5).

Bone collagen is often not well preserved, and a yield of less than 1 % is considered unreliable for isotope analysis (bone originally has more than 20 % collagen). The main issue for low yields is that degradation may have resulted in unequal breakdown and loss of the different amino acids, which individually have different isotope values because of the different chemical reactions involved in their initial production. The amount of carbon and nitrogen measured by the mass spectrometer, relative to the size of the sample put in for analysis, and the C to N ratio of the gases produced (which should be the same as in living organisms) are additional tests of reliability. Duplicate 1 mg samples of collagen pseudomorphs are placed in tin capsules and analyzed for $\delta^{13}C$ and $\delta^{15}N$ in continuous flow mode using a Carlo-Erba NA2500-II EA with a Costech Zero-Bank autosampler, coupled with a Thermo Delta + XL stable isotope ratio mass spectrometer in the Paleolab at USF (Fig. 6a).

Bone apatite and tooth enamel carbonate are prepared using procedures designed to remove non-biogenic carbon without altering the biogenic carbon isotope values (see Tykot 2004). About 10 mg of powdered sample is immersed in 2 % sodium hypochlorite to dissolve organic components (24 h for enamel, 72 h for bone

Bone Chemistry and Ancient Diet, Fig. 5 Chemical extraction of bone collagen for isotope analysis

apatite), followed by the removal of non-biogenic carbonates using 1.0 M buffered acetic acid for 24 h. The integrity of apatite and enamel samples is assessed through yields obtained in each stage of the pretreatment process and the CO_2 yield during the mass spectrometry analysis. More complex tests of sample reliability have been used in some laboratories, however, including Fourier transform infrared spectroscopy (FTIR). Apatite and enamel samples weighing 1 mg are isotopically analyzed on a second Thermo Delta + XL mass spectrometer equipped with a Kiel III individual acid bath carbonate system (Fig. 6b). It is a scientifically sound idea to perform repeat isotope analyses for outliers when testing a group of individuals thought to have had similar isotope values.

For both bone collagen and apatite/enamel carbonate analysis, reference gases and solid standard samples are analyzed to ensure reliability of the isotope results. The analytical precision for stable isotope ratio mass spectrometers is typically 0.1 ‰ or less for ^{13}C and ^{8}O and 0.2 ‰ for ^{15}N.

Estimating the percentage of C_4 plants in human diet is fairly straightforward for herbivores and human agriculturalists, if seafood was not available and animals were not consuming wild C_4 grasses, by simple interpolation between the endpoints of bone apatite for a pure C_3-based diet versus a pure C_4-based diet. Rather than using the average endpoints of −26.5 and −12.5 ‰, the specific carbon isotope values for the C_3 plants most likely consumed should be used, since they do vary between grasses (e.g., wheat, barley, rice) and legumes, etc., in order to set an accurate baseline. For a C_4 endpoint based on maize, the value would be about −10 ‰. So if the range was from −24 ‰ to −10 ‰ (14 ‰), then each per mil more positive than −24 ‰ would represent about 7 % maize in the overall diet.

When animal or aquatic foods are a significant part of the diet, the bone collagen carbon and nitrogen isotope data are necessary to include in the percentage calculations. Mathematical models have been developed to combine collagen and apatite isotope data for distinguishing between plant and animal food sources with similar isotopic signatures, most recently by using multivariate statistics (Froehle et al. 2012).

Trace Element Analysis

A number of studies have been done measuring and interpreting trace elements in bone mineral, including Sr, Ba, Fe, Cu, Mg, Mn, Zn, and Pb. Lead is a toxic element known to accumulate in bone due to the usage of lead-based drinking, cooking, and eating materials, as well as toy soldiers, paint chips, and other items children have ingested. Strontium and barium however are structural substitutes for calcium in bone apatite and tooth enamel and show significant trophic level variation in their concentrations. Both Sr and Ba are acquired by plants through the soil and are then passed on to their consumers, with a decrease in concentrations for each trophic level. This fractionation is even greater for Ba than Sr due to its chemical structure as barite ($BaSO_4$) in the soil. There is, however, considerable geological variability in the soils, so that archaeologists must be extremely careful in making interpretations about the relative importance of plants versus meat in the diet. Testing of herbivorous and carnivorous animals, and plant foods if available, from the same area is highly recommended. In addition, care is needed in taking samples from bone with minimal contamination or diagenesis (Burton & Price 2000). Most elemental analyses of bone have been done by ashing the sample, dissolution, and using an ICP spectrometer; some more recent

Bone Chemistry and Ancient Diet, Fig. 6 Stable isotope mass spectrometers in the Paleolab at the University of South Florida connected (**a**) to a CHN analyzer for collagen and other organic samples; (**b**) to a Kiel III acid bath for bone apatite, enamel, and other carbonate materials

studies where sample removal was not allowed have been done nondestructively using an X-ray fluorescence spectrometer (Fig. 7).

Trace element measurements of bone are reported relative to calcium, e.g., Sr/Ca and Ba/Ca, or logarithmically, e.g., log (Ba/Sr). Much greater differences in the Sr and Ba concentrations and in the Ba/Sr ratios have been observed for marine versus terrestrial diets.

Recent Applications

Stable isotope analysis of human remains to study ancient diets has expanded considerably in the last decade, with several dozen or more publications just in the last few years. The main issues being addressed include:

- Early hominin dietary practices
- Seafood consumption by near-coastal populations

Bone Chemistry and Ancient Diet, Fig. 7 Nondestructive trace element analysis of bone using a portable X-ray fluorescence spectrometer

- Dietary contribution of freshwater resources from lakes and rivers
- Mesolithic-Neolithic dietary changes
- Importance of millet in east Asia, Africa, and Europe
- The spread of maize in the New World
- Dietary differences based on sex and/or status
- Weaning practices
- Mobility and migration

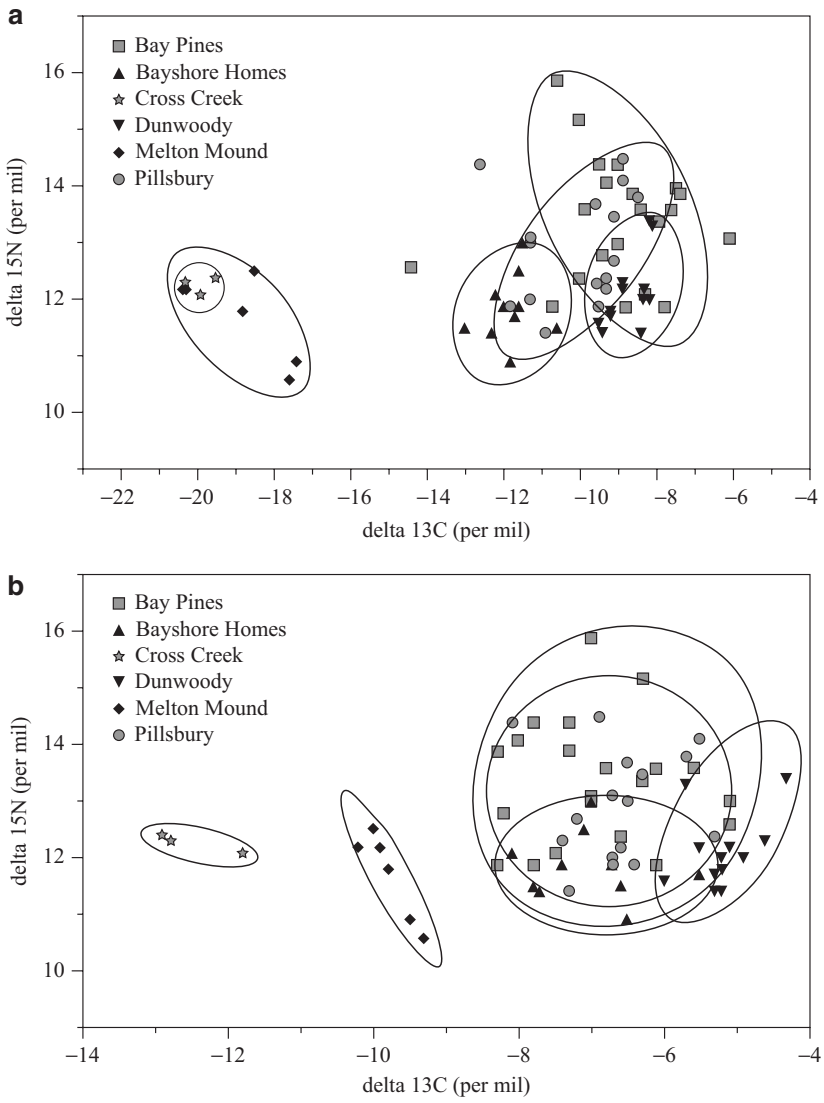
Following is an overview of two areas of my own research, specifically the importance of maize and seafood in different areas of peninsular Florida and the dietary practices of coastal and island inhabitants in the Mediterranean.

Florida

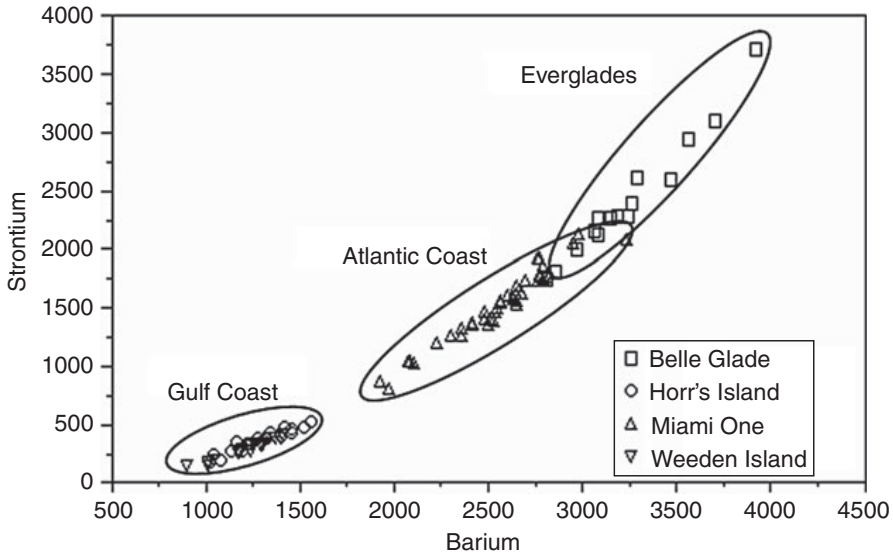
Bone collagen and apatite for more than 100 human individuals from 7 sites in peninsular Florida were analyzed by stable isotope mass spectrometry to test for the presence and increasing importance of maize in the southeastern United States (Tykot et al. 2005; Kelly et al. 2006). Previous studies, mostly on bone collagen, had clearly indicated maize was a dietary staple for the Mississippian culture by about 1000 CE, but it had been thought that maize may never even have reached southern Florida. At the inland site of Melton Mound, however, positive carbon isotope ratios especially for bone apatite and tooth enamel fully support that C_4 plants such as maize were a small but noticeable part of the diet by 600–800 CE. Four sites near the Gulf Coast were tested, with individuals from Bayshore Homes (Safety Harbor period) and Dunwoody (Late Caloosahatchee) averaging much lower $\delta^{15}N$ in bone collagen and more positive $\delta^{13}C$ for bone apatite, relative to the earlier Bay Pines (Weeden Island I) and Pillsbury (Manasota) sites, while the $\delta^{13}C$ results for bone collagen were similar for all four (Fig. 8). This strongly supports that it was a plant such as maize (with much less protein than fish but having an equal impact on apatite $\delta^{13}C$ values) did become a dietary staple in this region by the early 2nd millennium CE. For all sites, however, there was also a large range of variation among individuals, and at least at one site, it seems that males had higher nitrogen isotope values than females, perhaps due to greater fish consumption. This is being investigated further through elemental analysis of Ba and Sr, which in a separate study has shown a much greater importance of seafood at sites on the Gulf Coast when compared with those near Miami on the Atlantic side of Florida (Fig. 9).

The Mediterranean Region

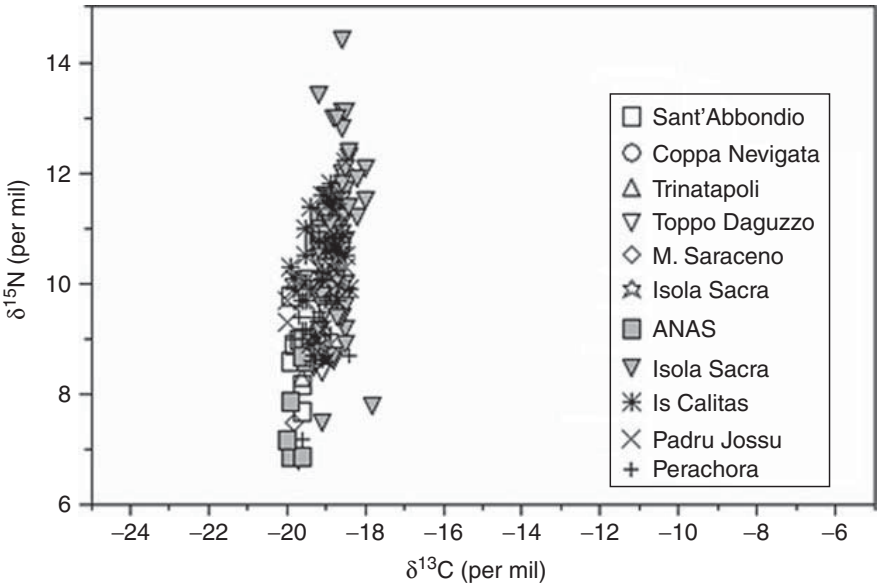
In the Mediterranean world, while seafood is a regular part of many modern people's diet, there is little in the way of shell mounds or other archaeological evidence that it was a staple in ancient times, even prior to the Neolithic. However, for Mesolithic sites on the Atlantic coast, including Portugal and Spain, isotope studies have shown that seafood was a major part of the diet (e.g., Richards & Hedges 1999). In contrast, an early study using both stable isotope and elemental analysis on Mesolithic coastal sites of Arene Candide in Liguria and Grotta dell'Uzzo in Sicily (Francalacci 1989; Mannino et al. 2011) suggested that seafood was



Bone Chemistry and Ancient Diet, Fig. 8 Stable isotope data for selected precontact archaeological sites in Florida. (**a**) $\delta^{15}N$ versus collagen $\delta^{13}C$; (**b**) $\delta^{15}N$ versus apatite $\delta^{13}C$



Bone Chemistry and Ancient Diet, Fig. 9 Barium versus strontium trace element data for selected Florida sites showing differences based on geographic regions



Bone Chemistry and Ancient Diet, Fig. 10 Human bone collagen $\delta^{13}C$ versus $\delta^{15}N$ for selected Bronze-Iron Age sites in the central Mediterranean

not a staple in the Mediterranean, and beginning in the Neolithic when there were domesticated sheep, goat, cattle, pig, wheat, barley, and other crops, studies by many scholars have shown it was negligible at sites in Italy, Croatia, Greece, Tunisia, and even on the islands of Sardinia (Lai et al. 2007), Malta, the Balearics, and Crete. There are some modest isotopic differences in diet based on status in the Late Bronze Age, e.g., at Mycenae (Richards & Hedges 2008), while freshwater fish become important at some sites, but even in Roman times when there was regular, large-scale maritime seafaring, it appears there was little seafood consumption even at the port site of Isola Sacra near Rome (Prowse et al. 2004; Killgrove & Tykot 2013) (Fig. 10). It is important, however, to have isotope data for fish and shellfish in the specific region of study since there are cases where their isotope values are rather negative and thus overlapping with terrestrial plant and animal values. African millet, a C_4 plant, was apparently present in Europe in the Neolithic, but not isotopically noticeable until the Bronze Age in northern Italy (Tafuri et al. 2009) and Iron Age at sites in Greece and Slovenia (Murray & Schoeninger 1988).

Future Directions

The analytical methods and examples presented above for human bone and other tissues do not include all of the scientific ways to study ancient diets. Stable isotope analyses have been done on individual amino acids in bone collagen, on cholesterol preserved in bones and teeth, on dental calculus, on carbonates and humic matter in soils, and organic residues (lipids) absorbed in pottery. Still being developed are other ways to study diet, including isotopic analyses of calcium, sulfur, and hydrogen, and decreasing even further the size sample necessary for analysis.

Acknowledgments I especially thank Nikolaas J. van der Merwe for getting me involved in isotope analyses more than 20 years ago, the dozens of colleagues I have worked with on six continents, and the many undergraduate and graduate students who have worked in my lab. For more than 10 years, most of my isotope samples have been analyzed in the Paleolab, managed by Ethan Goddard.

Cross-References

- ▶ Bioarchaeology in the Roman Empire
- ▶ Bioarchaeology, Human Osteology, and Forensic Anthropology: Definitions and Developments
- ▶ Bone: Chemical Analysis
- ▶ Isotope Geochemistry in Archaeology
- ▶ Isotopic Studies of Foragers' Diet: Environmental Archaeological Approaches
- ▶ Isotopic Studies of Husbandry Practices
- ▶ Maize: Origins and Development

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