Methodological Developments in the Use of Stable Isotope Analysis for Reconstructing Paleodiets

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RESEARCH PAPER APPROVAL

METHODOLOGICAL DEVELOPMENTS IN THE USE OF STABLE ISOTOPE ANALYSIS FOR RECONSTRUCTING PALEODIETS

By

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Understanding the composition of paleodiet is crucially important for the archaeological investigation of lifeways and subsistence practices within ancient societies. Among the numerous research methods capable of investigating various elements of paleodiet, the use of stable isotope analysis has become increasingly popular. Advantages of this research technique include its ability to recognize multiple types of dietary inputs through the examination of stable carbon and stable nitrogen isotopes, and to investigate dietary composition and variation at an individual level. This research paper examines how this methodology has developed through time. Specific attention will be directed toward both the procedural advances that continue to reshape the utility of this method, as well as changes in the theoretical assumptions that have guided its application.
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Reconstructing the diet of prehistoric peoples is an essential objective for archaeologists interested in understanding the lifeways and cultural dynamics of past human societies. A basic premise that has been reiterated through time by archaeologists interested in investigating paleodiet is that “we are what we eat” (Schwarcz 1991; Parkington 1991; Beehr and Ambrose 2007). From this assertion we can derive two interrelated research questions: what do we eat, and who are we? Answering these complex questions requires a multidimensional research approach that relies on various supporting lines of evidence. While stable isotope analysis, or any single method, should not be relied on to provide the only evidence in the reconstruction of a paleodiet, this method’s ability to measure dietary differences on an individual level has proven to be invaluable for the investigation of prehistoric foodways and paleodiets. In this paper I will trace the historical development of stable carbon and stable nitrogen isotope analyses within archaeology in order to demonstrate how this method has progressed through time, and the ways in which it continues to contribute to archaeological research. Research using stable isotope data continues to expand in new directions by incorporating a greater number of elements into research designs, such as strontium and oxygen, and examining new materials, such as ceramic residues, but these more recent developments will not be of primary concern in this paper.

While there are a number of methods that can provide insights into diet reconstruction (see Ambrose 1987), stable isotope analysis is often selected for its ability to directly examine specific types of dietary inputs. Initially, this method was often
applied to various permutations of the research question, “what do we eat?” Two of the most productive avenues of investigation to answer this question deal with measuring the proportion of C3 versus C4 plants in the diet of a population, and comparing marine versus terrestrial inputs into a diet (Pate 1994). Examining the contribution of C3 versus C4 plants to a prehistoric diet has been especially productive, as investigations into dietary dependence on maize, millet, and sorghum are all subsumed within this research objective. As these three domesticates all use the C4 pathway for photosynthesis, their appearance in the diet of individuals living in a predominantly C3 based ecosystem can be measured isotopically, as C4 plants leave a distinctly less negative δ\(^{13}\)C signature in human bone. Based on this isotopic difference, maize can be identified in the largely C3 eastern woodland environment, while millet and sorghum are distinguishable within a diet based on C3 domesticates such as rice, wheat, and barley. The success of this approach hinges on the observation that the isotopic composition of C3 and C4 plants are recognizably different, and that these differences can be identified in the bone chemistry of individuals feeding on these food sources.

A pioneering application of stable isotope analysis in archaeological research was conducted by van der Merwe and Vogel (1978), in an attempt to examine the proportion of maize in woodland North American paleodiets. By comparing the isotopic ratio of \(^{13}\)C in human bone collected from pre-maize Archaic and Woodland burials with burials from the maize-growing Mississippian period, these researchers were able to isotopically detect the introduction of maize into the eastern woodland diet. Other early work by van der Merwe and Medina (1991) identified the spread of maize into the Orinoco Valley in South America, and simultaneously described the canopy effect in the Amazon rainforest.
This study’s recognition of the canopy effect, which will be more fully described later, highlights the importance of understanding prehistoric foodwebs in the context of their total environment in order to more accurately understand the meaning of stable isotope data (van der Merwe 1982). Other novel applications of stable isotope analysis in archaeology are described by DeNiro (1987), such as the examination of carbonized organic residues on potsherds and the use of stable nitrogen isotopes to differentiate between marine and terrestrial food sources. While the use of potsherd residues for stable isotope analysis did not rise to popularity until significantly later in time, the use of stable nitrogen isotopes became rapidly integrated into isotopic analyses. This allowed archaeologists to construct a more complex understanding of paleodiets, as it provided information on food sources that were unidentifiable using carbon isotope data alone.

The study of nitrogen isotopes is able to differentiate between marine and terrestrial dietary inputs by relying on the premise that the bone chemistry of animals reflects the isotopic composition of their dietary inputs. In a study that incorporated 66 species of birds, plants and mammals, Schoeninger and DeNiro (1984) recognized that terrestrial animals had generally lower concentrations of $^{15}$N than those inhabiting marine environments. This conclusion allows for the differentiation of marine and terrestrial animals based on the ratio of $^{15}$N/$^{14}$N in their bone. Also, some differentiation can be recognized between herbivores and carnivores within a given environment, although the $\delta^{15}$N values of these groups can display some overlap, which is generally attributed to the nitrogen cycle of the local environment and individual species’ feeding strategies. These observations were then applied to the study of human bone from individuals living in societies utilizing a wide variety of subsistence strategies, from marine mammal hunters
and salmon fishers to agriculturalists, in order to examine the degree to which isotopic ratios in human bone reflect that of their food sources (Schoeninger et al. 1983). The study found that people who ate predominantly either marine or terrestrial foods had $\delta^{15}N$ values that are as differentiated as those of their food sources. Individuals eating a combination of marine and terrestrial foods had intermediate $\delta^{15}N$ values. These findings have been important for archaeologists, as they provide a method for identifying the relative contribution of marine and terrestrial food sources to a paleodiet.

Early applications of stable isotope analysis often focused on investigating when C4 plants began to significantly contribute to the diets of people living in predominantly C3 environments. This approach emphasized identifying diachronic changes in diet within a region in an effort to build dietary chronologies, through which phenomena such as the appearance and intensification of agriculture could be recognized. In North America, examinations focused on the development of maize agriculture throughout the Mississippi river valley, and associated river systems. Lynott et al.’s (1986) examination of the development of maize agriculture in southeast Missouri and northeast Arkansas is emblematic of these early studies as it surveys carbon isotope ratios from burials in the region that were dated from Archaic through historic time periods. By focusing the analysis exclusively on maize consumption, this study is not actually reconstructing a paleodiet, but rather is establishing a regional chronology for maize consumption, and by extension maize agriculture. Ambrose (1987) notes that only one of the five major early isotopic studies in eastern North America even examines nitrogen isotopes, which further demonstrates how research at the time was directed towards establishing a chronology for maize consumption. While other studies charted these changes in maize consumption in
reference to social or demographic variation through time (Buikstra et al. 1987; Buikstra and Milner 1991), few attempts were made, using stable isotope analysis, to investigate the relationship between diet and social, economic, or cultural processes. Even proposed future research at the time placed the “highest priority” on tracing the spread of cultigens (Ambrose 1987: 101), rather than investigating whole diets as they relate to the study of cultures and societies.

As studies in so-called “isotopic archaeology” began to expand beyond traditional applications of examining C4 domesticates within C3 environments, there was a subsequent increase in the abundance of studies that examined both carbon and nitrogen isotopes. Surveys of the field at this time highlight the cases in which stable carbon and stable nitrogen isotopic evidence was effectively synthesized in order to develop paleodietary models that were able to consider a variety of types of food inputs (Schwarcz and Schoeninger 1991; Pate 1994). While this shift did not change the fundamental research question of “what do we eat?”, it did broaden the interpretation of this question to allow for more complex, holistic answers. A methodological shift that coincided with the incorporation of multiple lines of isotopic data was the movement from the older practice of examining a single food source, such as maize, to the development of a synchronic foodweb. The foodweb concept maps the entire range of available food sources according to their stable carbon and stable nitrogen isotopic ratios. The isotopic ratios for each individual examined in the study are also charted on this map, establishing a spatial relationship between various food sources and the diet of the individual. Proximity to a food source reflects the relative dependence on that source,
thus illustrating the composition of the entire diet of an individual. When multiple individuals are plotted, the paleodiet of larger communities can be established.

One of the earliest cases employing the foodweb concept is Keegan and DeNiro’s (1988) reconstruction of paleodiets in the Bahamas using both stable carbon and stable nitrogen isotopic evidence. Constructing a foodweb was an especially appropriate methodological practice for this study, as the Lucayan Taínó, whose diet was being examined, consumed a large amount of marine resources. Through the comprehensive reconstruction of the local foodweb, including dozens of species of mammals, fish, mollusks, algae, cultigens and other possible dietary inputs, the Lucayan Taínó diet could effectively be contextualized within its local environment. Many of these resources have overlapping $^{13}$C ratios, but due to their various levels in the food chain, they have quite different $^{15}$N ratios. These various nitrogen stable isotope levels are reflected in the stable isotope analysis, thus allowing for the identification of various marine resources that would not have otherwise been able to be differentiated.

A second example of how multi-isotopic studies can broaden our understanding of paleodiets is in the examination diets containing beans. Beans are legumes and should therefore have lower $\delta^{15}$N values than other plants since the root systems of legumes contain nitrogen-fixing bacteria that take in atmospheric nitrogen ($N_2$) and turn it into a form that plants can use. By contrast, non-legumes must depend on nitrogen-fixing bacteria in the soil to produce nitrates, which can then be taken in by the plant. This longer process results in higher $\delta^{15}$N values in non-legumes, which should be passed along to consumers of these plants. Ideally, the variation in nitrogen isotope ratios between legume consumers and non-legume consumers would be recognizable in Fort
Ancient populations in the Ohio River Valley, and in the Northeast, where beans became integrated into local diets during late prehistoric times. However, there are no studies that have yet been conducted to test this idea. While Morton and Schwarcz (2004) were able to detect a difference in $\delta^{15}N$ value in experimental food residues composed of legumes and non-legumes, they were not able to detect a similar difference in $\delta^{15}N$ values for archaeological food residues or in the analysis of archaeological human bone. However, their analysis focused on remains recovered from a number of sites across southern Ontario, and still leaves many questions unanswered relating to the importance of beans in Fort Ancient and Northeastern diets.

The use of multiple lines of isotopic evidence has led to an increase in the complexity of paleodietary modeling and a more intricate answer to the question, “what do we eat?” However, methodological advances have also led to a concurrent increase in the limitations of this method. These issues are not insurmountable and can be accommodated in most situations, but each one of them emphasizes the point that we must be aware of exactly what we are measuring. The first of these limitations deals with the type of bone material that is being used for analysis. While the collagen component of bone was traditionally used to conduct stable isotope analysis, it has been found that collagen isotopic levels are controlled by dietary protein, while the carbonate component of bone, apatite, can better reflect the whole diet (Ambrose and Krigbaum 2003). This is because bodies will differentially preference proteins from animal sources for the production of collagen. Such a process can lead to a situation where collagen levels are not indicative of the entire diet, as the presence non-animal proteins that are less-depleted in $^{13}\text{C}$, such as those from maize, could be under represented in relation to carbon.
isotopes derived from animal proteins. Only in situations where non-animal proteins compose a significant component of the diet will the body select this protein source to be used for collagen production. Many studies have began to emphasize that examining both the collagen and carbonate components of bone, as well as tooth enamel, can provide a more accurate reflection of the total diet (Harrison and Katzenburg 1993).

Other limitations to the application of this method focus on how the meaning of stable isotope ratios depends on the isotopic character of the paleoenvironment being examined. Different ecosystems have unique isotopic relationships that must be taken into account when conducting this method of analysis, as a high value in one environment is not necessarily a high value in other environments. Van der Merwe and Medina’s (1991) recognition of the canopy effect, which was referenced earlier, is one example of how the unique characteristics of a local ecosystem can produce seemingly anomalous isotopic ratios. This is an effect found in forested environments in which δ¹³C values increase, i.e. become less negative, at higher levels from the forest floor to the canopy as a result of how CO₂ is recycled with this ecosystem. These varying levels of ¹³C are then passed along to the fauna within this system, including humans, thus leading to a more complex carbon cycle, which must be accounted for. Understanding the local carbon cycle is essential for accurately interpreting isotopic data from such an environment. Arid environments also have been found to display high levels of variability in the abundance of nitrogen isotopic values. Similar critiques have also been made for tropical reef environments, as they too can produce variation in isotopic levels that could only be accounted for through the reconstruction of the paleoenvironment (Keegan and DeNiro 1988). Ambrose (1991) advocates for the reconstruction of the
entire local foodweb, beginning with the soil composition in order to accurately differentiate between the influences of the local ecosystem and physiological processes. Essentially, in order to accurately reconstruct the paleodiet of a culture, this analysis must be contextualized within the local paleoenvironment.

One last limitation to this method is that in order to accurately attribute an isotopic trend to a dietary practice, every potential cause of such an isotopic signature must be accounted for and addressed. An isotopic ratio is not a discrete indicator that is capable of independently indentifying a specific dietary input. Instead, it is usually only able to indicate a general trend, such as an increased reliance on C4 plants. However, the identification of which C4 plant is being consumed depends on various lines of archaeological and paleoenvironmental evidence. Amaranth is one of only a few native North American plants that uses the C4 photosynthetic pathway, but the massive shift in $\delta^{13}C$ values in the North American Midwest around AD 850-950 was never attributed to an increase in amaranth consumption because there is no archaeological evidence for amaranth consumption. Instead, paleoenvironmental and archaeological evidence both indicated that this isotopic shift to more positive $\delta^{13}C$ values was caused by the introduction and adoption of maize agriculture within the region. While the method of stable isotope analysis is very effective at identifying changes in the isotopic composition of a population’s diet, the interpretation of this isotopic variation is dependent on various supporting lines of evidence.

In examining the historical application of stable isotope analysis to archaeological materials, I have traced the theoretical framework in which this method is applied from early applications that focus on establishing chronologies of maize consumption, to more
holistic examinations of dietary practices within the context of foodwebs and paleoenvironmental influences. This development has led to a change in the nature of the research questions being asked involving the use of stable isotope analysis from inquiring about “what do we eat?” to “who are we?” If we are what we eat, then what is our diet saying about us? New avenues of research have begun to examine the relationship between dietary practices and social organization, individual or collective identities, and social class. In this new theoretical model, the consumption decisions of a society are dynamic social processes. Cultures are seen as constructing their own diets and taking an active role in the exploitation and management of food resources. This new research direction investigating the meaning of diets also has expanded to not only include evidence from the isotopic analysis of bone, but also of food residues found on archaeological ceramics as well. While this application of stable isotope analysis is still in its infancy, there is an increasing corpus of work describing its practice (Beehr and Ambrose 2007; Boyd et al. 2008; Reber and Evershed 2004; Seinfeld et al. 2009).

Investigation into the relationship between dietary variation and social organization often examines how isotopic variation in bone from a number of individuals varies relative to their social status within a society. Unlike earlier studies, this line of inquiry relies on archaeological evidence, such as the analysis of mortuary treatment, to recognize social patterns, which can aid in the interpretation of meaningful, socially influenced patterns of diet. Although not the first to do so, Ambrose et al.’s (2003) investigation of differences in diet based on social status is typical of this new direction in the investigation of paleodiets. In this study, high status and low status burials were identified within Mound 72 at Cahokia on the basis of body location, prestigious
associated artifacts, and the presence of retainer burials. Based on the measurements of bone carbonate and bone collagen, there were significant differences in $\delta^{13}C$ values for high status and low status burials, with high status individuals having much lower $\delta^{13}C$ values, which would indicate a diet less reliant on maize than lower status burials. Additionally, elite individuals were found to have higher $\delta^{15}N$ values, which is indicative of a diet composed of greater proportions of animal protein, such as deer or freshwater fish, which would have had $^{15}N$ isotopic values similar to those recorded in humans. Based on this finding, it was proposed that lower status individuals had more limited access to high quality sources of protein, such as meat, than did high status individuals. This forced lower status individuals to rely primarily on lower quality sources of protein such as maize to meet their dietary demands. Archaeological evidence such as decapitation and binding of low status individuals, and the presence of cedar litters under high status burials further supports the conclusions drawn from this isotopic data by indicating that differences in diet were directly reflective of differences in social status. Lastly, discrete dental markers of low status female burials indicate that they were not a part of the same breeding population as high status individuals, suggesting that the differences in social class were indicative of a rigid structure of social organization. The various conclusions that can be drawn from this study illustrate how examining the relationship between diet and culture has provided archaeologists with a new method by which to examine social organization, class divisions and the construction of individual and group identities.

The use of stable isotope analysis in investigating archaeological questions has undergone rapid development since its early conception, both in the actual practice of the
method and in the theory upon which methodological decisions are based. Comparing an early application of stable isotope analysis, that of Lynott et al. (1986), with a more recent example, Finucane et al. (2006), highlights a number of methodological and theoretical transformations that stable isotope analysis has undergone during its relatively brief history in archaeology.

The previously mentioned study by Lynott et al. (1986) needs some further elaboration before it can serve as a basis for comparison. In this project, bone collagen was analyzed from 20 individuals that were collected from 14 sites throughout the Ozark highland and Mississippian alluvial valley in northeastern Arkansas and southeastern Missouri. These sites have associated dates that range in time from 3200 BC to AD 1880. The $\delta^{13}C$ values for these individuals were measured, and a marked change in bone composition towards more positive values was identified in individuals dated to around AD 1000 and later. The conclusion drawn was that this increase in $\delta^{13}C$ indicates a shift to maize agriculture, and although it is slightly later in time than Emergent Mississippian developments in other areas, the authors emphasized that regional variability during the formative period of the Mississippian culture’s development accounts for the timeline of maize agriculture that they present. A number of features of this study allow it to be identified as an early foray into the archaeological application of stable isotope analysis. On a methodological level, the authors only examine carbon isotopes, paying no attention to nitrogen, thus emphasizing their exclusive focus on maize. Furthermore, their use of only bone collagen in their study indicates an early methodological practice. Their use of early theoretical models is also evident in their decision to sample a small number of individuals over an extensive period of time, which
allows only for the recognition of a single dietary change over time. This emphasis on investigating chronology is a common feature of early applications of stable isotope analysis.

In contrast to this early example of the use of stable isotope analysis, its use in the examination of human and animal remains from the highland Peruvian urban site of Conchopata demonstrates a more contemporary application of this method (Finucane et al. 2006). The objectives of this study were to examine the role of maize agriculture in Andean states, and to better characterize the animal management practices of the Wari state, of which Conchopata was a part. This was done by measuring the $\delta^{13}$C and $\delta^{15}$N values for the collagen and carbonate parts of the bones and teeth of 38 individuals, as well as the bones of 40 animals from four species known to be sources of dietary protein in the area. Included in the animal sample were bones from llamas, alpacas, domestic guinea pigs, and the leaf mouse. Based on the $\delta^{13}$C values measured in human bone, it was concluded that people at Conchopata relied on maize as a dietary staple, as opposed to a subsistence pattern focusing on potatoes and tubers that would otherwise be expected in the highlands. This reliance on maize led the authors to conclude that the Wari state was supported by a maize-based subsistence economy. Furthermore, the analyses of camelid bone identified the presence of a high $\delta^{13}$C ratio camel population and a low $\delta^{13}$C ratio camel population. Such evidence indicates the practice of intentionally varied, specific feeding strategies for camelids, which the authors refer to as a maize-camelid, agro-pastoral complex. The significant methodological elements to note in this study include the incorporation of both carbon and nitrogen isotopes, as well as the examination of collagen and apatite components of bone and teeth in the analysis. The theory guiding
this research project also clearly emphasizes the examination of a comprehensive diet at a single time and place, as a way of understanding how the people of Conchopata constructed their diet.

The comparisons that can be drawn between these cases from the Ozark highlands and Conchopata clearly demonstrate how the application of this method in archaeology has changed through time. The theoretical implications of the first study are limited with a focus on investigating when maize can be detected in the Ozark highlands and adjacent Mississippi alluvial valley. The explicit emphasis of this research question is placed on dating when the Ozark peoples adopted maize agriculture, and excludes any consideration of how this change in subsistence practice may have affected local societies at this time. While the question of maize agriculture was also addressed in the Conchopata study, this issue was subsumed within larger questions dealing with economic models, animal management practices, and the development of state level societies. The theoretical foundation of these research questions clearly emphasizes the importance of investigating human behaviors and how people and societies construct their diet.

The implications of how these two different theories guide the reconstruction of a paleodiet are evident in the methodological practices employed in each of these studies. The Ozark study was only concerned with the presence or absence of maize consumption, and had no interest in developing an understanding of other cultural questions at any individual site. This can be seen in the methodological decision to investigate only the $\delta^{13}$C values of a relatively few individuals scattered across space and time. In contrast, the Conchopata study not only sampled twice as many humans as the Ozark study, but it
also included twice as many animal samples as the Ozark study included humans, all from the same site and dated to the same period. By using a more comprehensive sample, combined with the collection of both $^{13}$C and $^{15}$N data, this project was able to examine the presence of maize agriculture, as the Ozark study did, but it did so within the framework of investigating overarching social phenomenon. The breadth of data that this study collected enabled the reconstruction of an intricate paleodiet, and from that, models of economic, social and subsistence practices could be devised. While each of these studies are valid and effective within their own theoretical framework, this comparison clearly demonstrates how transformation in the use of stable isotope analyses has led to an increasing complexity of the theoretical models used to examine the relationship between paleodiet and culture.

The development of the stable isotope analysis has unquestionably led to a more complete and integrated understanding of the dynamic relationship between paleodiet and cultural phenomenon. As this method has evolved, it has in many ways been a microcosm, representative of the changes taking place within archaeological theory as a whole. Ranging from formative questions of culture-history and chronology to modern ideas of political economy and identity, this method has helped reshape archaeology’s conception of the meaning of food and food production in antiquity. While many supporting lines of evidence are necessary for the reconstruction of a paleodiet, it is unquestionable that stable isotope analysis is a crucial method for investigating whether we really are what we eat.
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