Stable Isotope Analysis and Ancient Diets: They Are What They Ate

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Introduction

Stable carbon, nitrogen, and oxygen isotope studies have become widely applied to studies of ancient subsistence patterns, providing complementary information to faunal, paleobotanical and other dietary indicators (**Figure 1**). Using small samples of bone, teeth, and/or hair for this inexpensive analysis, the study of many individuals has often revealed dietary differences based on age, sex, status, and other sociocultural factors; local ecology; seasonal variation; and chronological change. Presented here is an overview of sampling and analysis methods; examples of studies on maize in the New World, millet in the Old World, and the importance of seafood; and suggestions for future research.

Archaeological Evidence for Subsistence

Faunal remains
Macrobotanical remains
Coprolites or stomach contents
Pollen
Phytoliths
Starch grains
Shell middens
Bone chemistry
Skeletal pathology
Pottery residues
Artistic depictions
Historical documentation
Plant impressions on ceramics
Evidence of land clearance, irrigation

Figure 1. *Isotope analyses strongly complement other evidence about ancient dietary practices*

Isotope Basics

Isotopes are elements which vary in the number of neutrons in their nuclei (**Figure 2**). The multiple isotopes of carbon (12, 13, 14), nitrogen (14, 15), and oxygen (16, 17, 18) affects the energy used in chemical reactions, resulting in variation in isotope ratios in different materials (Tykot 2004). Except for carbon 14, these are all stable isotopes which do not decay over time.

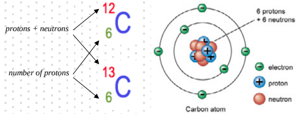


Figure 2. *Carbon atom and elemental signatures*

Carbon dioxide (CO_2) in the atmosphere consists of about 98.9% $^{12}CO_2$ and 1.1% $^{13}CO_2$ (plus radioactive $^{14}CO_2$ about 1 x 10^{-10} %). CO_2 is photosynthesized by plants and metabolized into complex molecular compounds that we categorize as **carbohydrates**, **proteins**, and **lipids**. Lighter isotopic compounds react faster, using less energy, resulting in changes in the $^{13}C/^{12}C$ ratio from the atmosphere to plants. This is called **isotopic fractionation**, and results in different carbon isotope ratios between plants with different photosynthetic pathways (C3, C4, and CAM), and this difference is passed on to their consumers (**Figure 3, right**).

Stable **nitrogen ($^{15}N/^{14}N$) isotopes** vary between nitrogen fixing and non-fixing plants, and along with **oxygen ($^{18}O/^{16}O$) isotope ratios** also vary based on climate and altitudinal effects and between terrestrial, freshwater, and marine ecosystems. When plant and animal foods are consumed, the metabolic processes involved fractionate the isotope ratios, increasing the proportion of the heavier carbon (and nitrogen) isotopes remaining in the body tissues. When carbon and nitrogen isotopes are graphed together, several different food groups may be differentiated (**Figure 4, right**).

Carbon, oxygen, and nitrogen isotope ratios are reported using the **delta (δ) notation**, in **parts per mil (‰)** relative to the PDB (C, O) and AIR (N) standards. Plant and animal compounds simply have less of the heavier isotopes than atmospheric CO_2, resulting in relative isotope ratios that are negative.

Figure 5a,b. *Isotope studies in the New World distinguish maize from other plant foods (above); in the Old World most studies focus on the importance of aquatic resources, and the use of millet and secondary animal products (below).*

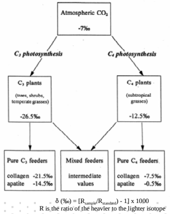


Figure 3. *Carbon isotope fractionation from atmospheric to plants to consumers*

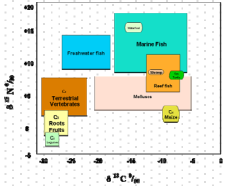


Figure 4. *Graph showing the carbon and nitrogen isotope values associated with several food groups. Using more specific values for particular localities is recommended.*

Stable Isotope Studies of Human Diets

Isotope analyses to reconstruct human dietary patterns have now been going on for over thirty years (Vogel & van der Merwe 1977; see Tykot 2006). Laboratory-based and other isotope studies have specifically determined that **bone collagen** (the organic portion, consisting of many different amino acids) is produced mainly from dietary protein, while **bone apatite** (the mineral portion, consisting of calcium hydroxyphosphate/carbonate) represents the whole diet (Ambrose & Norr 1993; Tieszen and Fagre 1993). Turnover rates for bone are slow, so that the isotope values obtained represent at least the last several years of an individual's life. **Tooth enamel and dentin**, while composed of the same material as bone apatite and collagen, respectively, do not turn over, so their isotope values reflect the specific time period of their formation regardless of the age at death (e.g. Wright 1999). Isotope studies on enamel have produced reliable data going back millions of years (e.g. Lee-Thorp 2000). **Hair** and teeth both grow sequentially, so that microsampling may be used to address short term isotopic variation (e.g. O'Connell & Hedges 1999; Balasse 2002).

Overall, stable isotope analysis is able to quantify the proportions of C3 and C4 plants (e.g. maize) and their consumers in human diets, compare terrestrial vs. freshwater vs. marine resources, and reveal whether there were systematic differences between individuals based on geography, chronology, social status, and/or sex, in both the New and Old Worlds (**Figure 5, left**).

Figure 6. *Sample selection and preparation in the Laboratory for Archaeological Science at the University of South Florida. One gram of bone is used to produce collagen samples for analysis, and less than 10 mg for apatite and tooth enamel .*

Sample Preparation and Stable Isotope Analysis

Less than **one gram** of bone is necessary to extract sufficient collagen (a 1% yield is considered reliable) for stable isotope analysis, and may generally come from any part of the skeleton. Much smaller samples of well-preserved tooth dentin may also be tested, while for apatite and enamel analyses only several **milligrams** are needed. Overall, isotopic analysis is **minimally destructive** to skeletal remains.

At the University of South Florida, sample preparation is done using well-established laboratory procedures (**Figure 6, above**). Bone collagen is extracted by demineralizing whole bone using 2% HCl for 72 hrs, dissolving base-soluble contaminants using 0.1 M NaOH (24 hrs before and after demineralization), and separating residual lipids with a mixture of methanol, chloroform and water for 24 hrs.

Collagen **pseudomorphs** are analyzed for carbon and nitrogen isotopes using a CHN analyzer coupled with a Finnigan MAT Delta Plus XL stable isotope ratio mass spectrometer set up with a continuous flow (**Figure 7, below**). Along with visual analyses and data from the sample preparation, **C:N ratios** of the analyzed gases are calculated to determine the preservation of collagen and the reliability of the isotope results.

Carbonate from apatite and enamel samples is also extracted using established techniques, with the removal of organic components using bleach (24 hrs for enamel, 72 hrs for apatite), and of non-biogenic carbonates using buffered 1 M acetic acid (24 hrs). These samples are introduced to a second Delta Plus XL mass spectrometer using an automated Kiel III device.

Figure 7. *Marine Sciences Laboratory at USF St. Petersburg. From left: one mass spectrometer, with CHN analyzer for organic samples; second mass spectrometer, with Kiel device for inorganic carbonate samples. Both systems allow the input of several dozen samples and standard reference materials*

Applications – Latin America

In the last fifteen years I have worked on a large number of isotope projects, on all continents. Illustrated here are just some examples of studies done in Belize, Mexico, Guatemala, Peru, Chile, Argentina, Florida, Italy, and China. Most of these have been published or are in press.

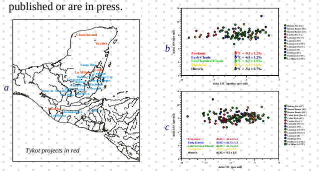
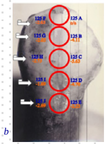
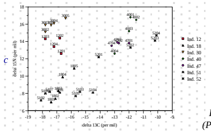
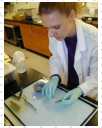


Figure 8a-c. *A large number of sites have been done now in Belize and Guatemala, but relatively few in Mexico and Honduras. A comparison of results from different time periods suggests a significant jump in maize in the overall diet by the Early Classic, and possibly as a protein source by the Postclassic.*



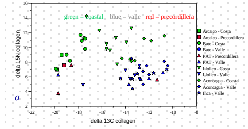
Micro-sampling of prehistoric Ecuador incisor shows 4‰ carbon range





Hair segments from Chongos (Peru) show C and N differences

Figure 9a-d. *In South America, a number of major isotope studies have been done in recent years, at least along the Andes and in Patagonia (a). Microsampling of teeth (b) from highland Ecuador, and of hair (c, d) from Peru show significant short-term dietary variation.*



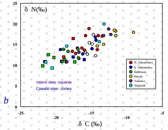


Figure 10. *A regional study in central Chile (a) shows geographic and chronological differences, with maize a staple in the PAT, Llolleo and Aconcagua periods. In prehistoric Patagonia (b), the relative importance of seafood and guanaco corroborates some ethnohistoric accounts.*

Applications – Old World

In Europe, it was only in the Iron Age that a C4 plant, millet, was introduced from Africa, so studies on earlier periods have focused entirely on the importance of seafood and other aquatic resources. In east Asia, another variety of millet was locally domesticated by the beginning of the Neolithic.

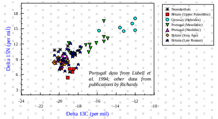


Figure 11. *Several studies have shown that in the Atlantic, seafood was only important in pre-neolithic times.*

Figure 12. *A number of projects done in the Mediterranean also suggest that seafood was never a dietary staple, even on islands.*

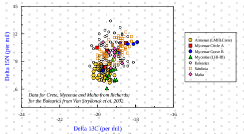


Figure 13. *Isotope data available for inland and coastal sites in Italy, and on Sardinia, Malta, the Balearics, and Crete, show little if any seafood consumption. For the inland site of Mycenae, however, the highest status individuals appear to have had some access to seafood.*

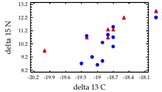


Figure 14. *On Sardinia, at the Bronze Age site of Padru Jossu, there appears to be small but measurable male-female differences in diet.*

Applications - North America

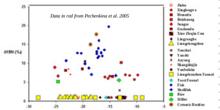


Figure 15. *In northern China, millet appears to have decreased in importance in later periods, perhaps with the expansion of rice from the south. The values shown for Liangchengzhen are based on apatite (hence no nitrogen isotope values).*

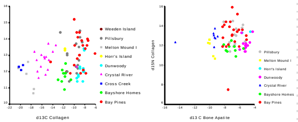


Figure 16. *Many studies have been done in North America, mainly looking at the spread of maize from the southwest to as far as Canada. One of the studies done at USF compared inland, riverine, and coastal sites in peninsular Florida, with the bone apatite values suggesting the consumption of maize by 600 AD.*

Other Stable Isotope Studies

Other isotope analyses recently applied to archaeology include specific studies on soils (e.g. Webb et al. 2004); movement and migration using oxygen (e.g. White et al. 2000) and strontium (e.g. Price et al. 2002) isotopes; and compound specific analysis of human bones (e.g. Howland et al. 2003) and pottery residues (e.g. Reber & Evershed 2004). All of these isotope methods may be used to address significant questions we have about ancient societies in many parts of the world.

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