ARTICLES

Stable Isotope Studies in Human Evolution

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The discipline of human evolution usually involves the evaluation of changes in gross and molecular morphology or changes in artifact assemblages. In contrast, stable isotope analysis is an indirect line of investigation. Understanding the human evolutionary sequence requires information on nutritional, biobehavioral, and general ecology. These are the kinds of information that stable isotope analysis can provide. Such studies may not identify the mechanisms for change, but their application serves to elucidate the situations under which change occurred.

Stable isotope analysis has been coopted from other sciences. Its theoretical base is in physics and its early applications were in geochemistry. Consequently, it melds aspects of an historical science (geology) with aspects of an experimental science (chemistry) and a more theoretical science (physics). Such cooption is common in the study of human evolution because its historical nature requires scholars to compile observations and to develop and apply new methods of investigation.

The purpose here is to introduce the reader to those areas of bioarcheology and paleoanthropology that have made use of stable isotope analyses.[1-7] Although there have been recent applications to the earlier part of the record, the majority of stable isotope studies have focused on the latter portion of human evolution and thus have been restricted to the bioarcheological and archeological literature. First, however, it is necessary to include an introduction to the processes through which isotopic inferences are made.

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Key words: Carbon, nitrogen, oxygen strontium, nutritional ecology, omnivory, paleoecology

ISOTOPES AND ISOTOPIC RATIOS

Organisms are composed of common elements such as hydrogen (H), carbon (C), nitrogen (N), oxygen (O), sulfur (S), fluorine (F), calcium (Ca), and phosphorus (P), along with less common ones like strontium (Sr). Many of these elements occur in more than one form that are called isotopes, i.e., ^{12}C and ^{13}C are carbon isotopes. Isotopes of an element all have the same number of electrons and protons, but differ in the number of neutrons in their nuclei. Among isotopes of a single element, those with even numbers of neutrons are much more abundant than are ones with odd numbers because of the way these isotopes are synthesized in stars. The percentages of elements shown in Table 1 are the natural abundance ratios found on earth. Although unstable radioactive isotopes also occur, stable isotopes of elements are my focus here. For elements like strontium and carbon, that have both stable and radioactive isotopes, I will discuss only the stable isotopes.

Different isotopes of an element share the same chemical properties because chemical reactions are determined largely by electron configuration. Isotopes differ from each other in mass, however, because of the different numbers of neutrons. ^{1}H refers to the stable isotope of hydrogen which contains one neutron; ^{2}H (commonly called deuterium and represented as D) contains two neutrons, has a greater mass, and is referred to as the "heavier" isotope. Among the elements with lighter overall masses (i.e., hydrogen, carbon, nitrogen, oxygen, and sulfur) the mass differences between isotopes translate to differences in rates of **reaction**. For example, ^{12}C reacts faster than ^{13}C. Chemical bonds of "lighter" isotopes break and form more rapidly than do bonds of "heavier" isotopes (e.g., ^{12}C–^{14}N bonds break and form more rapidly than do ^{12}C–^{15}N bonds). These **reaction rate** differences result in isotope ratios in a **product** that are different from those of the starting components or the **substrate** of a reaction. For example, bone collagen is a protein made up of individual amino acids, each of which is a product resulting from reactions on a substrate composed of food and breakdown products from an animal's own tissues. The $^{15}N/^{14}N$ ratio of nitrogen in bone collagen (the product) is different from the ratio in all the nitrogen in the animal's diet and breakdown products (the substrate). Bone collagen normally has relatively more ^{15}N than is in the diet because relatively more ^{14}N is excreted. Hence, bone collagen is enriched in ^{15}N relative to its substrate, the diet, whereas excretion materials are depleted in ^{15}N. Whether the product is enriched or depleted with regard to the heavier isotope depends on the particular set of reactions necessary for its synthesis. Similarly, the $^{18}O/^{16}O$ ratio in bone mineral (the product) differs from the $^{18}O/^{16}O$ ratio in the animal's body water which is the substrate of the oxygen in bone mineral.

In the cases of collagen and bone mineral, the difference in isotope ratio between product and substrate is called **fractionation**. For collagen synthesis, enzymatic control determines the magnitude of the fractionation; this is a case of **kinetic isotope**

TABLE 1. Stable Isotopes Used in Evolutionary Studies

Element	Isotopes	Natural Abundance (In Percent)	Isotope Ratio Measured
Hydrogen	^{1}H	99.985	D/H
	^{2}H (D)	0.015	
Carbon	^{12}C	98.900	$^{13}C/^{12}C$
	^{13}C	0.100	
Nitrogen	^{14}N	99.640	$^{15}N/^{14}N$
	^{15}N	0.360	
Oxygen	^{16}O	99.800	$^{18}O/^{16}O$
	^{17}O	0.040	
	^{18}O	0.200	
Strontium	^{84}Sr	0.560	$^{87}Sr/^{86}Sr$
	^{86}Sr	9.870	
	^{87}Sr	7.040	
	^{88}Sr	82.53	

Taken from Burlingame and Schnoes.[151]

fractionation. For bone mineral synthesis, the magnitude of fractionation is determined only by the temperature of the reaction; this is referred to as an **equilibrium isotope fractionation** [Box 1].

Among heavier elements like calcium and strontium, the mass difference between the isotopes is small compared to the overall mass of the element. Thus, the rate of reaction is essentially the same for all isotopes of these elements. Within strontium, ^{86}Sr and ^{87}Sr have the same rates of reaction and, unlike the situation with lighter elements, metabolic reactions such as photosynthesis or bone mineral synthesis do not change the $^{87}Sr/^{86}Sr$ ratio of the product (plant tissue or bone mineral). Instead, the ratio of $^{87}Sr/^{86}Sr$ in biological tissues directly reflects the substrate, which for strontium is a rock.

With the exception of strontium, processes that result in the transfer of elements from the geosphere to the biosphere as well as between different compartments of each sphere—for example, the transfer of carbon from the ocean to the atmosphere or from plant tissue to animal tissue—are associated with sequential changes from the natural abundance isotope ratios through kinetic and equilibrium isotope fractionation. These changes, however, are small so that direct reporting of isotope ratios is impractical. For this reason, isotope ratios are represented as δ values in which the isotope ratio within a sample is compared to the ratio of an internationally recognized standard [Box 2].

Stable isotope ratios serve as probes of various aspects of paleoecology and human behavior, and thus provide additional understanding of events that are documented by other paleoanthropological and bioarcheological studies. For example, the parameters of the temperature and humidity of an environment are encoded in the oxygen stable isotope ratios of bone mineral and in the hydrogen isotope ratios (D/H) of the nonexchangeable hydrogen atoms in bone collagen. The type of plant cover is reflected in the amount of carbon isotope ratios in paleosol carbonate nodules and soil organic matter. Human migration patterns can be monitored by using strontium isotope ratios. Human and other animal diets can be estimated using the carbon, nitrogen, and sulfur stable isotope ratios of various body tissues, although sulfur is seldom used as there is so little of it in bone tissue.[8]

PROPERTIES OF FIVE STABLE ISOTOPES

Carbon Isotope Ratios: $\delta^{13}C$

The majority of the world's active cycling carbon is sequestered in the ocean as dissolved carbonate. During the exchange of this oceanic carbon pool with atmospheric carbon dioxide (CO_2), ^{13}C in the atmosphere is depleted by equilibrium isotope fractionation.[2] The carbon source for all terrestrial plants is atmospheric CO_2. During the transfer of CO_2 from the atmosphere to plant tissue there is a further depletion of ^{13}C through kinetic isotope fractionation during photosynthesis. Plant $\delta^{13}C$ values are determined by the photosynthetic pathway as well as by source carbon. The pathways[9] are commonly referred to as C_3, C_4, and the less common Crassulacean Acid Metabolism (CAM), which was identified in and is mainly studied in succulents. Today, C_4 plants have $\delta^{13}C$ values that cluster around a value of −12°/oo, whereas most C_3 plant species have values around −26°/oo (Fig. 1). In temperate regions, the majority of plants are C_3 although some of the grasses may be C_4. In contrast, vegetation in tropical regions can be divided into C_3 herbaceous vegetation and trees, and C_4 grasses.[10] Certain C_4 plants, such as the tropical cultigens maize, millet, and sorghum, have been adapted to temperate regions through human manipulation.[11] CAM plants in hot, arid areas have $\delta^{13}C$ values like those of C_4 plants. Marine planktonic species tend to have $\delta^{13}C$ values that are intermediate between the extremes delineated by terrestrial C_3 and C_4 plants because their substrate carbon and their process of carbon uptake differ from terrestrial plants.[12] However, the $\delta^{13}C$ values of prehistoric plants are approximately 1.0% higher than those of modern plants because our current atmosphere, in relation to that prior to the 20th century, is enriched in ^{12}C. This change in $\delta^{13}C$ values is the result of large-scale combustion of C_3 biomass (trees) and fossil fuels by humans.[13–15]

The $\delta^{13}C$ values of animals are positively correlated with the values of their substrate diet.[16] Thus, the isotope signal from C_4 or C_3 plants is recorded in animals that feed on one or the other of these plant types. Marine vertebrates tend to have $\delta^{13}C$ values between these extremes (see Fig. 1). Similarly, the isotope signal from C_4 or C_3 plants may be directly recorded in human tissues by a diet of those plants, or indirectly by a diet that includes meat. Thus, if humans eat browsers there will be a C_3 isotope sig-

Box 1. Understanding Stable Isotopic Reactions

Substrate: the starting components or source of a chemical reaction involving stable isotopes of elements. For example, the food that an animal eats is a substrate; the ocean is the source of atmospheric carbon.

Reaction: The process by which stable isotopes are altered. For example, digestion and enzyme processes, photosynthesis, etc.

Reaction Rate: The speed at which chemical bonds break among isotopes of an element. The smaller the number of neutrons, that is, the lighter the mass of the isotope the faster the reaction rate. For example, ^{12}C reacts faster than ^{13}C.

Product: The result of a reaction on a substrate. For example, bone collagen (the product) is the result of enzyme reaction on food (the substrate).

Fractionation: The difference between stable isotopic ratios in the substrate and the product. That is, due to a reaction on the substrate, the isotopes of an element may be found in different proportions in the product.

Kinetic Isotope Fractionation: Fractionation in which the reaction is caused by the addition of biochemical properties.

Equilibrium Isotope Fractionation: Fractionation in which the reaction is caused by a physical property, such as temperature, evaporation, evapotranspiration.

nal; if they eat grazers, there will be a C_4 isotope signal. In addition, a marine signal is transferred to humans who eat marine organisms. Because marine organisms have values that are intermediate between terrestrial C_3 and C_4 values, the diet of prehistoric human populations that had access to marine foods as well as to C_3 and C_4 plants is indeterminate using $\delta^{13}C$ values alone.

Nitrogen Isotope Ratios: $\delta^{15}N$

The major nitrogen reservoir is the atmosphere rather than the ocean. The transfer of inorganic nitrogen (N_2) into the biological realm depends on specialized organisms such as those found in bacterial nodules on the roots of leguminous plants. Because this occurs with little or no fractionation, many legumes have $\delta^{15}N$ values that are similar to that in the atmosphere (i.e., 0.0°/oo). The majority of plants, however, take up soil nitrogen made available through bacterial degradation of organic material. This tends to generate $\delta^{15}N$ values in plants that are more positive than atmospheric nitrogen. Thus, terrestrial plants from natural habitats display a bimodal distribution of nitrogen stable isotope ratios[17] with nitrogen-fixing plants such as peas, beans, mesquite, and acacia forming one node and nonfixers forming the other. Marine organisms tend to have more positive $\delta^{15}N$ values than do terrestrial organisms (see Fig. 1) because the majority of usable nitrogen results from bacterial activity and because it is such a limiting nutrient in the ocean.

The $\delta^{15}N$ values in the tissues of animals are positively correlated with the values in their diets.[18,19] There also is a step-wise increase in delta values from one trophic level to another (see Box 3). In most areas of the world, marine vertebrates have $\delta^{15}N$ values that are 6 to 8°/oo more positive than are the values in terrestrial vertebrates at similar trophic levels[12] (see Fig. 1). Humans with a significant marine adaptation record the $\delta^{15}N$ marine signal in their tissues.[20] Exceptions to this general rule occur in desertic areas where water or caloric stress affects metabolism[12,21-23] and in warm-water reefs or areas with shallow coastal margins where blue-green algae directly fix atmospheric nitrogen.[24,25]

Hydrogen Isotope Ratios: D/H (δD)

Hydrogen is ubiquitous throughout the geosphere. The large relative difference in mass between its stable isotopes means that hydrogen exhibits the largest fractionations for the light stable isotopes.[2] Huge fractionations take place as a result of equilibrium isotope fractionation in the transfers of water from the ocean to the atmosphere and to precipitation. As a result, the δD of rainwater and environmental water vary in association with temperature: waters in the higher latitudes and altitudes are significantly depleted in D relative to waters in the tropics and low altitudes.[26] Plant tissues, although enriched in D relative to environmental water,[27] track these differences.[28] When plants of the same physiognomic group are compared, they are also found to track relative humidity.[29] The growth rings of bristlecone pine trees show a decrease in δD between 6,800y BP and 2,000y BP as a consequence of climatic cooling.[30] Further, the δD values of the nonexchangeable hydrogen atoms in deer bone collagen reflect the δD in plant cellulose, which, in turn, reflects temperature[31] when relative humidity is independently evaluated.[32]

The CAM δD values of plants such as cacti can differ in values from those of C_3 to C_4 plants growing in the same area.[29] Furthermore, the δD of bean shoots differs from that of maize shoots grown under identical conditions.[28] At this stage, the processes responsible for these differences are poorly understood; thus, the generality of these observations is unknown. If these differences prove to be general, it may be possible to distinguish between cactus and maize/bison dependence and to identify the use of beans as a protein source among prehistoric human populations in the southwestern United States.

Oxygen Isotope Ratios: $\delta^{18}O$

Oxygen is one of the world's most abundant elements. Of its three stable isotopes (see Table 1), the $^{18}O/^{16}O$ ratio is usually measured because it affords a greater mass difference than the others do and because ^{18}O is far more abundant than is ^{17}O. Like δD, $\delta^{18}O$ values of rainwater and environmental water vary due to temperature.[33] Carbonate and phosphate oxygen reflect these variations, as expected, by equilibrium isotope fractionation. In oceans, where the source water ratio is known, paleotemperature can be calculated from the oxygen isotope ratio in non-biological minerals and the biogenic minerals of animals that do not have a constant body temperature.[34,35] Within continental interiors, however, the composition of

Box 2. Measuring δ With Relation to an International Standard

Relational measurements are made using a mass spectrometer (for greater detail see Schoeninger and Moore[6]). For light elements, the material of interest (collagen, shell carbonate, soil organics) is either combusted at high temperatures or chemically treated to liberate a gas such as hydrogen (H_2), carbon dioxide (CO_2), or nitrogen (N_2). Strontium isotope ratios are measured on a different kind of mass spectrometer using a solid sample. In all cases, the isotope ratio of the sample introduced into the mass spectrometer is measured relative to a laboratory standard of the same chemical composition (a sample of CO_2 is measured against standard CO_2). Each laboratory has its own internal standards, which means that isotope ratios vary between laboratories. To standardize values so that measurements between laboratories can be directly compared, the laboratory standards are calibrated relative to internationally recognized standards for hydrogen, carbon, nitrogen, and oxygen. The international standard is seldom analyzed directly, but the mass spectrometers are attached to computers with the software necessary to perform calculations comparing the isotope ratios measured in the laboratory to the international standard. The value presented in manuscripts is a δ value defined as:

$$\delta = \left[\frac{R_{sample}}{R_{std}} - 1\right] \times 1000\,‰$$

where R is the isotope ratio (e.g., $^{13}C/^{12}C$) and the std is the internationally recognized standard.

A δ value that is negative indicates that the sample is depleted in the heavier isotope relative to the internationally recognized standard; one that is positive indicates that the sample is enriched relative to the standard. Due to historical accident, the international standard for carbon, Pee Dee Belemnite marine carbonate (PDB), has more ^{13}C relative to ^{12}C than is true for the vast majority of biological samples. Thus, biological samples have carbon δ values that are negative. The opposite is true for nitrogen: the majority of biological samples contain relatively more ^{15}N than does the standard, which is atmospheric nitrogen, formally referred to as Ambient Inhalable Reservoir (AIR). Thus, most biological samples have nitrogen δ values that are positive. For hydrogen, the standard is Standard Mean Ocean Water (SMOW). For oxygen, SMOW is the generally accepted standard, although values sometimes are given relative to PDB. The δ values are written for the heavier isotope as follows: δ D (for hydrogen or δ^2H), $\delta^{13}C$ for carbon, $\delta^{15}N$ for nitrogen, and $\delta^{18}O$ for oxygen. There is no internationally recognized standard for strontium; the values may be presented as ratios ($^{87}Sr/^{86}Sr$) or as $\delta^{87}Sr$ relative to an identified laboratory standard.

environmental water usually is unknown and organisms lacking constant body temperature are less useful. However, modern birds have a constant temperature during gestation, and eggshell carbonate $\delta^{18}O$ values vary across climate regimes.[36,37]

The $\delta^{18}O$ values in bone and tooth enamel phosphate also show promise. Among mammals that obtain their water by drinking, the oxygen isotope ratios in bone phosphate associate linearly with the ratio in rainwater.[38] In addition, ratios obtained from human tooth enamel have been shown to vary with latitude.[39] Further, modern animals that obtain a significant amount of their body water from leaves have $\delta^{18}O$ values that vary with average humidity.[40] A recent study that should stimulate further investigation used the combination of δD and $\delta^{18}O$ values in modern deer bone to estimate humidity.[32] The applications have been extremely limited thus far because of the difficulty of sample preparation. Once such technical barriers have been eliminated,[41] this promises to be an important means of climate reconstruction.

Strontium Isotope Ratios: $^{87}Sr/^{86}Sr$

The distribution of stable isotope ratios of strontium ($^{87}Sr/^{86}Sr$) differs from that of the more familiar strontium concentration or strontium/calcium ratio in biological systems. Whereas the latter two vary as a function of trophic position,[42,43] the ratio $^{87}Sr/^{86}Sr$ reflects the source strontium. Very old rocks contain significantly more ^{87}Sr than do rocks of recent origin because the isotope is a product of the long-term decay of the radioactive isotope ^{87}Rb. Rocks serve as the source of the strontium in soil and groundwater that is taken up by plants and plant tissue. The $^{87}Sr/^{86}Sr$ ratios in those plants reflect whatever rocks served as sources. The $^{87}Sr/^{86}Sr$ ratio in animal bone, which is the same as that of the plants in the animal's diet, also reflects the source rock because there is no biological fractionation of strontium isotope ratios. If children or adolescents moved from an area characterized by ancient rocks to one with recent rocks, their teeth, formed early in life, should have a different ratio than that in bone formed later in life, and would thereby record the migration.[44]

MATERIALS ANALYZED AND THE POTENTIAL FOR DIAGENESIS

Bones and teeth, because of their ubiquity in the fossil and prehistoric record, are the biological tissues most often analyzed. These complex tissues have two major components, the organic matrix and the inorganic mineral fraction. More than 90% of the organic matrix is the protein collagen. Noncollagenous proteins are also present,[45] but have been given limited attention[46] because they occur in such low quantities. The major fraction of bone and tooth mineral is calcium phosphate, although up to 8% by weight is carbonate.[47] Stable isotope studies use carbon, nitrogen, and nonexchangeable hydrogen in collagen, carbon bound within the crystal lattice of bone mineral, oxygen in phosphate and carbonate, and strontium bound to phosphate.

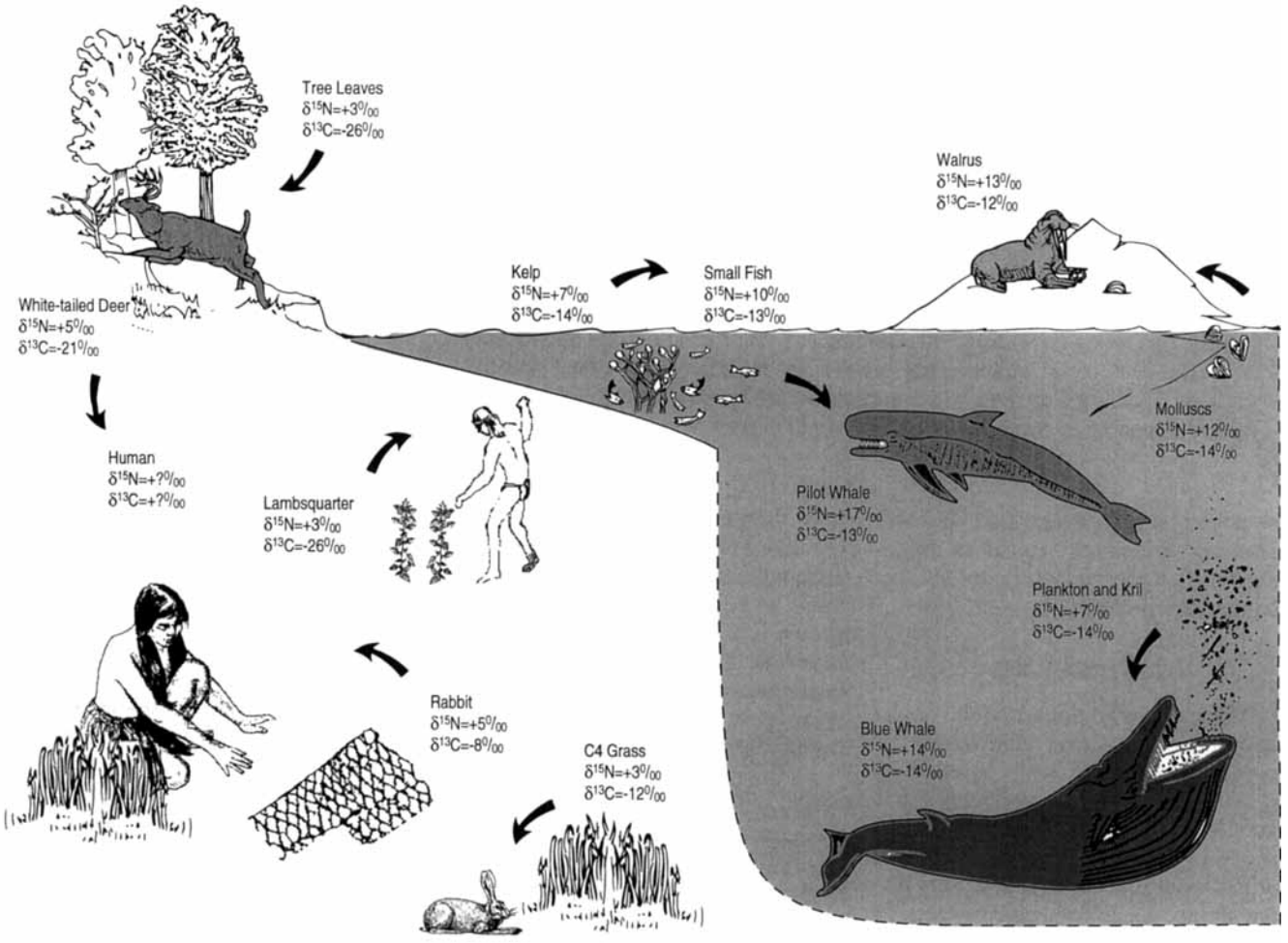

Figure 1. Average $\delta^{13}C$ and $\delta^{15}N$ values in tissue extracted from plants and animals in an ideal ecological system. Marine organisms differ from terrestrial organisms in both $\delta^{13}C$ and $\delta^{15}N$ values. There is also a step-wise increase in $\delta^{15}N$ values between levels in a trophic pyramid. Within the terrestrial system, $\delta^{13}C$ values distinguish between plants such as tropical grasses, which use the C_4 photosynthetic pathway, and herbaceous plants such as trees and lambsquarter, which use the C_3 photosynthetic pathway. These differences are passed to the animals that feed on them, as shown by the difference in $\delta^{13}C$ values of the deer and the rabbit. Maize, millet, and sorghum are examples of domesticated C_4 plants; wheat, rice, manioc, and rye are all C_3 plants. Humans are shown with question marks because their values will vary according to their diets.

The $\delta^{13}C$ values in bone collagen appear to record different aspects of diet than do those in the mineral fraction. Bone mineral carbonate is in equilibrium with CO_2 dissolved in blood and thus should reflect the total metabolic carbon pool which consists of diet plus tissue breakdown products. Collagen, however, should predominantly reflect the protein and, to a lesser extent, the carbohydrate fraction.[48] Recent results of feeding experiments largely support these expectations.[49,50] Tissues other than bone have been analyzed less often but can be very useful. Hair, fingernails, skin, and muscle—all proteins containing carbon, nitrogen, and hydrogen—can be analyzed in the same manner as is bone collagen.[51]

The carbon in soil carbonates and paleosol organic matter can serve as ecological indicators. Soil carbonate $\delta^{13}C$ reflects the dissolved carbonate components in groundwater, which, in turn, reflects the plant biomass at the time of soil formation.[52] Soil organics also record the carbon isotope ratio of the plant cover.[53]

All of these materials are subject to compositional changes (diagenesis) after formation. The processes involved, however, differ from material to material as well as between different elements of a single material. For example, the carbon and nitrogen atoms within intact collagen are not subject to exchange, but about 20% of the hydrogen atoms are exchangeable. Within the mineral phase, the carbonate fraction of bone is highly susceptible to diagenetic alteration,[54] but tooth enamel carbonate appears to have greater resistance.[55] On the other hand, the oxygen in tooth enamel carbonate may not be shielded from groundwater exchange.[56]

In most cases, the oxygen isotope record is better preserved in phosphates than in carbonates, although enamel must be screened to assure its integrity.[57] The strontium analyzed for ratios is subject to all the same diagenetic alterations that must be dealt with in trace element analysis.[58–61] At present there is no generally accepted method of assessing the integrity of the signal, although work on solubility profiles may ultimately provide one.[62] Soil carbonate nodules can be evalu-

Box 3. Nitrogen Uptake by Plants and Animals

In the transfer of nitrogen from plant to animal or from animal to animal, the amino acids of protein are always the substrate of nitrogen for its consumer's tissues. Because animals cannot metabolize N_2, inhaled nitrogen or nitrogen dissolved in water leaves no signature in an animal. Within individual systems, there is an increase in $\delta^{15}N$ values between levels in a trophic pyramid.[12] The tissues of herbivores that feed on plants are more enriched in ^{15}N than are plant tissues, and carnivore tissues are, in turn, more enriched in ^{15}N than is herbivore flesh. During metabolism, the bonds in amino acids containing ^{14}N break more readily than those containing ^{15}N. The result is that during energy metabolism a greater quantity of amino acids containing ^{14}N are broken down and the nitrogen is excreted as urea, which is depleted in ^{15}N.[149] As a consequence, the dietary amino acids available for protein synthesis are enriched in ^{15}N.

ated for potential alteration,[63] but soil organics are more subject to diagenetic alteration,[52] a fact that mandates caution in their use.

APPLICATIONS

Most, although not all, of the applications I will describe relate to diet reconstruction in one way or another. In part, this reflects the link between diet and tissue composition. It also reflects the recognition that the ways in which animals obtain access to necessary nutrients are the most basic aspects of behavior.

Introduction of Agriculture

Sidestepping the issue of domestication versus agriculture, carbon stable isotope analysis has revolutionized the study of the spread of maize, a C_4 plant, throughout the New World. Thanks to its many applications, there is a general understanding of the introduction and spread of maize. In Europe, Asia, and Africa, various combinations of $\delta^{13}C$ and $\delta^{15}N$ values have also been useful in addressing specific questions about domestication and agriculture.

MesoAmerica is the apparent site of the first domestication of maize in the New World, although its adoption as a staple varied by area. In the Tehuacan Valley of Mexico, the isotope data indicate that maize was used as early as 4,000 BC, even though midden analyses have indicated little dietary dependence on it.[18] In contrast, carbon isotopic results from the west coast of Panama dating to the same period indicate that maize was not a major diet item.[64] In Costa Rica, large-scale dependence on maize did not occur until approximately 300 AD.[64] Among the Maya of Belize, carbon isotopes have shown that the use of maize was less intensive than in Tehuacan throughout the entire prehistoric period[65] and apparently decreased during the Terminal Classic period, the time of maximum population size and maximum agricultural intensification.[66] On the west coast of Mexico, carbon isotopic investigations indicate that maize and alternative subsistence strategies are present in the same area.[67]

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In North America, where maize replaced previously domesticated C_3 plants, the timing of its adoption varied by region, probably reflecting the genetic alteration required for its adaptation to various climatic conditions. In the western interior at the Basketmaker II site of Cedar-Mesa in Utah, both the carbon isotope data and results from midden analyses argue for major dependence on maize by 2,000 years ago.[68] Applications have been hampered in this region because other food items such as bison meat, amaranth, and various types of cactus have $\delta^{13}C$ values that are similar to that of maize. This problem has been addressed at sites from the Late Archaic period of the Lower Pecos,[69] the Panhandle region of Texas and Oklahoma,[70] and at Pecos Pueblo in New Mexico.[71] In all of these, and in others where alternative sources have not been considered,[72] the $\delta^{13}C$ signal is consistently C_4.

In the eastern interior there are fewer confounding influences because marine foods and alternative C_4 plants were less available. For this reason, most studies have been done in combination with material from this area (see Schoeninger and Moore[6] for references prior to 1990). The carbon isotopic results from these studies indicate that maize was not a major diet item in the central portion of North America until about 900–1000 A.D. This contrasts with several accelerator mass spectrometry dates indicating its presence a thousand years earlier in the Middle Woodland period.[73] Perhaps earliest maize dependence is not recorded in the $\delta^{13}C$ signatures of collagen but only in those of carbonate.[74] Along the southeastern coast, $\delta^{13}C$ results show that maize was a staple at about 1,000 AD.[75,76] Little human dependence on maize occurred in New England until the time of European colonization.[25]

The $\delta^{13}C$-based information from South America also indicates variable timing in the shift to maize use. In the lowland regions of Venezuela, there was a significant change by 400 AD,[77] whereas in the Andean highlands of central Peru, maize was eaten as early as 400 BC,[78] but became a major crop only in the fifteenth century.[79] On the Peruvian coast, however, maize became a staple prior to 900 AD.[54,80] In coastal Ecuador,[81] poor bone preservation precluded application of isotopic techniques to the earliest periods, but data from the later Formative Period (1700 to 1100 BC) indicate that some maize was being used. The contemporaneous $\delta^{13}N$ values for the same region indicate marine dietary dependence. By the subsequent period (300 BC to 100 AD), the maize C_4 signal is strong and in association

with a marine signal.

In Europe, unlike the New World, both the majority of staple domesticates—barley, wheat, and rye—and the baseline environment are C_3. This has necessitated indirect and innovative measures to study the shift to agriculture. Along the coasts of Denmark, Portugal, and Britain, stable carbon and nitrogen isotope ratios document a change from marine fishing and gathering during the Mesolithic period to complete dependence on terrestrial food sources, presumably domesticated grains, during the Neolithic period and perhaps as early as 7,000 BP.[20,82-84] Millet, which is the only C_4 domesticate in the area, was documented by $\delta^{13}C$ measurements as human food in Iron Age Slovenia, a finding that is contrary to written records stating its use as animal fodder.[85]

Little work has been done in Asia, although carbon isotope data have corroborated archeological evidence for millet domestication in northern China as early as 7,000 BP.[86] This approach could prove useful in documenting the transition of subsistence based on tubers (C_3) to millet (C_4), then to rice (C_3) that is indicated in the prehistoric record, as well as for investigating social differences in access to rice.

The use of stable isotopes in monitoring domestication in Africa has not been extensive, mainly because the domesticates there include both C_3 and C_4 plants, as do the natural biomes. Carbon isotopic studies along the prehistoric Nile river have shown a switch from C_3 to C_4 plants in the human diet indicating the use of millet when the Nile was low (350 to 550 AD) and drought-like conditions favored this hardy grain.[51] Seasonal changes in diet are also recorded in the $\delta^{13}C$ signatures of hair.[51] In South Africa, isotope studies have documented dependence on C_4 crops like sorghum and millet, as well as on C_4-based domestic stock such as ovicaprines and cattle during the Iron Age.[87]

The promise of tracing the domestication of beans has not yet been fulfilled. Although legumes, in theory, should be depleted in ^{15}N relative to other terrestrial plants, in practice archeological beans have been reported to have $\delta^{15}N$ values similar to those of other plants.[5] Food residues on archeologically recovered cooking utensils have never been found to have $\delta^{15}N$ values of atmospheric nitrogen,[5] as would be expected if beans had been used. Bioarcheological studies that have interpreted decreases in $\delta^{15}N$ values of human bone collagen as indicating increasing dependence on beans[18,88] are highly speculative when applied to areas with soils rich in organics, the reason being that leguminous plants readily take up soil nitrogen when it is available.[89]

Box 4. Diet Isotopes and Amino Acids

The amino acids that make up dietary protein are either essential or nonessential to humans. Nonessential means that humans can synthesize them, whereas essential amino acids must come from diet. If nonessentials are available in the diet, they may not be synthesized either, but may be taken up directly from foods. Available data[150] indicate that increasing the protein proportion of a vegetarian diet does not result in a linearly increasing representation of protein in the consumers' bone collagen $\delta^{13}C$ values. In all likelihood the leveling of the curve is determined, at least in part, by the relative proportion of amino acids taken directly from diet versus those that are synthesized. Meat provides all the amino acids necessary for collagen synthesis, but plant proteins do not. Perhaps meat protein is overrepresented in bone collagen $\delta^{13}C$ and $\delta^{15}N$ values. In that case, these values may serve to monitor meat intake when corrections are made. More experimental work is required to fully realize this potential.

Social and Economic Implications of Diet Differences

Now that the general pattern of the introduction of maize into the New World has been established, other specific questions are being addressed by means of stable isotope studies. For example, differential access to maize has been documented within the social polity surrounding Cahokia, the large prehistoric complex near present-day St. Louis.[74] During the same period, chiefdom-level populations of the Ohio Valley were demonstrably more dependent on maize than were tribal-level populations in the same region.[90] A decrease in maize use accompanied the collapse of the polity controlled by prehistoric Moundville located in present-day Alabama.[91] Similarly, a switch to millet in prehistoric Egypt occurred at a time of political and climatic upheaval.[51]

Within-site differences in diet documented by isotopic studies have also proven useful as social and economic indicators. Among human skeletons from the civic-ceremonial center of Angel (1200–1450 AD) in southwestern Indiana, some burial positions have been associated with distinctive carbon stable isotope values.[92] One adult female, buried between the legs of an older adult male, displayed a pure C_3 signal in contrast to the strong C_4 signals of the remainder of the population. A similar juxtaposition has been reported from inland prehistoric Belize, where one male buried in a stone tomb displayed a nitrogen stable isotope signal indicative of marine foods.[65] Large ranges in both $\delta^{13}C$ and $\delta^{15}N$ have been reported for some prehistoric populations living at times when the archeological record indicates changes in subsistence that contrast with the lack of variation during periods of more stable adaptations.[75]

Use of Aquatic Resources

Several studies have used stable isotope analysis of carbon and nitrogen to assess dependence on marine and freshwater resources. The transition from the Mesolithic to the Neolithic periods in Europe occurred in association with a significant reduction in dependence on marine fish.[82] A similar change occurred on the east coast of North America when maize agriculture was introduced; another decrease in marine food use followed colonialization by the Spaniards.[75] In the coastal northeast of this continent,[25] and in the Bahamas,[24] isotopic ratios have been useful in distinguishing be-

tween the use of shallow-water fish and invertebrates versus deep-ocean fish and mammals.

Along the west coasts of North and South America, carbon and nitrogen isotope data document a decrease in dependence on marine foods as one moves inland from the coast.[80,81,93–95] Similarly, carbon isotope data from southern Australia have been interpreted as indicating less access to marine fish in noncoastal areas,[96] although some individuals may have had access to marine foods.[97] The same pattern occurs across the islands that make up present-day Japan, where the isotope signatures also show a greater use of marine foods on the northern islands than on the southern island of Honshu.[98–100] The dichotomy between coastal and inland data is repeated in South Africa, where there also is decreasing marine dependence through time.[101] An incidental finding is that those who ate larger amounts of seafood had a lower incidence of caries.[102] In contrast to South Africa, Britons of the Mesolithic period retained a strong marine signature even when their bodies were buried at some distance from the present coast.[83]

In some geographic areas, fresh water resources have also been monitored by the use of nitrogen stable isotopes. In prehistoric communities along the Great Lakes of North America,[103] the Black Warrior River in Alabama,[91] and near the Valdivia river in Ecuador,[81] the dependence on fish was detected because the fish in these waters had very high $\delta^{15}N$ values. In contrast, fish were eliminated as major food sources for recent prehistoric peoples in a swampy region of present-day Nevada and in northern Kenya near Lake Turkana[104,105] because the fish in these regions had $\delta^{15}N$ values that were too low to account for the values measured in human bone collagen.

Mobility Assessment

In some cases, stable isotope ratios have served to substantiate archeologically or ethnographically based expectations of population movements; in other cases they have served to question these expectations. An example of the latter is South Africa, where seasonal transhumance between the coast and inland areas was rejected on the basis of stable carbon isotope ratios.[101] Supporting evidence, however, was obtained from isotopic studies in South and East Africa, where pastoral adaptations were identified using nitrogen isotope ratios.[106] In southeastern Australia, an inland prehistoric population from a riverine habitat included four individuals whose $\delta^{13}C$ values indicated their access to the coast, or arid regions, of the continent's interior.[97]

The potential of strontium stable isotope ratios ($^{87}Sr/^{86}Sr$) has yet to be fully realized,[44] but preliminary results justify pursuing this line of inquiry. In a study using European material, two or possibly three prehistoric individuals buried in present-day Germany originated from a more southern area within Europe.[107] In a better controlled study, significant differences among individuals from two prehistoric sites in the United States southwest[108] support archeologically based hypotheses of migration into the region around 1400 AD. An intriguing finding is that one fossil hominid from Sterkfontein has a $^{87}Sr/^{86}Sr$ ratio similar to that of modern fauna living some distance away[109] although the omnipresent problem of diagenesis makes the implications of this discovery uncertain.

In some cases, stable isotope ratios have served to substantiate archeologically or ethnographically based expectations of population movements; in other cases they have served to question these expectations.

Diet Composition, Energetics, and the Question of Omnivory

The subsistence strategies of our predecessors were probably as varied as those reported for living human and nonhuman primates.[110] Because of the recognized associations among diet composition, subsistence strategy, and behavior, one goal of stable isotope studies has been to estimate the relative contribution of various foods in diet. The approaches used have been mass balance equations, comparisons of human $\delta^{15}N$ values with those of carnivores and herbivores, and determination of the relative spacing between the $\delta^{13}C$ value in bone collagen and that in bone apatite carbonate.

In its simplest form, a mass balance equation assumes that the δ value of the diet is equal to a weighted average of the δ values of the various diet components. Thus, a diet consisting of 30% squash and beans plus 70% maize would have a $\delta^{13}C$ value reflecting 30% C_3 and 70% C_4. In turn, the δ value (e.g., $\delta^{13}C$ or $\delta^{15}N$) in collagen is assumed to be linearly correlated with the δ value of the diet. In other words, the fractionation between diet and collagen is assumed to be constant across all types of diets. Recent studies have used carbon and nitrogen equations in concert to delineate dependence on maize, bison, and deer at Pecos Pueblo in New Mexico,[71] Great Lakes fish in Ontario,[103] maize in Belize,[88] and terrestrial, near-shore marine, and deep-sea resources on Nantucket island.[25] Complications have arisen, however, during attempts to determine exact percentages rather than relative amounts of each food item. For example, $\delta^{13}C$ values reported in human collagen from the Tehuacan valley,[16] the American plains,[111] and the southwestern United States[71] translate to 100% of the calories coming from maize if all the assumptions of the method are invoked. Recent laboratory feeding experiments[49,50,112] are refining our understanding of the specific dietary sources of nitrogen used in collagen synthesis and of carbon used in collagen and carbonate synthesis. Results from such studies suggest that the potential for mass balance equations will be greater than it was in the past.

The second approach involves the comparison of human $\delta^{15}N$ values with those of herbivores and carnivores. According to the model, human

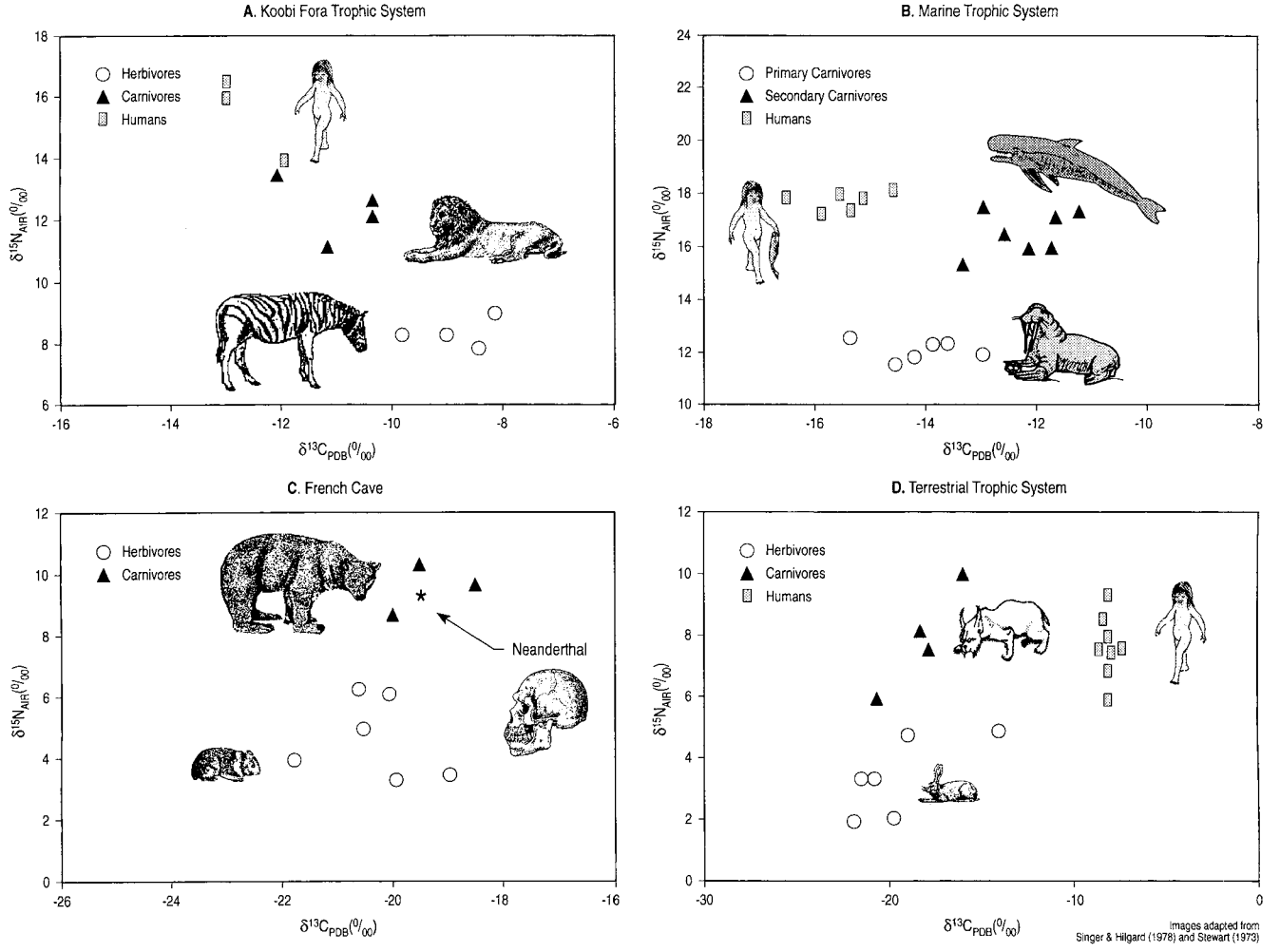

Figure 2. Presentation of three trophic systems in which human $\delta^{15}N$ values cluster with or are more positive than those of carnivores. These are compared with data from Bocherens et al.,[117] in which the same pattern indicates that "Neandethals" were similar to modern *humans as energy maximizers*. A: $\delta^{13}C$ and $\delta^{15}N$ values of species of carnivores and herbivores from the Koobi Fora trophic system near Lake Turkana in northern Kenya as compared with human pastoralists from east Africa. Data redrawn from Schoeninger[42] and Ambrose and DeNiro.[106] B: This marine trophic system includes data from Inuit and salmon fishers on the northwest coast of the United States which are plotted with data from marine fish and mammals collected along the California coast. Data are replotted from Schoeninger, DeNiro, and Tauber.[20] In this figure, primary carnivores refer to invertebrate feeders such as walrus; secondary carnivores refer to vertebrate feeders such as seals and toothed whales. C: French Cave. Data replotted from Bocherens et al.[117] D: This terrestrial trophic system is a pot of isotope data from recent prehistoric agriculturalists from one of the pueblos in the southwestern United States and of herbivorous and carnivorous fauna from the western portion of the United States. Data replotted from Schoeninger et al.[20]

herbivores are expected to have $\delta^{15}N$ values that are similar to those of nonhuman herbivores, whereas the values of human carnivores (such as precontact Inuits) should be similar to those of nonhuman carnivores. Several studies have indicated that humans have $\delta^{15}N$ values that are more positive than their diet. Nursing infants have more positive $\delta^{15}N$ values than their mothers do. Moreover, among several prehistoric groups, children under the age of four years have bone collagen $\delta^{15}N$ values that are significantly more positive, on average, than those of adults.[113–115] Based on these studies and the predictions of the model, $\delta^{15}N$ values of human omnivores who obtain protein from both plants and animals are expected to be determined by the amount of nitrogen provided by each source. As often happens in the scientific enterprise, the original expectations of the model have not been fulfilled, but other exciting possibilities have resulted. One test involved a single trophic system in northern Kenya (Fig. 2A) in which human pastoralists had $\delta^{15}N$ values that were greater than those of most carnivores rather than intermediate between those of herbivores and carnivores as was expected. In another system (Fig. 2B), the $\delta^{15}N$ values of Inuits were equivalent to secondary carnivores like killer whales rather than equivalent to those of primary carnivores as had been predicted. Finally, in an agricultural system in the southwest United States (Fig. 2D) where human diet was about 80% maize, humans had $\delta^{15}N$ values similar to those of carnivores. In all these cases, humans look much more carnivorous than was expected.

These results suggest that the $\delta^{15}N$ value of substrate proteins is not the only variable that influences the $\delta^{15}N$ values in human bone collagen, but that fractionation between diet and bone collagen is not constant across

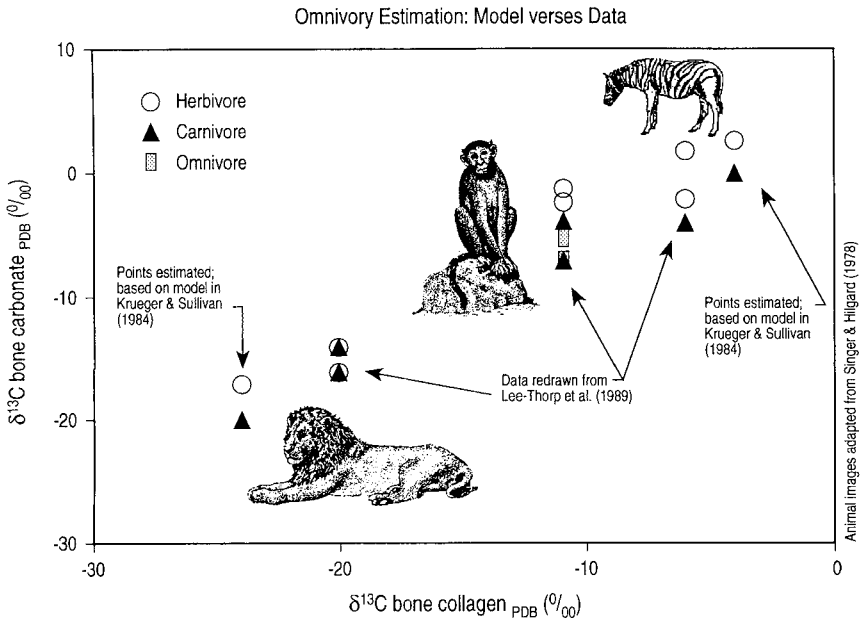

Figure 3. Comparison of data expected from the omnivory estimation model[48] with data actually collected.[120] The model predicts that herbivores and carnivores will differ in the spacing between the $\delta^{13}C$ value in bone carbonate and bone collagen. Herbivores are expected to have an offset between the two values of 8°/oo, whereas carnivores are expected to have an offset of 3°/oo. The C_3 and C_4 endpoints, which are plotted according to this expectation, show the effects of the differential offsets. Data collected in a large study of South African mammals and replotted in the same manner as those based on the model do not completely support this expectation. Values at the C_3 end show complete overlap between herbivores and carnivores; no omnivores were available for this section. The values from the middle of the range are somewhat more separated and the values at the C_4 end show an offset close to the expected values. Lee-Thorp and her colleagues suggested that the lack of separation might be due to the nature of the sample.[120] Limiting analyses to single trophic systems may permit application of this method.

diets. Metabolic changes caused by water and caloric stress have been implicated in elevated values in some regions,[22] but do not apply in the three cases cited. When these data are compared with results from other primate species, however, they suggest that energetics has an influence that is additional to that of the isotopic value in substrate protein, which is determined, in part, by trophic position (see Box 3). The hair of *Cebus*, an insect and fruit eater, has been found to have significantly more positive $\delta^{15}N$ values than does *Alouatta*, a leaf eater;[116] based on their trophic level offset, this difference was expected. However, *Brachyteles* hair had significantly more positive $\delta^{15}N$ values than did that of *Alouatta*, even though both are reported to have diets consisting of fruit and leaves. One difference between these two genera is that *Brachyteles* is an energy maximizer with larger day and home ranges than *Alouatta*, which is an energy minimizer. Nitrogen metabolism should be different between energy maximizers and minimizers, with absolutely greater amounts of nitrogen being ingested and excreted by the former. Such metabolism would result in retention of relatively more ^{15}N.

A similar aspect of energetics probably influences human nitrogen metabolism. This type of energy strategy was obviously in place by the time of Neanderthals (Fig. 2C), for they also appear to have been carnivorous[117] as compared with associated fauna, whereas, in comparison with other human groups, they were similar to the southwest agriculturalists (Fig. 2C). Furthermore, a wide range of $\delta^{15}N$ values has been observed in some populations (Fig. 2D), but not in others (Fig. 2B). The primate data cited earlier show very little variation, but a small number of chimpanzees[118] showed variation equivalent to that among the maize agriculturalists. Diets composed of highly varied protein sources may account for this, especially in dry, warm areas where plants often display a wide range of $\delta^{15}N$ values.[89]

Another method of estimating omnivory depends on the difference between the $\delta^{13}C$ values in the collagen and the carbonate of the same individual. This model is based on the assumption that, in carnivores, lipids provide energy and serve as the substrate for the carbon used in bone carbonate synthesis. This is in contrast to the situation of herbivores, in which carbohydrate provides energy and is the substrate. Because lipids and carbohydrates have significantly different $\delta^{13}C$ values,[119] carnivores and herbivores are expected to differ in their bone carbonate $\delta^{13}C$ values when these values are standardized by comparison to the values from bone collagen.[48] The offset is often represented as Δ^{13}Ccoll-carb. The values for pure C_3 and pure C_4 feeders as predicted by the model are plotted in Figure 3. A test of the model was promising, but did not completely meet expectations.[120] The expected separation of herbivores and carnivores appeared among C_4 feeders but not among C_3 feeders, and omnivores did not fall neatly in between. Two possible explanations have been proposed. First, the expected patterns may have been obliterated because the animals were chosen from several different trophic systems. Results from a recent study can be interpreted as supporting this explanation,[121] although no carnivores were analyzed. Alternatively, dietary quality may be a factor. Within a prehistoric skeletal series from South America, human agriculturalists with adequate intake of animal protein had Δ^{13}Ccoll-carb values similar to those of carnivores in the region.[80] Further, recent laboratory studies have found Δ^{13}Ccoll-carb values in herbivorous rats that approach the expected carnivore value.[49,50] Additional baseline studies are necessary to fulfill the tantalizing promises this approach holds.

Paleoclimate, Paleoecology, and Ecology

Previously, I have touched on the potential for using $\delta^{15}N$ values as energetic and diet breadth monitors and $\delta^{18}O$ values in bone phosphate as temperature and humidity indicators. However, another isotopic probe has actually reached the application phase. The carbon isotope ratios in

several different kinds of materials reflect various aspects of ecology and paleoecology, although, due largely to the paucity of such studies, their interpretations have been contested. For example, the $\delta^{13}C$ values in fossil tooth enamel carbonate and in carbonate nodules have been presented as supporting a shift from a C_3 biome to one including C_4 grasses in the late Miocene of India.[122] In contrast, results from animal species having a wide range of adaptations suggest that C_4 grasses were present by the middle Miocene in India[123] and east Africa.[52] Similarly, the $\delta^{13}C$ values in pedogenic carbonates and organic matter indicate a woodland or forest setting for the middle Miocene site of Fort Ternan,[63] whereas a previous study of soil profiles had concluded that it was more savanna-like.[124] The suggestion of diagenetic alteration[125] of samples taken for isotopic analysis has been refuted.[126]

Analyses of accompanying fauna may help resolve the disagreement about diagenetic alteration. Plants in modern closed-canopy forests are depleted in ^{13}C because of the recycling of carbon into CO_2 from organic detritus on the forest floor. This "canopy" effect is passed on to animals feeding in such regimes.[127] The $\delta^{13}C$ values in the hair of monkey species from Costa Rica and Brazil reflect the amount of forest cover in their habitat: open-forest species have significantly higher $\delta^{13}C$ values than do closed-forest species.[116] It is predicted that tooth enamel carbonate from arboreal monkeys at Fort Ternan will provide a monitor of forest cover and thus indicate the type of ecosystem characteristic of this important paleoanthropological site.

EXPECTATIONS AND PREDICTIONS

Much has been accomplished, but many areas still hold promise for the future. Among bioarcheologists interested in the prehistory of North and MesoAmerica, the analysis of additional prehistoric populations to determine their $\delta^{15}N$ and $\delta^{13}C$ values should continue to clarify the pattern of maize introduction, its association with fish use, and possibly, although less likely, its association with legume domestication. Oxygen isotope ratios in bone phosphate and δD values in collagen should pinpoint such climatic variations as the little ice age and, thus, determine its association, if any, with societal transitions. Much remains to be learned from the rest of the world. With the few exceptions noted earlier, South American maize agriculture and coastal marine dependence have not been isotopically traced. In Asia, the timing and overall dependence on millet domestication remains to be substantiated, as does the replacement of millet by rice. The adaptations of prehistoric Australians deserve attention because so little is known about them. A similar lack of information dictates greater attention to prehistoric Africa beyond that already done in the eastern and southern regions. Relatively little has been done in Europe, where the spread of millet and grazing domesticates could be monitored. This is also true across the Middle East into Asia.

Early Hominid Evolution

There is even greater potential for major contributions in the subdiscipline of paleoanthropology. Oxygen isotope ratios in bone phosphate should contribute to testing of the hypothesis that our lineage evolved in concert with changing climate[128] and advance our understanding of the ecology of the earliest hominids. Our early Miocene ancestors were forest dwelling C_3-feeding hominoids[129] with gastrointestinal tracts adapted for extracting nutrients from C_3 plants, mainly their fruit and young leaves. By the middle Miocene, several hominoid genera exhibited thick molar enamel,[130] a trait that among living primate species is associated with feeding on hard objects,[131] but apparently they retained the primitive pattern of fruit eating.[132]

By the Pliocene, our lineage had distinguished itself as being bipedal, with some genera adapted to the forest edge or savanna. The diet choices in the forest are quite different from those of the savanna[133] (Fig. 4). In terms of energy sources, the savanna does not have the abundance of fruit-producing trees but, in contrast, provides great numbers of tubers and grasses.[134] Many of the tuber plants eaten today by foraging people are leguminous,[134] but the protein contained in their beans would have remained essentially unavailable to hominids who lacked cooking techniques.[135] Thus, prior to cooking, only the tubers themselves would have provided nutrients. Nutrients from grasses are also problematic because humans and primates, in general, do not efficiently digest grasses or cereal fibers.[129] The few that do, such as baboons, can be viewed as "derived" relative to the "primitive" condition. Humans eat cereals that have been highly processed by cooking, grinding, alkali treatment, and predigestion by yeast, but early hominids lacked such techniques. With the possible exception of processing using pounding stones, they would have been limited to in-mouth processing. Tubers, however, are highly caloric and contain a high level of protein (up to 10%). Further, they can be harvested efficiently and in abundance with a digging stick. But the savanna offered an abundant, more highly concentrated source of easily digested protein: herd-dwelling, C_4-grass-feeding herbivores (such as today's zebra) or grazer/browsers (such as today's elephants and gazelles) with a mixed C_3/C_4 signal. Browsing herbivores having a pure C_3 signal also dwelled on the savanna, although in smaller groups; they constituted less of the overall faunal composition.

In combination with other lines of evidence, the expected stable isotope patterns for particular nutritional strategies of Plio-Pleistocene hominids can be proposed.[136] The main dietary evidence has come from the morphology of masticatory systems, which varied among hominid groups even though body sizes appear to have been roughly equivalent.[137] Of additional consideration are the established associations among relative brain size, energy requirements, relative sizes of portions of the gastrointestinal tract, and diets of living primate species.[138–140] All of the early hominid species probably required higher quality diets than those of extant folivorous primates.[141] Thus, the australopithecines, considered to have been "robust" and "gracile" groups, probably were more analogous to living chimpanzees than to living mountain gorillas in terms of their

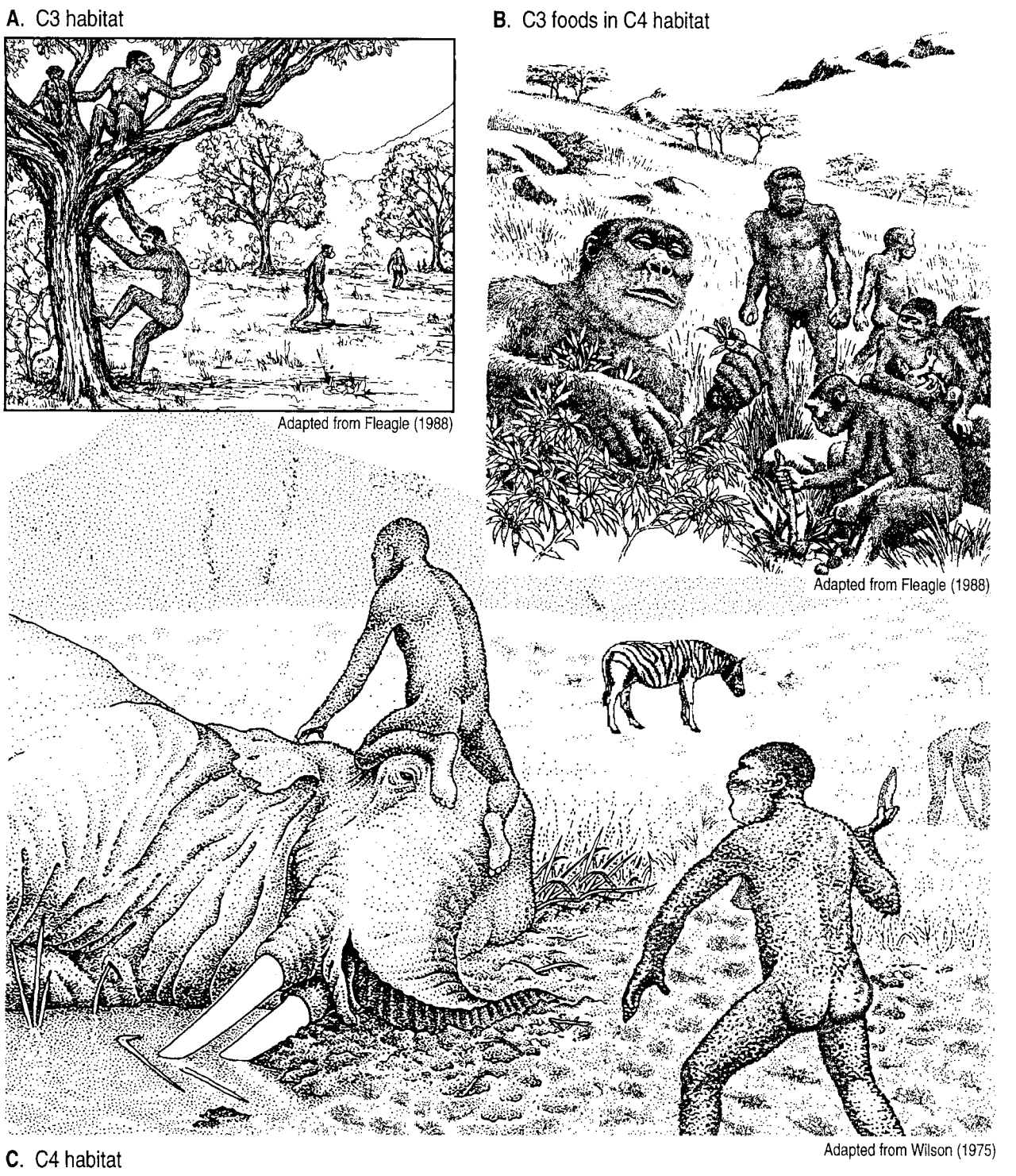

Figure 4. Idealized nutritional habitats of *Australopithecus afarensis* (4A) with C_3 leaves, nuts, and fruits, robust *Australopithecus* (4B) with C_3 berries and tubers in a C_4 grassland, and *Homo habilis* (4C). The latter shows the butchery of an animal which would have had a mixed C_3/C_4 signal, while an animal with a pure C_4 signal grazes in the background.

diet. The earliest members of the genus *Homo*, which, in comparison with the two australopithecine groups, had larger brains, used a greater proportion of resting metabolic energy, and had smaller absorptive areas in their gastrointestinal tract, would also have required a higher quality diet.[141,142]

Diet and Behavior of "Robust" Australopithecines

The "robust" australopithecines had extremely thick dental enamel

and crack-stopping structures that were resistant to fracture under masticatory loads.[143] Dental microwear studies indicate that these species ate substantially more hard foods than did the "gracile" australopithecines.[144] In addition, the "robust" australopithecine premolars and molars were larger relative to their anterior teeth than is true for the "gracile" group and the teeth are larger than expected from body weight. Taken together, these observations suggest that the "robust" species may have eaten a greater amount of protein and calorie-rich seeds and nuts or other items requiring extensive in-mouth processing, whereas the "gracile" species may have included more leaves and softer fruits. Alternatively, the thick enamel should have been more resistant to wear resulting from a diet that included grit-covered tubers. Tubers and hard objects such as nuts have a characteristic C_3 $\delta^{13}C$ signal. Berries, which also have a C_3 carbon isotopic signal, could have been an important source of energy, although their lack of protein and seasonal availability suggest that they could have been a significant food item for only part of the year.

The South African "robust" species displays a carbon isotope signal in the apatite carbonate of tooth enamel, which, using a strict mass balance interpretation,[145] translates to 75% C_3 and 25% C_4. Thus, resolution of the current debate about the ecology of the fossil settings is key: if the site was closed woodland, tubers are unlikely, if it was savanna, nuts are less likely. The predominance of grazing fauna at Swartkrans indicates open savanna, providing indirect support for the use of digging sticks by the early australopithecines.

An additional probe may be provided by nitrogen isotopes, on the remote chance that protein is retained in enamel or bone. A narrow range of $\delta^{15}N$ values and a low overall value would indicate small ranging patterns, limited numbers of food items, low protein intake, and an energy minimization strategy. Other possible probes are oxygen isotopes. If the oxygen isotope ratios of "robust" australopithecines are found to be similar to those of animals that rely on drinking water for their body water, this would indicate that these species did not obtain the majority of their body water from food, a pattern typical of forest dwelling hominoids today. By inference, this would suggest that they were savanna dwellers. On the other hand, a $\delta^{18}O$ signal similar to that of browsers would indicate that the C_3 signal in the "robust" species derived from nut eating in the closed forest and that the recovery of their bony remains from more open sites indicates death, not subsistence, in the area.

Grass seeds, a potential food, have a C_4 carbon isotopic signal and fit the masticatory morphology.[146] The "robust" species appear to have had the large gut necessary for processing and breaking down cereal fibers.[141] It seems a remote possibility that these species, with such thick enamel, large guts, and small brains, regularly ate meat. Another form of omnivory, which includes social insects and small animals in the diet, along with the use of teeth for bone breakage and marrow extraction is a possibility.[145,147] Assuming such a scenario, however, requires ways in which a 40-kg animal, lacking sophisticated trapping technology, can efficiently collect enough insects and rodents to make them a dependable component of its diet.

Diet and Behavior of "Gracile" Australopithecines

The tooth enamel of the "gracile" Australopithecines is thin relative to that of the "robust" species, although it is thick compared to the enamel of living pongids.[148] It has therefore been suggested that these australopithecines ate more leaves and fruits than did the "robust" species. A C_3 $\delta^{13}C$ signature would indicate forest feeding: the leguminous acacias of the savanna have secondary compounds that discourage primate feeding. A large variation in $\delta^{15}N$ values would indicate a large ranging pattern and the consumption of a variety of food items similar to the diet of chimpanzees today. Because they retained large guts and lacked the level of encephalization in *Homo*, it is likely that the main protein source for gracile Australopithecines was young leaves, although the emphasis on fruits suggests that they were energy maximizers as compared with the "robust" species. A $\delta^{18}O$ value in phosphate similar to that in browsers would indicate that the majority of these species' body water came from fruits and young leaves. The wear striations on their teeth do not support the interpretation that they fed on grit-covered tubers. The only other major food source on the savanna that they could have processed with their relatively small teeth would have been meat. Thus, a C_4 signal would indicate meat eating, a most interesting finding if it were found to occur.

Diet and Behavior of Homo *Species*

Within the *Homo* lineage, a C_4 signal would indicate hunting or active scavenging of savanna-dwelling grazers. The species included within *Homo* display the level of encephalization that is expected to be accompanied by reduced digestive systems. Such reduction would eliminate grass seeds and stems as foods. The reduction in their masticatory system relative to the Australopithecines also argues for easily digested foods. If their food was meat, we would expect to find a high $\delta^{15}N$ value indicating excess protein. In contrast, a C_3 signal would suggest dependence on tubers and, possibly, some browsing-fauna. A mixed signal would most likely indicate that they ate a combination of meat and tubers, as tropical woodland-dwelling foragers apparently do today. That signal should be coupled with a large range of $\delta^{15}N$ values indicating a large ranging pattern or diet breadth. The $\delta^{18}O$ value would most likely be similar to that of animals that must drink, because more water would be required to eliminate the excess nitrogen derived from meat eating. In addition, a hominid that was diurnally active on the open savanna would require more water for heat dissipation than could be provided by the water in food.

CONCLUSIONS

Several previously intractable problems of interest to bioarcheologists and paleoanthropologists have been innovatively addressed through stable isotope analysis. The pattern of maize introduction throughout the New World is becoming clear. It is now pos-

sible to trace the development of fishing industries. Unique adaptations of such varied foods as cactus and reef fish versus deep ocean fish have been identified. Seasonal changes in diet habits and migratory behavior can be investigated. Various aspects of climate and ecology can be quantified. Rough estimates of ranging patterns and diet breadth may be possible. Diet quality and metabolic efficiency may be traceable. In the future, isotopic investigations may even provide critical information about the diet and the ecological setting of our earliest ancestors. An unnamed sage once said: give the child a hammer and the whole world becomes poundable. Stable isotope analysis has proven to be a premier hammer.

ACKNOWLEDGMENTS

The majority of the studies cited were funded by the Anthropology Program of the National Science Foundation. Without that support, we would understand the geochemical cycling of isotopes but lack the knowledge for application and interpretation of isotopic signatures in bioarcheology and paleoanthropology. In addition, my thanks go to Dr. John Fleagle for inviting me to write this paper, as well as for his encouragement during its revision. I also thank Dr. Jeffrey Bada (University of California at San Diego), Dr. Henry Bunn (University of Wisconsin), the late Dr. James A. Gavan (University of Missouri), Dr. Kathleen Gordon (Smithsonian Institution), Ms. Jill Heilman (Arizona State University), Ms. Kris Hallin (University of Wisconsin), and the conscientious reviewers, all of whom took their valuable time to discuss the ideas presented here and to comment on previous drafts of this manuscript.

REFERENCES

1 Melander L, Saunders LH (1980) *Reaction Rates of Isotopic Molecules*. New York: John Wiley and Sons.

2 Hoefs J (1987) *Stable Isotope Geochemistry*. New York: Springer-Verlag.

3 Rundel PW, Ehleringer JR, Nagy KA (eds) (1989) *Stable Isotopes in Ecological Research*. New York: Springer-Verlag.

4 Buncel E, Saunders WH Jr. (eds) (1992) *Heavy Atom Isotope Effects*. New York: Elsevier.

5 Schwarcz H, Schoeninger MJ (1991) Stable isotope analyses in human nutritional ecology. Yrbk Phys Anthropol *34*:283–321.

6 Schoeninger MJ, Moore K (1992) Bone stable isotope studies in archeology. J Wor Preh *6*:247–296.

7 Ambrose SH (1993) Isotopic analysis of paleodiets: Methodological and interpretive considerations. In Sandford MK (ed), *Investigations of Ancient Human Tissue: Chemical Analyses in Anthropology*. Langhorne, PA: Gordon and Breach Science Publishers, pp 59–130.

8 Krouse HR, Iyer SS (1994) Sulphur and hydrogen isotope studies relevant to paleodiet reconstruction. Fourth Advanced Seminar on Paleodiet, Banff, Alberta, Canada.

9 O'Leary MJ (1988) Carbon isotopes in photosynthesis. BioSci *38*:328–336.

10 Tieszen LL (1991) Natural variations in the carbon isotope values of plants: Implications for archaeology, ecology, and paleoecology. J Arch Sci *18*:227–248.

11 van der Merwe NJ (1982) Carbon isotopes, photosynthesis, and archaeology. Am Sci *70*:596–606.

12 Schoeninger MJ, DeNiro MJ (1984) Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochim Cosmochim Acta *48*:625–639.

13 Wahlen M (1994) Carbon dioxide, carbon monoxide and methane in the atmosphere: Abundance and isotopic composition. In Lajtha K, Mitchener RH (eds), *Stable Isotopes in Ecology and Environmental Science*. Oxford: Blackwell Scientific Publications, pp 93–113.

14 Tieszen LL, Fagre T (1993) Carbon isotopic variability in modern and archaeological maize. J Archeol Sci *20*:25–40.

15 Schoeninger MJ, Schurr M (1994) Interpreting carbon stable isotope ratios. In Johannessen S, Hastorf C (eds), *Corn and Culture In The Prehistoric New World*. Boulder: Westview Press, pp 55–66.

16 DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta *42*:495–506.

17 Virginia RA, Delwiche CC (1982) Natural 15N abundance of presumed N2-fixing and non-N2-fixing plants from selected ecosystems. Oecologia *54*:317–325.

18 DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta *45*:341–351.

19 Hare PE, Fogel ML, Stafford TW Jr, Mitchell AD, Hoering TC (1991) The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. J Archeol Sci *18*:277–292.

20 Schoeninger MJ, DeNiro MJ, Tauber H (1983) Stable nitrogen isotope ratios of bone collagen reflect marine and terrestrial components of prehistoric human diet. Science *220*:1381–1383.

21 Ambrose SH, DeNiro MJ (1986) The isotopic ecology of East African mammals. Oecologia *69*:395–406.

22 Heaton THE, Vogel JC, von la Chevallerie G, Gollett G (1986) Climatic influence on the isotopic composition of bone nitrogen. Nature *322*:822–823.

23 Ambrose SH (1991) Effects of diet, climate and physiology on nitrogen isotope abundances in terrestrial foodwebs. J Archeol Sci *18*:293–317.

24 Keegan WF, DeNiro MJ (1988) Stable carbon- and nitrogen-isotope ratios of bone collagen used to study coral-reef and terrestrial components of prehistoric Bahamian diet. Am Antiquity *53*:320–336.

25 Little EA, Schoeninger MJ (1995) The late Woodland diet on Nantucket Island and the problem of maize in coastal New England. Am Antiquity *60*:351–368.

26 Gonfiantini R (1986) Environmental isotopes in lake studies. In Fritz P, Fontes JC (eds), *Handbook of Environmental Isotope Geochemistry*. Amsterdam: Elsevier, pp 113–168.

27 White JC (1989) Stable hydrogen isotope ratios in plants: A review of current theory and some potential applications. In Rundel PW, Ehleringer JR, Nagy KA (eds), *Stable Isotopes in Ecological Research*. New York: Springer-Verlag, pp 142–162.

28 Ziegler H (1989) Hydrogen isotope fractionation in plant tissues. In Rundel PW, Ehleringer JR, Nagy KA (eds), *Stable Isotopes in Ecological Research*. New York: Springer-Verlag, pp 105–123.

29 Sternberg LdSL (1989) Oxygen and hydrogen isotope ratios in plant cellulose: Mechanisms and applications. In Rundel PW, Ehleringer JR, Nagy KA (eds), *Stable Isotopes in Ecological Research*. New York: Springer-Verlag, pp 124–141.

30 Feng X, Epstein S (1994) Climatic implications of an 8000-year hydrogen isotope time series from bristlecone pine trees. Science *265*:1079–1081.

31 Cormie AB, Schwarcz HP, Gray J (1994) Relation between hydrogen isotopic ratios of bone collagen and rain. Geochim Cosmochim Acta *58*:377–391.

32 Cormie AB, Luz B, Schwarcz HP (1994) Relationship between the hydrogen and oxygen isotopes of deer bone and their use in the estimation of relative humidity. Geochim Cosmochim Acta *58*:3439–3449.

33 Gat JR, Gonfiantini R (eds) (1981) *Stable Isotope Hydrology: Deuterium and Oxygen-18 in the Water Cycle*. International Atomic Energy Agency, Vienna Technical Reports.

34 Kolodny Y, Luz B, Navon O (1983) Oxygen isotope variations in phosphate of biogenic apatites. I. Fish bone apatite—rechecking the rules of the game. Earth Planet Sci Lett *64*:398–404.

35 Shackleton NJ (1989) The Plio-Pleistocene ocean: Stable isotope history. In Rose, Schluchter (eds), *Quaternary Type Sections: Imagination or Reality?* Rotterdam: Balkema, pp 11–24.

36 Folinsbee RE, Fritz P, Krouse HR, Robblee AR (1970) Carbon-13 and oxygen-18 in dinosaur, crocodile, and bird eggshells indicate environmental conditions. Science *168*:1353–1356.

37 Sarkar A, Bhattacharya SK, Mohabey DM (1991) Stable-isotope analyses of dinosaur eggshells: Paleoenvironmental implications. Geology *19*:1068–1071.

38 Longinelli A (1984) Oxygen isotopes in mammal bone phosphate: A new tool for paleohydrological and paleoclimatological research? Geochim Cosmochim Acta *48*:385–390.

39 Fricke H, O'Neil JR, Lynnerup N (1995) Oxygen isotope composition of human tooth enamel from medieval Greenland: Linking climate and society. Geology *23*:869–872.

40 Ayliffe LK, Chivas AR (1990) Oxygen isotope composition of the bone phosphate of Australian kangaroos: Potential as a palaeoenvironmental recorder. Geochim Cosmochim Acta *54*:2603–2609.

41 O'Neil JR, Roe LJ (1994) A rapid and precise method of oxygen isotope analysis of biogenic phosphate. Isr J Earth Sci *43*:203–212.

42 Schoeninger MJ (1989) Prehistoric human diet. In Price TD (ed), *Chemistry of Prehistoric Human Bone*. Cambridge: Cambridge University Press, pp 38–67.

43 Sillen A, Lee-Thorp JA (1994) Trace element and isotopic aspects of predator-prey relationships in terrestrial foodwebs. Palaeo Palaeo Palaeo *107*:243–255.

44 Ericson JE (1989) Some problems and potentials of strontium isotope analysis for human and animal ecology. In Rundel PW, Ehleringer JR, Nagy KA (eds), *Stable Isotopes in Ecological Research*. New York: Springer-Verlag, pp 251–259.

45 Masters PM (1987) Preferential preservation of noncollagenous protein during bone diagenesis: Implications for chronometric and stable isotope measurements. Geochim Cosmochim Acta *51*:3209–3214.

46 Tuross N (1993) The other molecules in ancient bone: Noncollagenous proteins and DNA. In Lambert J, Grupe G (eds) *Prehistoric Human Bone: Archaeology at the Molecular Level*. Berlin: Springer-Verlag, pp 275–292.

47 Pate FD (1994) Bone chemistry and paleodiet. J Archeol Meth Theory *1*:161–209.

48 Krueger HW, Sullivan CH (1984) Models for carbon isotope fractionation between diet and bone. In Turnlund JR, Johnson PE (eds), *Stable Isotopes in Nutrition*. Washington, DC: American Chemical Society Symposium Series, pp 205–220.

49 Tieszen LL, Fagre T (1993) Effect of diet quality and composition of the isotopic composition of respiratory CO_2, bone collagen, bioapatite, and soft tissue. In Lambert J, Grupe G (eds) *Prehistoric Human Bone: Archaeology at the Molecular Level*. Berlin: Springer-Verlag, pp 121–156.

50 Ambrose SH, Norr L (1993) Relationship of carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. In Lambert J, Grupe G (eds) *Prehistoric Human Bone: Archaeology at the Molecular Level*. Berlin: Springer-Verlag, pp 1–38.

51 White CD, Schwarcz HP (1994) Temporal trends in stable isotopes for Nubian mummy tissues. Am J Phys Anthropol *93–:165–188*.

52 Kingston JD, Marino BD, Hill A (1994) Isotopic evidence for Neogene hominid paleoenvironments in the Kenya Rift Valley. Science *264–955–959*.

53 Ambrose S, Sikes N (1991) Soil carbon isotope evidence for Holocene habitat change in Kenya Rift Valley. Science *253*:1402–1405.

54 Schoeninger MJ, DeNiro MJ (1982) Carbon isotope ratios of apatite from fossil bone cannot be used to reconstruct diets of animals. Nature *297*:577–578.

55 Lee-Thorp JA, van der Merwe NJ (1991) Aspects of the chemistry of modern and fossil biological apatites. J Archeol Sci *18*:343–354.

56 Wang Y, Cerling TE (1994) A model of fossil tooth and bone diagenesis: Implications for paleodiet reconstruction from stable isotopes. Palaeo Palaeo Palaeo *107*:281–289.

57 Bryant JD, Luz B, Froelich PN (1994) Oxygen isotopic composition of fossil horse tooth phosphate as a record of continental paleoclimate. Palaeo Palaeo Palaeo *107*:210–212.

58 Nelson BK, DeNiro MJ, Schoeninger MJ, DePaolo DJ (1986) Effects of diagenesis on strontium, carbon, nitrogen, and oxygen concentration and isotopic composition of bone. Geochim Cosmochim Acta *50*:1941–1949.

59 Tuross N, Behrensmeyer AK, Eanes ED, Fisher LW, Hare PE (1989) Molecular preservation and crystallographic alterations in a weathering sequence of wildebeeste bones. Appl Geochem *4*:261–270.

60 Sealy JC, van der Merwe NJ, Sillen A, Kruger FJ, Krueger HW (1991) $^{87}Sr/^{86}Sr$ as a dietary indicator in modern and archaeological bone. J Archeol Sci *18*:399–416.

61 Koch PL, Halliday AN, Walter LM, Stearley RF, Huston TJ, Smith GR (1992) Sr isotopic composition of hydroxyapatite from recent and fossil salmon: The record of lifetime migration and diagenesis. Earth Planet Sci Lett *108*:277–287.

62 Sillen A, LeGeros R (1991) Solubility profiles of synthetic apatites and of modern and fossil bones. J Archeol Sci *18*:385–397.

63 Cerling TE, Quade J, Ambrose SH, Sikes NE (1991) Fossil soils, grasses, and carbon isotopes from Fort Ternan, Kenya: Grassland or woodland? J Hum Evol *21*:295–306.

64 Norr L (1991) Nutritional Consequences of Prehistoric Subsistence Strategies in Lower Central America. Ph.D. Dissertation, University of Illinois, Urbana.

65 White CD, Schwarcz HP (1989) Ancient Maya diet: As inferred from isotopic and elemental analysis of human bone. J Archaeol Sci *16*:451–474.

66 White CD, Healy PF, Schwarcz H (1993) Intensive agriculture, social status, and Maya diet at Pacbitun, Belize. J Anthropol Res *49*:347–355.

67 Blake M, Chisholm BS, Clark JE, Voorhies B, Love MW (1992) Prehistoric subsistence in the Soconusco region. Curr Anthropol *33*:83–94.

68 Matson RG, Chisholm B (1991) Basketmaker II subsistence: Carbon isotopes and other dietary indicators from Cedar-Mesa, Utah. Am Antiquity *56*:444–459.

69 Huebner JA (1991) Cactus for dinner, again! An isotopic analysis of late Archaic diet in the Lower Pecos region of Texas. In Turpin SA (ed), *Papers on Lower Pecos Prehistory. Studies in Archeology*. Austin: University of Texas at Austin, pp 175–190.

70 Habicht-Mauche JA, Levendosky AA, Schoeninger MJ (1994) Antelope Creek phase subsistence: The bone chemistry evidence. In Owsley DW, Jantz RL (eds), *Skeletal Biology in the Great Plains: A Multidisciplinary View*.

71 Spielmann KA, Schoeninger MJ, Moore K (1990) Plains-Pueblo interdependence and human diet at Pecos Pueblo, New Mexico. Am Antiquity *55*:745–765.

72 Ezzo JA (1992) Dietary change and variability at Grasshopper Pueblo, Arizona. J Anthropol Archeol *11*:219–289.

73 Riley T, Walz GR, Bareis CJ, Fortier AC, Parker KE (1994) Accelerator mass spectrometry (AMS) dates confirm early *Zea Mays* in the Mississipipi River valley. Am Antiquity *59*:490–498.

74 Buikstra JE, Milner GR (1991) Isotopic and archaeological interpretations of diet in the central Mississippi Valley. J Archeol Sci *18*:319–329.

75 Schoeninger MJ, van der Merwe NJ, Moore K, Lee-Thorp J, Larsen CS (1990) Decrease in diet quality between the prehistoric and the contact periods. In Larsen CS (ed), *The Archaeology of Mission Santa Catalina De Guale: 2. Biocultural Interpretations Of A Population In Transition*. New York: American Museum of Natural History, pp 78–93.

76 Larsen CS, Schoeninger MJ, van der Merwe NJ, Moore KM, Lee-Thorp JA (1992) Carbon and nitrogen stable isotopic signatures of human dietary change in the Georgia Bight. Am J Phys Anthropol *89*:197–214.

77 van der Merwe NJ, Roosevelt AC, Vogel JC (1981) Isotopic evidence for prehistoric subsistence change at Parmana, Venezuela. Nature *292*536–538.

78 Burger RL, van der Merwe NJ (1990) Maize and the origins of highland Chavin civilization: An isotopic perspective. Am Anthropol *92*:85–95.

79 Hastorf CA (1985) Dietary reconstruction in the Andes. Anthropol Today *6*:19–21.

80 Ericson JE, West M, Sullivan CH, Krueger HW (1989) The development of maize agriculture in the Viru Valley, Peru. In Price TD (ed), *The Chemistry of Prehistoric Human Bone*. Cambridge: Cambridge University Press, pp 68–104.

81 van der Merwe NJ, Lee-Thorp JA, Raymond J (1993) Light, stable isotopes and the subsistence base of formative cultures at Valdivia, ecuador. In Lambert JB, Grupe G (eds) *Prehistoric Human Bone: Archaeology at the Molecular Level*. Berlin: Springer-Verlag, pp 63–97.

82 Tauber H (1981) ^{13}C evidence for dietary habits of prehistoric man in Denmark. Nature *292*:332–333.

83 Clutton-Brock J, Noe-Nygaard N (1990) New osteological and C-isotope evidence on Mesolithic dogs: Comparisons to hunters and fishers at Star Carr, Seamer Carr and Kongemose. J Archeol Sci *17*:643–653.

84 Lubell D, Jackes M, Schwarcz H, Knyf M, Meiklejohn C (1994) The Mesolithic-Neolithic transition in Portugal: Isotopic and dental evidence of diet. J Archeol Sci *21*:201–216.

85 Murray M, Schoeninger MJ (1988) Diet, status, and complex social structure in Iron Age Central Europe: Some contributions of bone chemistry. In Gibson B, Geselowitz M (eds), *Tribe and Polity in Late Prehistoric Europe*. New York: Plenum Press, pp 157–178.

86 Cai L, Qiu S (1984) Carbon-13 evidence for ancient diet in China. Kaogu (Archaeology) *10*:949–955.

87 Lee-Thorp JA, Sealy JC, Morris AG (1993) Isotopic evidence for diets of prehistoric farmers in South Africa. In Lambert J, Grupe G (eds) *Prehistoric Human Bone: Archaeology at the Molecular Level*. Berlin: Springer-Verlag, pp 99–120.

88 Schwarcz HP (1991) Some theoretical aspects of isotope paleodiet studies. J Archaeol Sci *18*:261–275.

89 Shearer G, Kohl DH (1992) Information derived from variation in the natural abundance of ^{15}N in complex biological systems. In Buncel E, Saunders WHJ (eds), *Heavy Atom Isotope Effects*. Elsevier Science Publishers, pp 191–237.

90 Schurr MR, Schoeninger MJ (1995) Associations between agricultural intensification and social complexity: An example from the prehistoric Ohio Valley. J Anthropol Archeol *14*:315–339.

91 Schoeninger MJ, Schurr MR (n.d.) Human subsistence at Moundville: The stable isotope data. In Knight VJ, Steponaitis VP (eds), *Moundville Revisited*.

92 Schurr M (1992) Isotopic and mortuary variability in a Middle Mississippian population. Am Antiquity *57*:300–320.

93 Chisholm BS, Nelson DE, Schwarcz HP (1982) Stable carbon isotope ratios as a measure of marine versus terrestrial protein in ancient diets. Science *216*:1131–1132.

94 Lovell N, Chisholm BS, Nelson DE, Schwarcz HP (1986) Prehistoric salmon consumption in interior British Columbia. Can J Archeol *10*:99–106.

95 Walker PL, DeNiro MJ (1986) Stable nitrogen and carbon isotope ratios in bone collagen as indices of prehistoric dietary dependence on marine and terrestrial resources in southern California. Am J Phys Anthropol *71*:51–61.

96 Collier S, Hobson KA (1987) The importance of marine protein in the diet of coastal Australian Aborigines. Curr Anthropol *28*:559–564.

97 Pate FD (1995) Stable carbon isotope assessment of hunter-gatherer mobility in prehistoric South Australia. J Archeol Sci *22*:81–87.

98 Chisholm B, Koike H, Nakai N (1988) Study of paleodiet using stable-carbon isotope analysis. Archaeol Nat Sci *20*:7–16.

99 Minagawa M, Akazawa T (1991) Dietary patterns of Japanese Jomon hunter-fisher-gatherers: Stable nitrogen and carbon isotope analyses of human bones. In Aikens CM, Rhee SN (eds), *Pacific Northeast Asia in Prehistory: Recent Research into the Emergence of Hunter-Fisher-Gatherers, Farmers, and Socio-Political Elites*. Seattle: University of Washington Press.

100 Roksandic Z, Minagawa M, Akazawa T (1988) Comparative analysis of dietary habits between Jomon and Ainu Hunter-gatherers from stable carbon isotopes of human bone. J Anthropol Soc Nippon *96*:391–404.

101 Sealy JC, van der Merwe NJ (1986) Isotope assessment and the seasonal-mobility hypothesis in the Southwestern Cape of South Africa. Curr Anthropol *27*:135–150.

102 Sealy JC, Patrick MK, Morris AG, Alder D (1992) Diet and dental caries among later Stone Age inhabitants of the Cape Province, South Africa. Am J Phys Anthropol *88*:123–134.

103 Katzenberg MA (1989) Stable isotope analysis of archaeological faunal remains from southern Ontario. J Archeol Sci *16*:319–329.

104 Schoeninger MJ (1991) Subsistence strategies of Holocene period populations from East Turkana, Kenya. Am J Phys Anthropol Suppl *12*:159.

105 Schoeninger MJ (n.d.) Dietary reconstruction in the prehistoric Carson Desert: Stable carbon and nitrogen isotopic analysis. In Hemphill BE, Larsen CS (eds), *Understanding Prehistoric Lifeways in the Great Basin Wetlands: Bioarchaeological Reconstruction and Interpretation*. Salt Lake City: University of Utah Press.

106 Ambrose SH, DeNiro MJ (1986) Reconstruction of African human diet using bone collagen carbon and nitrogen isotope ratios. Nature *319*:321–324.

107 Price TD, Grupe G, Schröter P (1994) Reconstruction of migration patterns in the Bell Beaker period by stable strontium isotope analysis. Appl Geochem *9*:413–417.

108 Price TD, Johnson CM, Ezzo JA, Ericson J, Burton JH (1994) Residential mobility in the prehistoric southwest United States: A preliminary study using strontium isotope analysis. J Archaeol Sci *21*:315–330.

109 Sillen A, Hall G, Armstrong R (1995) $^{87}Sr/^{86}Sr$ in modern and fossil foodwebs of the Sterkfontein Valley. Paleoanthropology Society, Oakland, CA.

110 Harding RSO, Teleki G (eds) (1981) *Omnivorous Primates: Gathering and Hunting in Human Evolution*. New York: Columbia University Press.

111 Bumsted MP (1984) *Human Variation:* $\delta^{13}C$ in Adult Bone Collagen and the Relation to Diet in an Isochronous C_4 (Maize) Archaeological Population. Los Alamos, NM: Los Alamos National Laboratory.

112 Schoeninger MJ, Iwaniec UT, Spielmann KA, Treichel IW, Cook ME, Crenshaw TD (1993) Carbon stable isotope values in archaeological populations: Are they telling us what we *think* they are? Am J Phys Anthropol Suppl *16*:175.

113 Tuross N, Fogel M, Owsley DW (n.d.) Duration of breast feeding among hunter-gatherers and maize agriculturalists. Am J Phys Anthropol, in press.

114 Katzenberg MA, Saunders SR, Fitzgerald WR (1993) Age differences in stable carbon and nitrogen isotope ratios in a population of prehistoric maize horticulturalists. Am J Phys Anthropol *90*:267–281.

115 Schurr MR (n.d.) Stable isotopes as evidence for the age of weaning at the Angel Site: A comparison of isotopic and demographic measures of weaning age. In (ed), *Occasional Paper*. Carbondale: Center for Archaeological Investigations, Southern Illinois University.

116 Schoeninger JJ, Iwaniec UT, Glander KE (1994) Stable isotope variation in Costa Rican primates: Implications for diet selectivity. Am J Phys Anthropol Suppl *18*:113.

117 Bocherens H, Fizet M, Mariotti A, Lange-Badre B, Vandermeersch B, Borel JP, Bellon G (1991) Isotopic biogeochemistry (^{13}C, ^{15}N) of fossil vertebrate collagen: Application to the study of a past food web including Neandertal man. J Hum Evol *20*:481–492.

118 Fitzer PS, Schoeninger MJ, Sept JM (1993) Stable isotope variation in chimpanzees: Implications for diet selectivity in early hominids. Am J Phys Anthropol Suppl *16*:86–86.

119 DeNiro MJ, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. Science *197*:261–263.

120 Lee-Thorp JA, Sealy JC, van der Merwe NJ (1989) Stable carbon isotope ration differences between bone collagen and bone apatite,and their relationship to diet. J Archaeol Sci *16*:585–599.

121 Bocherens H, Fizet M, Mariotti A (1994) Diet, physiology, and ecology of fossil mammals as inferred from stable carbon and nitrogen biogeochemistry: Implications for Pleistocene bears. Palaeo Palaeo Palaeo *107*:213–225.

122 Cerling T, Wang Y, Quade J (1993) Expansion of C4 ecosystems as an indicator of global ecological change in the Late Miocene. Nature *361*:344–345.

123 Morgan ME, Kingston JD, Marino BD (1994) Carbon isotopic evidence for the emergence of C4 plants in the Neogene from Pakistan and Kenya. Nature *367*:162–165.

124 Retallack GJ, Dugas DP, Bestland EA (1990) Fossil soils and grasses of a Middle Miocene East African grassland. Science *247*:1325–1328.

125 Retallack GJ (1992) Comment on the palaeoenvironment of *Kenyapithecus* at Fort Ternan. J Hum Evol *23*:363–369.

126 Cerling T, Kappelman J, Quade J, Ambrose S, Sikes N, Andrews P (1992) Paleoenvironment of *Kenyapithecus* at Fort Ternan. Reply. J Hum Evol *23*:371–377.

127 van der Merwe NJ, Medina E (1991) The canopy effect, carbon isotope ratios and foodwebs in Amazonia. J Archaeol Sci *18*:249–259.

128 Vrba ES (1985) Ecological and adaptive changes associated with early hominid evolution. In Delson E (ed), *Ancestors: The Hard Evidence*. New York: Alan R. Liss, pp 63–71.

129 Milton K (1987) Primate diets and gut morphology: Implications for hominid evolution. In Harris M, Ross EB (eds), *Food and Evolution*. Philadelphia: Temple University, pp 93–115.

130 Andrews P, Martin L (1991) Hominoid dietary evolution. Philos Trans R Soc London B *334*:199–209.

131 Kay RF (1981) The nut-crackers: A new theory of the adaptations of the Ramapithecinae. Am J Phys Anthropol *55*:141–151.

132 Teaford MF, Walker AC (1984) Quantitative differences in dental microwear between primates species with different diets and a comment on the presumed diet of Sivapithecus. Am J Phys Anthropol *64*:191–200.

133 Sept J (1989) Archaeological evidence and ecological perspectives for reconstructing early hominid subsistence strategies. In Schiffer MB (ed), *Archaeological Method and Theory*. pp 1–56.

134 Vincent A (1984) Plant foods in savanna environments: A preliminary report of tubers eaten by the Hadza of northern Tanzania. World Archeol *17*:132–148.

135 Stahl AB (1989) Plant-food processing: Implication for dietary quality. In Harris DR, Hillman GC (eds), *Foraging and Farming: The Evolution of Plant Exploitation*. London: Unwin Hyman, pp 171–194.

136 Gordon K (1993) Reconstructing hominid diet in the Plio-Pleistocene. Revista di Anthropologia (Roma) *71*:71–89.

137 McHenry HM (1991) Femoral lengths and stature in the Plio-Pleistocene hominids. Am J Phys Anthropol *85*:12134.

138 Kay RF (1975) The functional adaptations of primate molar teeth. Am J Phys Anthropol *43*:195–216.

139 Chivers DJ, Hladik CM (1984) Diet and gut morphology in primates. In Chivers DJ, Wood BA, Bilsborough A (eds), *Food Acquisition and Processing in Primates*. New York: Plenum Press, pp 213–230.

140 Martin RD (1984) Body size, brain size and feeding strategies. In Chivers DJ, Wood BA, Bilsborough A (eds), *Food Acquisition and Processing in Primates*. New York: Plenum Press, pp 73–103.

141 Aiello LC, Wheeler P (1995) The expensive-tissue hypothesis: The brain and the digestive system in human and primate evolution. Curr Anthropol *36*:199–221.

142 Leonard WR, Robertson ML (1994) Evolutionary perspectives on human nutrition: The influence of brain and body size on diet and metabolism. Am J Hum Biol *6*:77–88.

143 Grine FE, Martin LB (1989) Enamel thickness and development in Australopithecus and Paranthropous. In Grine FE (ed), *Evolutionary History of the "Robust" Australopithecines*. New York: Aldine de Gruyter, pp 3–40.

144 Grine FE, Kay RF (1988) Early hominid diets from quantitative image analysis of dental microwear. Nature *333*:765–768.

145 Lee-Thorp JA, van der Merwe NJ, Brain CK (1994) Diet of *Australopithecus robustus* at Swartkrans from stable carbon isotopic analysis. J Hum Evol *27*:361–372.

146 Jolly CJ (1970) The seed-eaters: A new model of hominid differentiation based on a baboon analogy. Man *5*:5–26.

147 Sillen A (1992) Strontium-calcium ratios (Sr/Ca) of *Australopithecus robustus* and associated fauna from Swartkrans. J Hum Evol *23*:495–516.

148 Martin L (1985) Significance of enamel thickness in hominoid evolution. Nature *314*:260–263.

149 Steele KW, Daniel RM (1978) Fractionation of nitrogen isotopes by animals: A further complication to the use of variations in the natural abundance of 15N for tracer studies. J Agric Sci Cambridge *90*:7–9.

150 Iwaniec UT, Schoeninger MJ, Cook M, Treichel I, Crenshaw T (1994) The source of the dietary carbon isotope signal. Am J Phys Anthropol Suppl *17*:

151 Burlingame AL, Schnoes HK (1969) MS in organic geochemistry. In Elington G, Murphy MTJ (eds), *Organic Geochemistry*. Heidelberg: Springer-Verlag, pp 96–104.