The reconstruction of past human diet is one of the most basic concerns of the archaeologist. Food production and distribution, besides being essential to survival, are defined by, and help to define, a broad range of social, cultural, and political processes. In almost every society, food forms a key part of social and economic relations at multiple levels, as well as serving as a symbol related to different aspects of culture (e.g., ethnicity, political power, religion) and as an expression of forms of social identity (for a review on this topic see, for example, Ashley et al. 2004; Farb and Armelagos 1983; Harris 1985; Mintz and Du Bois 2002). In particular, the use and control of food can become an arena where power and prestige are negotiated (Appadurai 1981; Danforth 1999; Hastorf and Johannessen 1993; Ross 1987). Thus, it is important to recognize that food is a dynamic entity in a wide range of social relations in both private and public spheres.

Within the cultural historic framework of Caribbean archaeology, however, most studies have treated diet, subsistence, and food only as fossil indexes of chronological or cultural periods. Rainey (1935, 1940) was the first to do so when he used diet as a marker for shifts in ethnic identity in Puerto Rico. During his excavation in Ponce, Rainey noted that Cedrosan Saladoid subseries assemblages contained high concentrations of crab claws, while those from the later Elenan and Chican Ostionoid subseries contained high quantities of shellfish instead. This led him to define these deposits as belonging to successive peoples of the “Crab” and “Shell” cultures, respectively. In more recent years, deFrance (1989) conducted a more detailed analysis of Cedrosan Saladoid and Elenan Ostionoid assemblages from Maisabel in order to investigate and test Rainey’s dichotomy of Crab and Shell cultures. In the 1980s and 1990s, Yvonne Narganes Storde (1985,
1993a, 1993b) also began to conduct more refined zooarchaeological studies intended to identify differences in the diet of distinct cultural groups (Hueco and Cedrosan) in the early Ceramic age. In all of these cases as well, food remains were used as a diagnostic to define the different assemblages of different, but in this case contemporary, “peoples.” While valuable, these studies have failed to view food as an active element in the structuring of interpersonal relationships of the society in which it was being consumed. This reduces food to little more than a passive marker of people, rather than a potentially useful tool for the reconstruction of past social processes.

In order to distill meaningful and accurate insights about the broader social dimensions of food in any given past society, one must first employ an analytical methodology that is capable of reconstructing past subsistence patterns with sufficient fidelity and at a satisfactory resolution. Traditionally, paleodiets reconstruction has involved either a reliance on possibly inaccurate or slanted historical sources or the indirect archaeological study of diet through the analysis of food remains (zooarchaeology, paleoethnobotany) or nutrition-related pathologies in human skeletal remains (paleopathology). While these techniques are all capable of providing valuable dietary insights, all also possess theoretical and/or methodological shortcomings that can result in overly coarsely grained and/or misleading reconstructions of past human diet.

The study of food remains (either faunal or floral) is, perhaps, the most common means of paleodiets analysis. When carried out diligently, such techniques can provide detailed information on the different types of food resources exploited, quite often with species-specific resolution. This resolution is extremely useful since habitats and possible methods of exploitation can be inferred from the identification of particular species, and when combined with other types of information, such analyses can provide insights in matters such as the control of labor. However, the use of techniques involving the indirect study of diet through food remains can produce nonrandomly biased or misleading results because of the differential survival and preservation of different classes of food (Pearsall 2000). In the Caribbean, as in the Neotropics in general, where many important foodstuffs (e.g., manioc) are composed mainly of soft plant tissues that survive poorly or not at all, this is of particular concern (Newsom 1993). As floral and faunal evidence is rarely recovered in the relative quantities in which it was consumed, the conclusions derived from such evidence are necessarily qualitative rather than quantitative (Ambrose 1993:59). Moreover, it is important to remember that the scale of the data derived from the study of food remains is at the level of a community or group rather than that of an individual, that the temporal resolution provided by such evidence can be quite variable, and that the data it can produce can be misleading due to many cultural and natural formation processes that may have
affected its evidentiary basis (Barberena and Borrero 2005). Thus, indirect means of dietary reconstruction will tend to fail in the detection of subtle differences in diet between individuals. Put differently, the study of the actual food remains provides us with the menu rather than the meal (Ambrose 1993:82, 85).

The study of health indexes in human bones is another means of acquiring information about past peoples’ diets. Since many aspects of health are related to nutrition, skeletal health indicators and pathologies can provide indirect information about the types and quantities of foods consumed. Prevalence rates for skeletal pathologies such as cribra orbitalia, porotic hyperostosis, and linear enamel hypoplasias; the overall disease load in a population; and broader indicators such as stature have all been argued to serve as effective proxies for nutritional well being (Buikstra 1992; Goodman et al. 1988; Roberts and Manchester 1999). While absolute statements about nutrition are not possible as a result of this type of analysis, relative statements based on changes through time or differences between individuals resulting from differences in status are possible. The inferences derived from such studies are necessarily broad, however, and can be fraught with potential difficulties given the multiple etiologies of the pathologies under discussion and the fact that the individuals under study may not be representative of the population at large (Ambrose 1993; Wood et al. 1992). Furthermore, such analysis can really only be used to infer nutritional inadequacy rather than the presence or relative contribution of any actual dietary items (Schwarcz and Schoeninger 1991:284).

In light of the methodological shortcomings of the techniques most often employed in the study of diet in the prehistoric Caribbean, this chapter presents the results of a pilot study undertaken to assess the feasibility of instead using biogeochemical analytical techniques to reconstruct the subsistence patterns of the prehistoric inhabitants of Puerto Rico. The analysis of the stable isotopes of two light elements (carbon and nitrogen) in both the organic and mineral phases of human bone was employed to facilitate the direct dietary reconstruction of four individuals interred within the Ceremonial Center of Tibes. This work forms part of a larger ongoing project employing stable isotope analysis of Puerto Rican skeletal materials of varying ages and depositional histories that aims to define the relationship between subsistence and the in situ development of social complexity in prehistoric Puerto Rico. It is hoped that the use of this stable isotope technique, when combined with multisource mixture modeling, will produce data of sufficient resolution and quality to allow for the reconstruction of the possible dietary dimensions of fine-grained social processes. If successful, it is hoped that the application of this technique will allow for the detection of subtle, individual-level changes in diet possibly reflective of a diachronic shift in sociocultural organization.
Dietary Reconstruction Using Stable Isotopes

At its simplest, stable isotope analysis of paleodiet is predicated on the notion that you are, isotopically, what you eat. Stable isotope analysis of prehistoric human diets involves the comparison of carbon and nitrogen isotope ratios from human consumer tissues (in this case the organic and inorganic phases of bone) with those of the edible portions of possible food sources. While the relationship between the isotopic values of consumer and source tissues is far from one-to-one due to fractionation factors, the results of this analysis can allow one to determine, with a healthy degree of imprecision, the relative importance that various foodstuffs may have had in a past individual’s diet.

Stable isotopes are nonradioactive variants of an element differing from the most abundant isotope in the number of neutrons in the nucleus (Fry 2006). Due to the differences in their atomic masses, different isotopes of the same element can behave differently both kinetically and thermodynamically during chemical reactions, thereby resulting in a characteristic patterning of these isotopes in organisms and their tissues (Fry 2006; Schwarcz and Schoeninger 1991; Urey 1947). For the purposes of dietary reconstruction, the stable isotope ratios of two elements, carbon and nitrogen, are of principal interest, because these elements are taken up by plants from soil, water, and air and are passed on in a known fashion through various consumers in a food web (Ambrose 1987; Bender et al. 1981; Delwiche et al. 1979; DeNiro 1987; DeNiro and Epstein 1978, 1981; Schoeninger 1985; Schoeninger and DeNiro 1984; van der Merwe 1982; Vogel and van der Merwe 1977).

The reconstruction of prehistoric subsistence through stable isotope analysis is today a well-established technique, with some four decades of supporting literature. The technique has been employed in ecological studies since the 1960s (Parker 1964), and the possibility of its usefulness for the study of past human diets was realized soon after its inception (Hall 1967). While techniques have been refined and improved since their earliest practical applications (Bender et al. 1981; Chisholm et al. 1983; Chisholm et al. 1982; Delwiche et al. 1979; DeNiro 1985; DeNiro and Epstein 1978, 1981; Klepinger 1984; Schoeninger 1985; Schoeninger and DeNiro 1984; Schoeninger et al. 1983; Sullivan and Krueger 1981, 1983; Tieszen et al. 1983; van der Merwe 1982; van der Merwe et al. 1981; Vogel and van der Merwe 1977), the theoretical basis for the analysis remains the same: predictable variation in the isotopic signatures of certain classes of plant and animal foodstuffs (resulting from their preferred sources of food [and thus carbon and nitrogen] and their position in their respective food webs) can make possible the reconstruction of the relative quantitative contribution each class of foodstuff made to the diet of an analyzed consumer. More recent studies (e.g., Ambrose and Norr 1993) have refined the technique, as well as our understanding of the underlying
processes thereof, to the extent that now both protein and energy (carbohydrate, lipid) portions of an individual’s diet can be reconstructed more or less independently.

In stable isotope studies, isolated and purified samples (preparation methods are discussed below) are converted to a gaseous form and analyzed using an isotope ratio mass spectrometer (Ambrose 1993:67–71; Brenna et al. 1997). The results of this analysis are expressed as the ratio of the heavier to the lighter isotope (\(^{13}\)C to \(^{12}\)C in the case of carbon, \(^{15}\)N to \(^{14}\)N in the case of nitrogen) and are typically presented using a delta notation (\(\delta\)) in parts per thousand (per mil or ‰) relative to an international environmental standard. Sample delta values for carbon and nitrogen are determined using the following formulas:

\[
\delta^{13}C = \left( \frac{\left( ^{13}C/^{12}C \right)_{\text{sample}} - \left( ^{13}C/^{12}C \right)_{\text{PDB}}}{\left( ^{13}C/^{12}C \right)_{\text{PDB}}} \right) \times 1000
\]

and

\[
\delta^{15}N = \left( \frac{\left( ^{15}N/^{14}N \right)_{\text{sample}} - \left( ^{15}N/^{14}N \right)_{\text{AIR}}}{\left( ^{15}N/^{14}N \right)_{\text{AIR}}} \right) \times 1000
\]

The standard employed for carbon is the Vienna Pee Dee marine fossil formation (PDB, Craig 1957), which contains far more \(^{13}\)C than most human foodstuffs or tissues, thus rendering most archaeological \(\delta^{13}C\) values as negative numbers. The nitrogen standard is atmospheric nitrogen (AIR, Mariotti 1983), which is more depleted in \(^{15}\)N than most archaeological samples, with the result that most reported values in archaeological studies are positive numbers.

Broadly, \(\delta^{13}C\) values in flora discriminate exclusively between plants of the so-called C3 (trees, fruits, tubers, temperate grasses) and C4 (maize, sugarcane, sorghum, amaranths) photosynthetic pathways, with so-called CAM (Crassulacean Acid Metabolism, e.g., cacti or pineapple) and marine plants occupying the middle ground between the C3–C4 isotopic extremes (Sage et al. 1999; Smith and Epstein 1971). The cause of this isotopic difference can be found in the varying degree to which the different plant classes fix \(^{13}\)C available from atmospheric sources of CO2 (\(\delta^{13}C = -7\%o\)). As a result of these isotopic/photosynthetic differences, C3 plants have \(\delta^{13}C\) values averaging \(-26\%o\), whereas C4 plants average \(-12\%o\) (Bender et al. 1981; Smith and Epstein 1971). The tissues of the consumers of these plants, be they animals or humans, continue to reflect the isotopic signatures of their plant-based diet onward through the food chain, with, for example, the tissues of exclusive consumers of C4 plants having a much more enriched (less negative) \(\delta^{13}C\) value than that of an exclusive C3 consumer.

Values of \(\delta^{13}C\) can also be used as a marker of the consumption of marine
foods, as the bicarbonate ingested by most marine species is enriched by roughly 7‰ over atmospheric carbon dioxide, with the exception of reef and seagrass dwellers, which are enriched even more (4–7‰ greater still) than their pelagic marine neighbors (Chisholm et al. 1982; DeNiro and Epstein 1978; Fry et al. 1982; Schoeninger and DeNiro 1984; Schoeninger et al. 1983). Given the proximity of their δ¹³C values, diets that incorporate both marine foods and C₄ terrestrial foods can result in problematic interpretive issues.

Signatures of δ¹⁵N, on the other hand, can differentiate between legumes (averaging a δ¹⁵N of 1‰) and non-nitrogen fixing plants (an average of 3‰) (Dellwiche et al. 1979; Schoeninger and DeNiro 1984). Nitrogen isotope signatures also pattern strongly with trophic level, with a stepwise increase of approximately 3–5‰ between the tissues of consumers at successive trophic levels (herbivores, primary carnivores, secondary carnivores) (Schoeninger 1985; Schoeninger and DeNiro 1984). This latter effect is of particular use for archaeological studies of past diet as it allows for the discrimination of marine vs. terrestrial diets. This determination is possible as a result of the relatively “longer” trophic chains seen in marine ecosystems, which result in the fact that marine species tend to exhibit more enriched δ¹⁵N signatures than do their terrestrial equivalents (Bösl et al. 2006:297).

Most of the early archaeological applications of stable isotope analysis focused solely on the isotopic makeup of collagen, the organic (proteinaceous) fraction of human bone. This focus was a result of the persistence of the linear mixing model, which posited that consumed materials, and thus consumed isotopes, were incorporated in equal amounts in any and all tissues of the consumer (van der Merwe 1982; White and Schwarz 1989). Since, under this model, all tissues were believed to reflect faithfully the isotopic makeup of one’s diet, collagen from archaeological samples, because it presented fewer problems in terms of diagenetic alteration, became the preferred material for analysis. However, more recent research, including controlled diet experiments, has made clear that a different model, which combines portions of linear mixing with a macronutrient routing model (Chisholm et al. 1982; Krueger and Sullivan 1984), better reflects the way in which the constituent portions of foodstuffs are incorporated into a consumer’s tissues. Ambrose and Norr’s landmark 1993 study on this matter verified that different components of a consumer’s diet were preferentially incorporated into and reflected by different tissues of that consumer. Thus, collagen in human bone primarily reflects the protein portion of that individual’s diet (not the whole diet as had been previously assumed), whereas the apatite (mineral phase) of the same human bone reflects the entire diet, including both dietary protein and the dietary energy component (carbohydrates and lipids). Additionally, Ambrose and Norr (1993) found that the spacing between δ¹³C values in collagen and apatite, Δ¹³C_{ap-co}, can be of great utility in understanding the amount of protein in the diet as well as the relative amounts and isotopic signature of energy and protein foodstuffs.
A final key result of Ambrose and Norr’s (1993) study was their demonstration of the inappropriateness of applying a supposed universal fractionation factor to $\delta^{13}$C values (normally +5‰) of consumer collagen in an attempt to determine the carbon isotope value for that consumer’s proteinaceous food sources. While, on the one hand, $\Delta^{13}C_{ap-diet}$ is effectively constant at or around +9.4‰, based on the isotopic differences or similarities within and between the energy and protein portions of a consumer’s diet (e.g., C4-derived protein and C3-based energy), the degree of fractionation between consumer collagen and dietary protein (or $\Delta^{13}C_{co-protein}$) can range from +12.6‰ to −0.5‰ (Ambrose and Norr 1993). Thus, it is not possible to simply subtract 5‰ from consumer collagen $\delta^{13}$C to arrive at the $\delta^{13}$C value of the foods they ate. Nonetheless, with a detailed knowledge of the isotopic character of potential food sources, and in light of controlled diet experiments, it is still possible to reconstruct both the protein (meat) and energy (carbohydrate) components of a consumer’s diet independently and with notable accuracy as long as caution is exercised in the application of correction/fractionation factors.

**Previous Caribbean Isotopic Studies**

To date, only six isotopic studies have been undertaken of prehistoric subsistence in the Caribbean (Keegan 1985; Keegan and DeNiro 1988; Norr 2002; Schoeninger et al. 1983; Stokes 1998; van Klinken 1991). The four earliest studies were conducted when the linear mixing model (see above) was still en vogue and thus relied on the analysis of bone collagen alone for the reconstruction of the whole diet. This methodological shortcoming serves to substantially, if not totally, mitigate the usefulness of their results. Moreover, van Klinken (1991) included reported results for samples whose collagen C:N ratios (discussed below) were suggestive of a high degree of postmortem diagenetic alteration. Fortunately, Stokes (1998) included in her dissertation a reanalysis of many of the same individuals previously analyzed by Keegan, DeNiro, and van Klinken, in addition to a large number of individuals from other sites throughout the Caribbean. Stokes’s work thereby greatly augments and normalizes the comparative data set for the present study. Norr (2002) adds to this comparative corpus the results of an analysis of more than 20 individuals from the Tutu site in the U.S. Virgin Islands.

**Methods and Materials**

In August 2004, with funding from the H. John Heinz III Foundation, samples of human bone and teeth were extracted from skeletal material from four prehistoric Puerto Rican sites: Maruca, Punta Candelero, Paso del Indio, and Tibes (Table 10.1). Each site is located in a geologically and ecologically distinct portion of the island and, in general, the materials from them belong to different
cultural/chronological periods. Individuals were included in this study only when both age and sex could be assessed reliably, and the skeletal elements selected for sampling were required to be robust and dense. Any evidence of heat alteration, discoloration, preservative agents, consolidants, or fungal activity disqualified individuals or elements from the present study. Gloves and masks were worn throughout the sample extraction process, and sampling instruments were cleaned between individuals with dilute bleach in order to decrease the potential for cross-contamination of samples. While sampling requirements have since been revised, at the time of this pilot project two teeth and approximately 5 g of bone per individual were extracted. Samples were later subdivided for three types of analysis (stable isotope, trace element, and mtDNA) in a laboratory of the Anthropology Department of The Field Museum of Natural History, Chicago.¹ Funding for the pilot study limited sample size to four individuals per site (for a total of 16 individuals), rendering the resultant data too limited for anything but the most tentative conclusions, but useful for assessing the feasibility of further study. In the context of the present work, however, it was decided to go marginally further and use the present, admittedly limited, data set to formulate some working hypotheses about trends in Puerto Rican paleodiet by which we might direct our future research on the topic.

Human bone samples from the 16 individuals (including the four individuals from Tibes) were analyzed at the Environmental Isotope Paleobiogeochemistry
Laboratory of the Department of Anthropology at the University of Illinois Urbana-Champaign in 2004 and 2005 using the sample preparation and analytical methods described in Ambrose et al. (2003). Broadly, this process involves the mechanical cleaning and grinding of samples, followed by a division of the samples into fractions used for separate analysis of the mineral and collagenous portions of the bone. These split samples are subsequently isolated and purified (a process that involves demineralization, dehumification, filtration, and gelatinization for collagen and organic and labile carbonate removal for apatite) prior to their separate combustion and analysis via mass spectrometry.

As the principal goal of the pilot study was to assess the usefulness of stable isotope analysis for studying paleodiet in prehistoric Puerto Rico, assurance was required that samples under analysis were sufficiently unaltered following their prolonged period of burial. As the chemical interactions of bone with other body tissues and burial soils (diagenesis) can alter bone isotopic values and thus produce misleading dietary results, it is necessary in any instance of stable isotope analysis to assess the character and quality of the preserved osseous materials. To this end, percentage yield collagen, percentage yields carbon and nitrogen, and carbon to nitrogen ratio (C:N), the most commonly employed sample fitness standards, were calculated for all samples in order to assess their suitability for further study (Ambrose 1990; DeNiro 1985; van Klinken 1999).

The use of a continuous flow isotope ratio mass spectrometer allowed for the detection of both $\delta^{13}C$ and $\delta^{15}N$ values for bone collagen and $\delta^{13}C$ values for bone apatite from all samples in question. The results of this step of the analysis provide one with the isotopic signature of a consumer, but in order to arrive at the isotopic value of that individual's diet, one must employ correction factors to compensate for the isotopic changes brought about by chemical fractionation between consumer and consumed. Fractionation appears as an enrichment or depletion of the isotopic signature of a consumer’s tissue relative to the isotopic value of the foods being consumed, with different tissues and different organisms fractionating their dietary isotopes differently.

As discussed above, in the case of collagen $\delta^{15}N$ and apatite $\delta^{13}C$, the process of correcting for fractionation is a fairly simple one. To arrive at the $\delta^{15}N$ of an individual’s diet, one can simply subtract 3‰ from the $\delta^{15}N$ value derived from the collagen in order to account for the trophic level increase between the human under analysis and his/her diet, although published estimates do range between 1.6‰ and 5‰ (Ambrose 2000; Ambrose et al. 2003; Bocherens et al. 1995; Fry 1991; Hedges and Reynard 2007; Schoeller 1995; Schoeninger 1985; Schoeninger and DeNiro 1984; Schwarcz 1991; Schwarcz and Schoeninger 1991; Tuross et al. 1994). Similarly, in the case of apatite $\delta^{13}C$, a $-9.4‰$ correction factor to the consumer $\delta^{13}C$ signature allows one to arrive at the $\delta^{13}C$ value of the consumer’s whole diet (Ambrose and Norr 1993). However, in the case of bone collagen $\delta^{13}C$,
the matter of correcting for fractionation is substantially more complex. This complexity is the result of a compound relationship between the isotopic value of dietary protein, energy, and the relative amounts of each in the consumer’s diet, with all of these playing a role in determining the resulting spacing between analyzed collagen $\delta^{13}C$ and the $\delta^{13}C$ value of dietary protein. For the purpose of the present analysis, we have employed a very conservative collagen to protein spacing value of $+1.1\%$. The use of this spacing value was determined to be appropriate for the present study based on the observed strong negative correlation ($-1.0$) in the published data of Ambrose and Norr (1993) between $\Delta^{13}C_{ap-co}$ and the isotopic spacing from the $\delta^{13}C$ of collagen to that of dietary consumables. We used this observed correlation to generate a predictive formula for deriving an appropriate collagen–diet spacing value based on the $\Delta^{13}C_{ap-co}$ of a given sample. Use of this formula resulted in the $+1.1\%$ value employed herein. The use of a $+1.1\%$ spacing will, if anything, tend to underestimate the contribution of traditionally underappreciated foodstuffs (freshwater and terrestrial fauna) to the Tibes individuals’ diets. Even so, as is discussed below, the results of this analysis suggest that such sources actually dominated the protein portion of these diets, and thus the $+1.1\%$ value employed would appear to represent an extremely conservative estimate of $\delta^{13}C$ collagen to protein spacing.

In order to establish a set of baseline values for the dietary options to which the prehistoric human consumers of Tibes may have had access, it was also necessary to compile isotopic data for a range of potential foodstuffs. As the pilot study did not include funds to analyze any nonhuman (floral and faunal) materials, isotope values for possible foodstuffs were compiled from previously published results (Keegan 1985; Keegan and DeNiro 1988; March and Pringle 2003; Newsome et al. 2004; Stokes 1998). Due to discrepancies in the analytical methodology employed by these various studies, the lack of published data for many potential foodstuffs from the immediate area of Tibes, and the inclusion in the present study of isotopic data from species and individuals that may not faithfully represent the values of similar foods consumed at Tibes, the data compiled in this exemplar food web are best viewed as tentative and preliminary.

A correction factor of $+1.5\%$ $\delta^{13}C$ was applied to any modern food samples used in the construction of the ancient food web in order to account for the enrichment of atmospheric $^{12}C$ brought about by the recent burning of fossil fuels (Keeling et al. 1979; Marino and McElroy 1991). As well, corrections for the isotopic differences between consumable (flesh) and tested (bone or shell) tissues (for fish, $-3.7\%$ $\delta^{13}C$ and $+1.7\%$ $\delta^{15}N$; for all other animals, $-2.3\%$ $\delta^{13}C$ and $+0.6\%$ $\delta^{15}N$) have been applied as appropriate (DeNiro and Epstein 1978, 1981; Keegan and DeNiro 1988; van der Merwe 1982).

To further enhance the usefulness of stable isotope analysis for the reconstruc-
tion of the protein portion of the diet of the Tibes individuals, we also made use of a sophisticated multisource mixing analysis software called IsoSource (Phillips and Gregg 2003; Phillips et al. 2005). This software has only recently begun to be used in the reconstruction of past dietary patterns (Bocherens et al. 2005; Drucker and Henry-Gambier 2005; Newsome et al. 2004). Originally developed by researchers at the U.S. Environmental Protection Agency for ecological and geological applications, this software resolves the complicated computational issues that lie behind analysis of mixing equations where the number of potential sources is greater than \( n + 1 \), where \( n \) equals the number of informative isotope ratios (e.g., \( \delta^{13}C \)) possessed for both a consumer and all its potential food sources (Phillips and Gregg 2003; Phillips et al. 2005). The application of this technique, while failing to provide a silver bullet or definitive dietary combination for a past consumer, determines the range of (mathematically) feasible contributions of each potential food source to a given individual’s diet. This is accomplished through a process of simulation wherein all possible combinations of proposed foodstuffs, and their accompanying isotopic signatures, are tested against the observed isotopic values of the consumer under analysis: “In this method, all possible combinations of each source contribution (0–100%) are examined in small increments (e.g., 1%). Combinations that sum to the observed mixture of isotopic signatures within a small tolerance (e.g., “0.1‰) are considered to be feasible solutions, from which the frequency and range of potential source contributions can be determined” (Phillips and Gregg 2003:261).

Thus, while without this software the two isotope systems used in dietary reconstruction (carbon and nitrogen) might allow for the determination of the relative contributions of three \(( n + 1, \text{ where } n = 2)\) different foodstuffs, the use of multisource mixture modeling facilitates the determination of the dietary contributions of many more food sources, a situation that better mirrors the dietary variety available in the protein portion of the prehistoric Puerto Rican food web.

The percentage of \( \text{C}_4 / \text{CAM} \) plants in the diets of the individuals under study was calculated using the following formula (Ambrose et al. 1997; Schwarcz and Schoeninger 1991):

\[
\% \text{C}_4 = \left( \frac{-25 - (\delta^{13}C_{ap} - 9.4‰)}{15} \right) \times 100
\]

Due to uncertainty introduced by individual variation, instrumentation, and temporal factors, as well as the possibly confounding effect brought about by the presence in the diet of substantial quantities of marine foodstuffs, the accuracy of the results of this calculation of dietary percentage \( \text{C}_4 / \text{CAM} \) should be considered to be ±10 percent (Ambrose et al. 2003).

Finally, in order to assess the chronological dimensions of dietary change (or
the lack thereof) at Tibes, collagen samples from all of the individuals discussed herein were AMS dated at the Accelerator Mass Spectrometry Laboratory at the University of Arizona, Tucson.

**Results**

Of the samples extracted from the four individuals buried at Tibes, three (E-48, E-13, and CE-5) contained a satisfactory amount of collagen (averaging 1.7 wt%) and possessed C:N (carbon to nitrogen) ratios (3.44 average) at or exceeding acceptable standards (Table 10.2). In addition, the fourth individual (ES-3) presented a collagen yield of 0.48 wt% and a C:N ratio of 3.63, both of which deviate only minimally from desired/accepted levels. Due to the small degree of the departure of ES-3 values from accepted standards, we believed the inclusion of this individual in the present preliminary study to be warranted. These results demonstrate that the Tibes samples were sufficiently unaltered by diagenetic processes and that their resulting isotopic signatures can be considered to be the result of bona fide dietary intake rather than taphonomic alteration. Overall, 75 percent of the samples from the four sites under study (Maruca, Paso del Indio, Punta Candelero, and Tibes) met or exceeded standards for sample fitness, giving us great hope for the future expansion of this study.

The isotopic composition of bone collagen and apatite from the Tibes individuals and the predicted isotopic values for the protein and energy portions of their diets are provided in Table 10.3, as are the results of AMS dating of bone collagen from these individuals. The $\delta^{15}N_{\text{co}}$ values for these consumers were moderately enriched, ranging from 9.07‰ to 10.37‰, while the $\delta^{13}C_{\text{co}}$ values extended from $-17.15‰$ to $-17.72‰$. Both of these isotopic indicators would appear to indicate the possibility of an unanticipated source or sources of dietary protein for the individuals interred at Tibes. Moreover, the $\delta^{13}C_{\text{ap}}$ values for these individuals (which range from $-8.01‰$ to $-9.36‰$) are notably enriched, and the $\Delta^{13}C_{\text{ap-co}}$
Table 10.3. Analyzed stable isotope ratios of individuals interred at Tibes, predicted isotopic values of diets, and AMS dates

<table>
<thead>
<tr>
<th>Individual</th>
<th>$\delta^{15}$N co (‰)</th>
<th>$\delta^{15}$N protein (‰)</th>
<th>$\delta^{13}$C co (‰)</th>
<th>$\delta^{13}$C protein (‰)</th>
<th>$\Delta^{13}$C ap-co (‰)</th>
<th>$\delta^{13}$C diet (‰)</th>
<th>% C4/CAM</th>
<th>$^{14}$C Age, Years B.P.</th>
<th>Calibrated Dates (1σ age range) Area under Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-13</td>
<td>10.37</td>
<td>7.37</td>
<td>-17.21</td>
<td>≤-18.31</td>
<td>8.16</td>
<td>-9.05</td>
<td>-18.45</td>
<td>43.67</td>
<td>(cal A.D. 641: cal A.D. 691) 0.893963 (cal A.D. 750: cal A.D. 762) 0.106037</td>
</tr>
<tr>
<td>ES-3</td>
<td>10.32</td>
<td>7.32</td>
<td>-17.15</td>
<td>≤-18.25</td>
<td>7.79</td>
<td>-9.36</td>
<td>-18.76</td>
<td>41.6</td>
<td>(cal A.D. 615: cal A.D. 664) 1</td>
</tr>
<tr>
<td>CE-5</td>
<td>9.07</td>
<td>6.07</td>
<td>-17.72</td>
<td>≤-18.82</td>
<td>9.71</td>
<td>-8.01</td>
<td>-17.41</td>
<td>50.6</td>
<td>(cal A.D. 599: cal A.D. 653) 1</td>
</tr>
<tr>
<td>Mean</td>
<td>9.89</td>
<td>6.89</td>
<td>-17.36</td>
<td>≤-18.46</td>
<td>8.48</td>
<td>-8.88</td>
<td>-18.28</td>
<td>44.78</td>
<td>n/a</td>
</tr>
</tbody>
</table>
spacing values (all greater than 7.7‰) are fairly high, perhaps as the result of a large amount of C4/CAM-derived dietary carbohydrates, a possibility detailed below.

The predicted isotopic signatures for the diets of the Tibes individuals can be compared to the isotopic values of possible dietary elements using the individual carbon and nitrogen isotope values of a wide range (n = 83) of potential foodstuffs, as gleaned from published sources. The values provided in Table 10.4 reflect the isotopic values of these potential foodstuffs with appropriate correction factors for their respective dates and tissue type already having been applied. Given the enormous number of potential individual foods, for the purpose of dietary reconstruction, the individual species or genera of foodstuffs have been grouped by bio-ecological niche in order to produce the 11 categories of consumables represented in Table 10.4.

**Discussion**

Focusing first on the results of the analysis of bone collagen, both the carbon and nitrogen results require discussion. The moderately enriched δ¹⁵N signatures (averaging 9.89‰) observed in the collagen of the Tibes human samples speak to the high trophic level that these individuals must have occupied within the region’s prehistoric food web. In fact, the δ¹⁵N values of the human samples were on par with the upper range of values observed in typically high trophic level species of marine and freshwater fish and were outstripped only by those of the marine seals (Figure 10.1). This is not to say that the Tibes humans were as carnivorous as...
barracuda, sharks, or seals, as a little meat goes a long way isotopically, but rather that there was a substantial portion of (high trophic level) animal protein in the Tibes humans’ diets (Ambrose et al. 2003; Ambrose and Norr 1993). The potential source or sources for this high trophic level protein is clarified by the results of the $\delta^{13}C_{co}$ analysis, which produced surprisingly depleted (negative) carbon isotope ratios, averaging $-17.36\%o$. From an examination of the proteinaceous foodstuffs potentially available to the Tibes consumers, it is evident that their $\delta^{13}C$ values were likely pulled toward more negative values by the inclusion in the diet of some substantial amount of high trophic level (high $^{15}N$) and $^{13}C$-depleted protein. Based on the reconstruction of the local food web provided in Table 10.4, the list of likely candidates for this protein source includes freshwater fauna (mean $\delta^{15}N$ 7.19, mean $\delta^{13}C$ $-18.88$), birds (mean $\delta^{15}N$ 7.19, mean $\delta^{13}C$ $-18.5$), terrestrial fauna (mean $\delta^{15}N$ 6.61, mean $\delta^{13}C$ $-23.76$), and pinnipeds (mean $\delta^{14}N$ 19.1, mean $\delta^{13}C$ $-19.2$). By utilizing only traditional means of isotopic dietary reconstruction, there remains a notable lack of precision in determining the relative amount of protein that may have been derived from each of these potential sources. All that can be said is that any or all of these sources played an important role in the provision of dietary protein.

To some degree, this imprecision is the result of the breadth of the food web
groupings employed in this analysis and the resulting overlap of the contrived biocological food groups (Table 10.4). The variation in isotope values within each grouping is likely the result of a number of factors (inappropriate grouping, heterogeneity in geography, environment, or diet for specimens analyzed) and is likely further compounded by small sample size. In any instance of isotopic analysis of diet wherein the various potential food sources show substantial overlap, some degree of ambiguity will necessarily result. However, another source of uncertainty in the reconstruction of the protein portion of the Tibes individuals’ diets, that which results from the sheer multitude of possible food sources, can be minimized by employing multisource mixture modeling software such as IsoSource. As discussed above, this method allows for the resolution of individual source contributions when the number of potential sources is greater than \( n + 1 \), where \( n \) equals the number of informative isotope ratios (Phillips and Gregg 2003; Phillips et al. 2005). While such techniques do not provide absolute solutions for the relative makeup of an individual’s diet, they do allow determinations of major and minor contributors to be made.

The bounded results of the IsoSource analysis of the Tibes individuals’ diets are presented in Table 10.5 (as above, this analysis was undertaken using the very conservative collagen to protein spacing value of +1.1‰). For these purposes, the 1st percentile values are taken to represent a reasonable minimum amount of dietary protein derived from each individual source, the 99th percentile values represent a maximum potential contribution from each source, and the mean values represent the most likely amount of protein derived from each source in that individual’s diet. The mean values of the relative contributions of each of the potential sources (summing to 100 percent) are also represented graphically in Figure 10.2. All told, the results of this mixture modeling paint a rather surprising picture of prehistoric Puerto Rican protein consumption. First, the results suggest a large degree of dietary breadth for the Tibes individuals, in that many ecological niches (rivers, land, ocean, even the air, in the form of birds) were exploited as fruitful sources of dietary protein. Second, all four of the Tibes individuals exhibit an unexpectedly high reliance on dietary protein derived from freshwater fauna (river fish, shrimp, and crab) and terrestrial fauna (hutia, land crab, iguana), with smaller, but still significant, contributions coming from pinnipeds (seals), marine fish, and birds. In all four of the Tibes individuals, protein from freshwater and terrestrial sources accounts for at least 45 percent (summed means) of each individual’s total dietary protein. Third, for all four individuals, multisource mixture modeling reveals an unexpectedly low reliance on mollusks (both bivalves and gastropods) as a source of dietary protein. In all four individuals, mollusks account for, at most, 11.2 percent of dietary protein (summed means).

That the Tibes individuals exhibited a large degree of dietary breadth is perhaps the least surprising finding of this attempt at modeling. Given Tibes’s po-
Table 10.5. First percentile, mean, and 99th percentile values for multisource mixture reconstruction of Tibes's paleodiet

<table>
<thead>
<tr>
<th>Individual</th>
<th>Value</th>
<th>Freshwater Fauna</th>
<th>Terrestrial Fauna</th>
<th>Birds</th>
<th>Turtles</th>
<th>Marine Fish</th>
<th>Marine Crabs</th>
<th>Gastropods</th>
<th>Bivalves</th>
<th>Pinnipeds</th>
</tr>
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<tbody>
<tr>
<td>E-48</td>
<td>1st percentile</td>
<td>0.0</td>
<td>20.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Mean (%)</td>
<td>14.1</td>
<td>35.0</td>
<td>11.8</td>
<td>6.8</td>
<td>3.8</td>
<td>7.9</td>
<td>5.6</td>
<td>5.6</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td>99th percentile</td>
<td>50.0</td>
<td>50.0</td>
<td>30.0</td>
<td>20.0</td>
<td>10.0</td>
<td>25.0</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
</tr>
<tr>
<td>E-13</td>
<td>1st percentile</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Mean (%)</td>
<td>15.3</td>
<td>27.1</td>
<td>12.1</td>
<td>4.4</td>
<td>3.2</td>
<td>14.4</td>
<td>4.1</td>
<td>5.3</td>
<td>14.1</td>
</tr>
<tr>
<td></td>
<td>99th percentile</td>
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<td>40.0</td>
<td>55.0</td>
<td>15.0</td>
<td>10.0</td>
<td>40.0</td>
<td>15.0</td>
<td>10.0</td>
<td>20.0</td>
</tr>
<tr>
<td>ES-3</td>
<td>1st percentile</td>
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<td>0.0</td>
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<td>Mean (%)</td>
<td>24.6</td>
<td>22.3</td>
<td>14.6</td>
<td>4.6</td>
<td>3.5</td>
<td>8.5</td>
<td>3.1</td>
<td>5.8</td>
<td>13.1</td>
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<tr>
<td></td>
<td>99th percentile</td>
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<td>50.0</td>
<td>25.0</td>
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<td>35.0</td>
<td>10.0</td>
<td>25.0</td>
<td>20.0</td>
</tr>
<tr>
<td>CE-5</td>
<td>1st percentile</td>
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<td>10.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td></td>
<td>Mean (%)</td>
<td>17.1</td>
<td>35.4</td>
<td>16.8</td>
<td>5.7</td>
<td>2.5</td>
<td>8.9</td>
<td>4.6</td>
<td>4.6</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>99th percentile</td>
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<td>45.0</td>
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<td>10.0</td>
<td>25.0</td>
<td>15.0</td>
<td>15.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>
sition in an ecotone between the coastal plains and the uplifted central high-
lands, many ecological niches would have been available for exploitation within
more-or-less easy reach. Moreover, the unexpectedly high reliance on freshwater
fauna suggested by IsoSource does not seem wholly unreasonable given the prox-
imity of the site of Tibes to the Portugués River, from which such foodstuffs were
likely derived. The size and apparent prehistoric abundance of the island’s fresh-
water shrimp (of which there are three genera, the largest of which can weigh up-
wards of 0.5 kg), fish, and crabs make this possibility seem all the more likely
(DeFrance et al., this volume). On the other hand, the relative unimportance of
mollusks is still surprising, given that mollusk shells form such an enormous por-
tion of the remains typically recovered from Puerto Rican archaeological sites,
Tibes included. Such apparent ubiquity aside, the results of the present study
suggest that mollusks would seem to have contributed only a small fraction (av-
eraging less than 10 percent, reconstructed IsoSource mean values) of the protein
portion of the diet of individuals interred at Tibes. This result does, however, mesh
with both archaeological and ethnographic observations of a significant discon-
nect between the apparent observed quantity and the actual dietary contribution
of mollusks in sites dominated by shell middens (e.g., Osborn 1977).
In terms of dietary protein, then, what is presented herein represents a significant departure from traditional archaeological reconstructions of the diet of ancient Puerto Ricans (e.g., Rainey 1940). Such reconstructions depicted the Puerto Rican paleodiet as focused primarily on the exploitation of marine crabs and mollusks, neither of which factor large in our reconstruction of the protein food web of Tibes. In fact, our research suggests the inverse—that more proximate freshwater and terrestrial resources formed the major portion of the protein diet of the individuals buried at Tibes, with marine and other sources playing a more minor role. This subsistence strategy meshes well with models of locally focused resource exploitation such as that suggested in Wing (1989).

Turning to the energy portion of the human diet, the results are somewhat straightforward, if still not wholly expected. As stated above, all four of the Tibes individuals possessed notably enriched $\delta^{13}C_{ap}$ signatures. Despite their diversity, plants fall into three isotopically distinct groupings as a consequence of their distinct photosynthetic pathways. In Puerto Rico, this can even be simplified further into just two groupings, as Puerto Rican CAM plants tend to appear, isotopically, like C4 plants. Consequently, in Puerto Rico, there are two distinct groups, C3 plants with a far more negative $\delta^{13}C$ signature (averaging $-25.05\%$) and C4/CAM plants with a more enriched signature (averaging $-9.52\%$). Based on the enriched $\delta^{13}C$ values derived from the apatite of the Tibes human samples and utilizing the formula provided above for the determination of percentage C4/CAM in a given diet, the important role that C4/CAM plants must have played in the energy portion of the diet of all four Tibes individuals is evident (Table 10.3). From these calculations, C4/CAM plants were found to have accounted for an average of 44–78 percent of the dietary energy consumed by the Tibes individuals under analysis.

It must be made explicit that in suggesting that a major portion of dietary energy was C4/CAM-derived we are not therefore implying that the specific source of dietary energy was maize, the most ubiquitous C4 cultigen in the New World. Macrobotanical evidence for maize from Puerto Rico is nonexistent, with En Bas Saline in Haiti and the Tutu site on St. Thomas being the nearest sites at which such evidence has been found (Newsom 1999; Newsom and Wing 2004; Pearsall 2002), and there are numerous other tropically distributed and edible C4 plants (e.g., amarants and chenopods), the consumption of which may help to account for the calculated strong reliance on C4/CAM foodstuffs (Sage et al. 1999). That being said, in light of the recent recovery of maize starch grains from archaeological sites in Puerto Rico (Pagán Jiménez et al. 2005), the possibility that maize consumption may have played some significant role in the production of the observed enriched $\delta^{13}C_{ap}$ values of the Tibes consumers cannot be rejected out of hand.

Given the temporal (more than two centuries at the limits of the $2\sigma$ calibration) and possible sociocultural variation among the four individuals under
analysis, it is somewhat surprising that they form as tight an isotopic cluster as they apparently do. This apparent similarity does belie certain potentially significant isotopic and dietary differences among the individuals under study. While I am loathe to assign too much weight to differences observed among and between individuals in such a small sample, two aspects of these differences will nonetheless be briefly discussed here. First, the females from the Tibes sample, individuals E-48 and CE-5, differ substantially from the site’s males, E-13 and ES-3, in terms of the character of the protein portion of their diet. Both females possess markedly lower $\delta^{15}N$ values and slightly more depleted $\delta^{13}C$ collagen values than their male counterparts. These results would appear to suggest that the site’s female inhabitants consumed less seal and marine fish than their male counterparts and instead relied to a greater degree on terrestrial fauna for their dietary protein needs. While it is too early to draw any strong conclusions from these differences, they would appear to offer some suggestion of sex-based variation in diet among the members of the Tibes population.

Similarly intriguing, and equally speculative, are the aberrant isotopic results for both the dietary protein and dietary energy of individual CE-5. This individual would seem to have been consuming the least high trophic level protein (as reflected in the lowest observed $\delta^{15}N$ of the four individuals), the most terrestrial fauna and birds (as demonstrated by the most depleted $\delta^{13}C$ collagen values), and the most C4/CAM energy (reflected in elevated $\delta^{13}C_{ap}$ values and the largest $\Delta^{13}C_{ap-co}$ spacing). While this apparent variation may be the result of the small sample under study or due to temporal differences (individual CE-5 is the earliest of the four individuals), it, as well as the differences in diet based on sex, may also be the result of exactly the sort of socially driven dietary inequality that the present study was conceived to examine. In fact, the exact type of dietary difference observed in individual CE-5, less protein and more C4/CAM energy, has been associated with low social status burials in other, admittedly geographically removed, complex societies (Ambrose et al. 2003).

Taken as a whole, these preliminary results can perhaps also be used to provide some sort of insight into the broader nature of human activity at Tibes between A.D. 400 and A.D. 1200. Given that both the protein and energy portions of the diet, as we have reconstructed them here, deviate so notably from the faunal and botanical assemblages found at the site (as discussed by Newsom and DeFrance et al., this volume), the possibility must be raised that the individuals buried at Tibes may not have been consuming all of their meals at the site. In fact, based on our results, it seems likely that only a small portion of the individuals’ meals (those focused on mollusks) were consumed and evidenced within Tibes and that a much larger portion (the freshwater fauna, terrestrial fauna, pinniped, and bird meals) were consumed elsewhere, at some removed domestic setting. This raises the pos-
Bone Chemistry and Paleodiet at the Ceremonial Center of Tibes / 229

sibility that the site was, in fact, a vacant ceremonial center and that the archaeological remains recovered there only reflect some small subset of the cultural practices of the people eventually buried therein.

Conclusions

In sum, the present study both shows the promise of the application of stable isotope analysis to the resolution of paleodiet in Puerto Rico and provides some surprising insights into the diet of individuals interred at the Ceremonial Center of Tibes. The individuals buried at Tibes were consuming a substantial amount of high trophic level meat, as much as half of which was coming from traditionally underappreciated sources such as the nearby river and terrestrial ecosystems. In addition, they were supplementing their diets with marine elements (both fish and mammal) and birds, and they were only minimally reliant on more commonly suggested sources of food (crab and mollusks). The results of the study of the energy portion of their diets were similarly intriguing, as they indicated a large reliance on C4 or CAM plant materials, neither of which factor prominently in traditional reconstructions of the diet of the island’s prehistoric inhabitants.

With these results in hand, I am extremely positive about the upcoming analysis of much larger samples from Tibes and three other precontact sites. Using stable isotope analysis of paleodiet, a high resolution and higher-fidelity technique than those traditionally employed in the reconstruction of paleodiet, I am extremely hopeful that greater insights into the nature of the site of Tibes and the development of in situ social complexity at that site can be better understood.

Acknowledgments

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Note

1. While the present chapter focuses on the largely encouraging results of the stable isotope analysis, it deserves to be noted that the other types of analysis employed in the pilot study, namely trace element and mtDNA, yielded what are, at best, mixed results. Specifically, trace element analysis revealed higher than acceptable
levels of diagenetic alteration of the mineral phase of the bone samples, although the tooth samples were found to be sufficiently unaltered by such processes. The mtDNA analytical methods employed were also found to be of limited efficacy, as they encountered numerous difficulties in the amplification of bona fide ancient DNA, with success in both amplification and sequencing achieved in only 1 of the 16 samples (Laffoon et al. 2006).