AN ABSTRACT OF THE THESIS OF

Diana Milantia Roy for the degree of Master of Arts in Applied Anthropology presented on 23 September 2002.

Title: Using Stable-Isotope Analysis to Obtain Dietary Profiles from Hair

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Abstract approved _____

Robson Bonnichsen

Stable isotope analysis of human tissue can provide information about diet independent of artifactual remains. Food is broken down and used in the synthesis of body tissue, so the isotopic composition of hair keratin reflects the isotopic composition of foods consumed. Therefore, the analysis of hair can provide a window into broad dietary practices, and this view can supplement the information that is inferred from artifacts such as hunting tools and hearths.

This project details the use of historic Plains Indians hair as a sample material for carbon and nitrogen isotope analysis. A minimum specimen size of a 2-cm (100-150 μg) segment of a strand was established. This indicates that small hair fragments found in archeological excavations can be informative. It also allowed the testing of up to 12 sequential segments from strands up to 24 cm long. Since hair grows about 1 cm per month, a 24-cm strand provided about a 2-yr record of isotopes and diet. The isotopic variations along some strands were as high as 0.49‰ for $\delta^{15}N$ and 1.05‰ for $\delta^{13}C$, exceeding the background analytical uncertainty of 0.22‰ for $\delta^{15}N$ and 0.21‰ for $\delta^{13}C$. Differences between individuals and between population groups also exceeded this background level, validating the use of this isotope technique in discriminating isotopic differences between hairs and between people.

No isotopic differences were found between males and females, and no isotopic differences were found based on the age of the individual. This suggests that there are no physiological differences by gender or age affecting isotope metabolism, which means that should a study find an isotopic difference between men and women, it would reflect dietary differences, not physiological ones.

Isotope testing produced distinct isotope profiles ($\delta^{15}N$ vs. $\delta^{13}C$) for two cultural groups, the Lower Brule reservation Sioux of 1892 and the reservation Blackfoot of 1892 and 1935. The resultant dietary profiles indicate a higher consumption of meat by the Blackfoot and a higher consumption of corn by the Lower Brule. The two groups of Blackfoot fit into the same profile despite the passage of several decades. This raises the possibility that stable isotope analysis can also be used to identify members of the same cultural population.

Using Stable-Isotope Analysis to Obtain Dietary Profiles from Old Hair

by
Diana Milantia Roy

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Arts

Presented 23 September 2002
Commencement June 2003

Master of Arts thesis of Diana Milantia Roy presented on 23 September 2002.

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I understand that my thesis will become part of the permanent collection of the Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Diana Milantia Roy, Author

ACKNOWLEDGMENTS

I wish to acknowledge the members of my committee for their guidance in my epic thesis journey: to Rob Bonnichsen for the original spark and impetus; to Roberta Hall for statistics tutorials, insight into the Boas data collection, and all-around advice; and to Alan Mix for his geochemical expertise and bold, unfaltering leap from oceanography to anthropology. A special nod of appreciation goes to Bill Rugh for the hours he spent helping me with the arcana of mass spectoscopy and in overseeing the testing of my samples.

I most sincerely thank the following museums and their staff members who helped me locate, identify, and acquire hairs from their collections for my study: Ken Mowbray at the National Museum of Natural History in New York City (and additional help from Richard Jantz, University of Tennessee); and Douglas Owsley, David Hunt, and Kari Bruwelheide at the Smithsonian Institute in Washington, D.C.

I am indebted to those individuals from the Lower Brule and Blackfoot reservations a century ago who provided samples of their hair to anthropologists collecting anthropometric data. I am also indebted to the anthropologists who, working under the guidance of Franz Boas, collected these hair and data samples and documented them for posterity.

The laboratory analyses were supported by a grant for ancient molecular archeological research from Jean and Ray Auel to Robson Bonnichsen.

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CHAPTER 1
INTRODUCTION

As eating is a fundamental human need, so is diet a fundamental trait of culture. In fact, in a subsistence, pre-industrial society, the quest for food and its preparation are central to the structure of life and lifestyle. Diet strongly influences both cultural evolution and biological evolution, which together are the essence of anthropology. While biological evolution takes place over the very long term (such as the evolution of teeth for meat-eating or the evolution of enzymes for digesting milk after infancy), cultural evolution is more rapid. Cultural changes range from the technological (such as the development of tools for food preparation and storage, hunting, and fishing) to the social (such as the development of networks to trade food and goods). Naturally, technical and social achievement have applications beyond the acquisition of food. Therefore, information about diet can provide anthropologists and archeologists a key to the understanding of a people.

When archeologists study past populations, most of their methods for assessing diet use *indirect* evidence: tools, seeds and pollen, fossil remains of animals, and evidence of cooking. *Direct* methods of investigation, on the other hand, analyze the remains of the humans themselves, such as their bone, hair, and feces. Of these three, skeletal remains have been the most studied, but hair provides great unexploited potential. The average person sheds 50-100 hairs a day, and human hair can remain intact for thousands (at least 5200) of years (Macko et al. 1999a).

In many ways, we—including our hair—are what we eat. Research since the 1970s has shown that the protein in body tissues, including hair, maintains the carbon and nitrogen stable-isotope ratios present in the foods consumed. Certain broad classes

of food can be distinguished by their carbon isotope ratios (C3 vs. C4 photosynthesis in plants), nitrogen isotope ratios (animal vs. plant protein), and sulfur isotope ratios (marine vs. terrestrial foods). From these nitrogen and carbon isotope ratios in bone or hair, one can draw corresponding conclusions about the protein sources in a person's diet. Although these conclusions are necessarily broad, they can provide an important supplement or confirmation of other, more indirectly obtained evidence.

While the use of isotope chemistry to infer diet is well-established, there are important aspects of my research that are not. Published work on hair to date has used either modern hair or mummy hair, yet hair from most archeological contexts would be gleaned as single hairs only. What is the minimum quantity of hair that can yield meaningful results? By testing segments of different weights from the same person, I can find a practical lower limit.

Individual strands of hair can be cut into short segments, which are kept in order from the newest (most proximal) to the oldest (most distal). Since human hair grows at the approximate rate of 1 cm/month, it is possible to track variations in diet by testing segments along a strand of hair. If the segments can be short enough, this has the potential of finding short-term changes in diet, such as those due to seasonal subsistence change.

This project also studies whether sex and age affect the isotopic composition of hair. Sex and age could affect isotope composition in two ways: 1) via metabolic differences between the sexes and at different ages, which affect how the carbon and nitrogen in food are processed in the body; and 2) via actual differences in dietary intake. If no differences are found, then all members of a population can be considered together in developing a population profile based on nitrogen and carbon isotope compositions.

On the theory that members of the same cultural group have similar diets, a cultural group should produce a characteristic population isotope profile. While there will

be differences in isotopic composition from individual to individual, even within the same population, there should be differences *between* populations that exceed the variation *within* populations. In addition, significant cultural change, such as that occurring between pre-reservation and reservation Indians, should also change diet and, hence, isotope profiles. This can be investigated by testing hair from these two different time periods.

I obtained three sets of hair from museum collections. The sets represent three North American Great Plains populations (cultures) from the late pre-reservation and early reservation time periods (roughly 19^{th} to early 20^{th} centuries). I analyzed and compared these hairs to test the above propositions, and to test the validity of stable-isotope chemistry on small specimens of hair. Other studies have analyzed modern hairs from living people to test this methodology, which has the advantage of being able to directly (through interviewing) ascertain the individuals' diets. However, the use of older, historic hair has the advantage of exploring more traditional and subsistence-based diets. At the same time, however, the cultural origins of these hairs are known, providing a context for the experimental results. For the purposes of establishing this methodology, the museum hairs serve as historic analogs for the eventual testing of prehistoric hairs that could be found at archeological sites.

Finally, a major objective of this project is to use an interdisciplinary approach to solve problems in physical anthropology. Much has been learned in the past from morphological and descriptive analyses in physical anthropology, but much more can be learned in the future by adopting techniques from chemistry and biology to expand anthropological perspectives to include insights from disciplines such as physiology and genetics.

CHAPTER 2
BACKGROUND AND THEORY

The use of stable isotope analysis to study the diets of past populations dates back to the 1970s, when Vogel and van der Merwe (1977) showed that carbon isotope ratios in prehistoric human bone could confirm the onset of significant maize consumption (and, therefore, farming) in New York in the 11th century. Since then, the selection of isotopes yielding dietary information has expanded to include nitrogen and sulfur. In addition, physical anthropologists and archeologists have begun to explore the use of hair for these studies instead of bone, since hair remains are potentially more numerous and easier to test.

STABLE ISOTOPE RATIOS

Many of the most common elements in the body, including hydrogen, carbon, nitrogen, and oxygen, occur in more than one form, called isotopes. Isotopes are versions of the same element that differ only in the number of neutrons. This does not affect chemical properties (which are determined primarily by their electron configurations), but it does affect mass. An isotope with more neutrons, like ^{13}C, is slightly heavier than the more common isotope, ^{12}C, and tends to react with other compounds more slowly. Unlike radioactive isotopes (e.g. ^{14}C), stable isotopes do not decay over time, and so the ratio of one stable isotope to another (such as ^{13}C:^{12}C) in a given compound remains constant over time. It is this constancy that allows stable isotope ratios to serve as signatures of the origins of a substance. Carbon and oxygen stable-isotope ratios have been used, for example, to source ancient Mediterranean marble (Herz 1990).

In a similar way, the tissues in our bodies are composed of isotopes of carbon and nitrogen (among other elements) in specific ratios that reflect our raw materials: what we have eaten. The isotope values of human tissues directly relate to the

isotope values of food in the diet (Katzenberg and Krouse 1989). However, because living systems carry out chemical reactions (metabolism), isotope *fractionation* occurs in them. Fractionation is the result of the lighter isotope and the heavier isotope undergoing a reaction at different rates, so that a product has a different isotope ratio than its reactants. Fractionation is due to a kinetic effect, because the heavier isotope diffuses more slowly (Klepinger and Mintel 1986).

When a plant converts carbon dioxide to glucose, for example, the relative amounts of ^{13}C and ^{12}C are not the same in the CO_2 and the glucose. However, the fractionation of the isotopes is constant for a given chemical reaction. Fractionation occurs in both plants and animals, and varies with the specific metabolic pathway. Consequently, stable-isotope analysis traces not just the source of foods, but also the metabolic processes within the organism (Koch et al. 1994). The two most commonly investigated elements in stable-isotope analysis for dietary information are carbon and nitrogen.

Standard Notation and Universal Standards

The magnitude of the fractionations of carbon and nitrogen in biologically significant contexts is very small, and so the values of carbon and nitrogen ratios (^{13}C:^{12}C and ^{15}N:^{14}N) are expressed not as absolute values, but as values relative to (or deviations from) a universal standard that is arbitrarily assigned a zero value. The difference between the sample and the standard is designated δ (*delta*) relative to the heavier isotope, and is expressed in parts per thousand, written as ‰ (*per mil*). In my project, the measurements of interest are $\delta^{13}C$ and $\delta^{15}N$.

If a substance has a lower isotope ratio than the standard, the δ value will be negative. This is the case with most $\delta^{13}C$ values, because the universal standard for carbon is a marine carbonate from the PeeDee Belemnite (PDB) formation in South Carolina, and marine carbonates have higher ^{13}C:^{12}C ratios than almost any other

natural material (van der Merwe 1982). Atmospheric CO_2 in rural areas has a $\delta^{13}C$ of about –7‰. It is said to be *depleted* in ^{13}C relative to the standard.

The universal standard for nitrogen is an atmospheric nitrogen (N_2) sample referred to as AIR (ambient inhalable reservoir) (Richards et al. 1998). Most natural substances have positive $\delta^{15}N$ values, so they are said to be *enriched* in ^{15}N relative to the standard.

Mass Spectrometry

The stable-isotope ratio of a substance is measured in a mass spectrometer. A combusted gas of the specimen is introduced into the source of a mass spectrometer, which first ionizes the gas, then focuses it into a beam and accelerates it into a mass analyzer. The mass analyzer magnetically separates the one ion beam into several beams based on mass. Finally, the ion collector measures the relative intensities of the arriving ion beams, and reports these values as isotope ratios (Katzenberg 2000).

It is important to note that this is a destructive test, so the specimen used is gone forever. For archeology and physical anthropology, the use of very small specimen sizes is critical. Also, a specimen cannot be tested twice for a true test of reproducibility.

Fractionation of Carbon

Of primary dietary interest is the fractionation that carbon isotopes undergo in terrestrial plants as the result of photosynthesis, the fixation and metabolism of atmospheric carbon dioxide by the plant. There are two main photosynthetic pathways, called C3 and C4, and one minor one, called CAM (Crassulacean acid metabolism) (DeNiro 1987). C3 and C4 photosyntheses fractionate carbon differently, providing an isotopic distinction. The resulting products of C3 and C4

plants have non-overlapping $\delta^{13}C$ values. (CAM plants, which include cacti and bromeliads, have $\delta^{13}C$ values overlapping C3 and C4 foods.)

C3 (Calvin) photosynthesis is so called because the initial product of CO_2 fixation (using the enzyme ribulose bisphosphate carboxylase) is a three-carbon intermediate. C3 plants are more common than other types, as they comprise most temperate and tropical grasses, most shrubs, and all trees. C3 plants have $\delta^{13}C$ values ranging from –33 to –22‰, averaging –27‰ (DeNiro 1987). Most food plants (and almost all in North America) are C3 plants, including wheat, rice, beans, tubers, vegetables, most fruits, honey, nuts, and most grasses.

C4 (Hatch-Slack) photosynthesis creates a four-carbon intermediate via the enzyme phosphoenol pyruvate carboxylase. This form of photosynthesis predominates in hot, arid, sunny climates (including salt marshes), and produces the food plants maize, amaranth, sugar cane, sorghum, chenopods, some millets, and tropical grasses (Ambrose 1993). C4 plants have $\delta^{13}C$ values ranging from –16 to –9‰, averaging –12.5‰ (DeNiro 1987). C4 plants are enriched in ^{13}C relative to C3 plants because C4 photosynthesis is a more efficient process, using less time and experiencing less fractionation and less water loss.

In marine environments, carbon fractionation has an entirely different pattern than on land, though the food web is also based mainly on C3 plants. Marine carbon is ultimately derived from dissolved carbonates ($\delta^{13}C$~0‰), as opposed to atmospheric CO_2 ($\delta^{13}C$~–7‰). Though the average marine $\delta^{13}C$ value of organic matter is –19‰, the range of $\delta^{13}C$ values is very wide, and depends strongly on the specific environment (water depth, water temperature) (Ambrose 1993).

Because marine $\delta^{13}C$ values overlap those $\delta^{13}C$ values produced by both C3 and C4 photosynthesis, carbon isotope ratios cannot be used to discriminate marine foods unless 1) C4 foods are absent; and 2) one can test local food specimens for their isotope values.

There is a slight increase in $\delta^{13}C$ values with trophic level (moving up the food chain). Consequently, mammals (marine and terrestrial) have more enriched (less negative) $\delta^{13}C$ values than do plants. The enrichment of ^{13}C in human tissue protein relative to dietary intake is slight, and it may depend on the tissue type (DeNiro and Epstein 1978; Wada et al. 1991). However, the fractionation values reported in the literature vary enough that this is not certain. The enrichment of hair keratin's $\delta^{13}C$ compared to diet is about 1-2‰, similar to some reports for bone collagen (Wada et al. 1991; DeNiro 1987).

Table 2.1 shows some typical carbon isotopic compositions of foodstuffs from Nakamura et al. (1982). The $\delta^{13}C$ values represent the protein fractions only. The main difference between American and German meat and dairy products is the livestock feed: in the U.S., corn is a primary feedstock, while in Germany, grass is more important. The differences between American and German plant foods illustrate the variability of isotope compositions of similar plants from different environments.

Table 2.1. Examples of $\delta^{13}C$ Values of Foods, Protein Fraction

Foodstuff	$\delta^{13}C$	Foodstuff	$\delta^{13}C$
Beef (USA)	-13.9‰	Beef (Germany)	-23.2‰
Pork (USA)	-13.6‰	Pork (Germany)	-24.8‰
milk (USA)	-18.5‰	milk (Germany)	-24.8‰
eggs (USA)	-14.7‰	eggs (Germany)	-16.6‰
beans, peas (USA)	-23.9‰	beans, peas (Germany)	-25.1‰
corn (USA)	-12.0‰	corn (Germany)	-13.4‰
wheat (USA)	-24.1‰	wheat (Germany)	-26.6‰
carrots (USA)	-26.2‰	carrots (Germany)	-28.6‰
apples (USA)	-26.8‰	apples (Germany)	-26.8‰
tuna (USA)	-16.8‰		

Fractionation of Nitrogen

Nitrogen isotope compositions clearly track the trophic level of the consumer. Various researchers have found that $\delta^{15}N$ values increase 3-4‰ for each step up the food chain, from plants to herbivores to carnivores to nursing babies (Ambrose 1993; DeNiro and Epstein 1981; Katzenberg and Pfeiffer 1995). The fractionation of nitrogen isotopes in human bone collagen and hair are quite similar, enriching the $\delta^{15}N$ from the diet about 3-4‰ (Wada et al. 1991; Minagawa 1992).

Nitrogen isotope ratios can distinguish two types of plants: legumes and non-legumes. Legumes fix atmospheric nitrogen with almost no fractionation, so they have $\delta^{15}N$ values close to zero. Non-legumes use soil nitrogen that has been fractionated (and enriched in ^{15}N) by bacteria. In the past, non-legumes had $\delta^{15}N$ values averaging about 9‰, but today these values are lower due to the use of chemical fertilizers with $\delta^{15}N$ near zero (DeNiro 1987).

Marine organisms generally have higher (more enriched) $\delta^{15}N$ values than terrestrial organisms, since marine sources of nitrogen are derived from bacterial activity, which fractionates the atmospheric nitrogen isotopes. Marine vertebrates have $\delta^{15}N$ values 6 to 8‰ more positive than terrestrial animals at the same trophic level. Marine plants also have more positive $\delta^{15}N$ values than terrestrial ones do, except for blue-green algae, which are nitrogen fixers like the terrestrial legumes.

Table 2.2 shows some typical nitrogen isotopic compositions of foodstuffs from Minagawa (1992). The $\delta^{15}N$ values are from Japanese foods, but serve to illustrate the trophic effect of $\delta^{15}N$.

Table 2.2. Examples of $\delta^{15}N$ Values of Foods

Foodstuff	$\delta^{15}N$	Foodstuff	$\delta^{15}N$
Beef	7.7‰	wheat	4.1‰
Pork	6.6‰	potato	0.2‰
milk	6.6‰	apples	0.7‰
eggs	4.9‰	tuna	19.0‰
soybeans	1.5‰	salmon	11.4‰
cowpeas	2.4‰	carp	9.4‰
corn	-1.1‰	clam	7.2‰

Environmental Variability

In order to quantify the contribution of specific foodstuffs in the diet based on stable isotope ratios, one would need to test the candidate foodstuffs from the same time and place as the consumer. This is because isotope values can vary with climate and ecosystem (Ambrose 1993; Sealy et al. 1987). For instance, atmospheric CO_2 today is about 1‰ more negative than it was before the Industrial Revolution, due to the combustion of fossil fuels, which are strongly depleted in ^{13}C (DeNiro 1987). The air under forest canopies is depleted in ^{13}C due to the recycling of ^{13}C-depleted CO_2 respired from the plants (Tieszen 1991).

North America has both C3 and C4 grasses, and the proportion changes with latitude. At higher latitudes, C3 grasses predominate (Katzenberg and Krouse 1989). This will affect the interpretation of diet based on $\delta^{13}C$, so it is important to know the provenance of the material to be tested. For instance, Spielmann et al. (1990) found that old bison bone from New Mexico yielded $\delta^{13}C$ values as high as maize (a C4 plant), though bison graze on grasses and sedges, not maize. These grasses must have been C4 grasses. In the northern Great Plains, on the other hand, C3 grasses predominate, although C4 grasses increase in the summer (Tieszen 1991).

Under hot and arid conditions, especially drought, water-stressed animals undergo a change in physiology that affects their fractionation of nitrogen isotopes. By conserving water but excreting ^{15}N-depleted urea, such animals increase the $\delta^{15}N$ values of their tissues. Therefore, it is difficult to interpret diet from the $\delta^{15}N$ values of animals from water-stressed environments (Ambrose 1993).

Protein stress has a similar effect, in that inadequate protein intake leads to the breakdown and recycling of existing tissue proteins, further enriching ^{15}N levels by preferentially excreting ^{14}N (Katzenberg 2000). So, ironically, a grossly inadequate intake of protein could lead to elevated $\delta^{15}N$ values.

Dietary Significance

In terms of human diet, carbon isotope ratios in human tissue tell us something primarily about plant food sources: C3 vs. C4 foods. Maize is one of the few important C4 plant foods in North America, so this isotope signal has been used to discern if a prehistoric group has adopted (maize) farming (Vogel and van der Merwe 1977; van der Merwe et al. 1981). Because the $\delta^{13}C$ value is carried up the food chain with little further fractionation, the $\delta^{13}C$ value of a human tissue also reflects the plants eaten by the animal foods consumed.

While carbon isotope ratios in human tissue have been used to discern marine contributions to the diet, this can only be done where there are no C4 plants in the diet (Tauber 1981), due to overlapping $\delta^{13}C$ values.

Nitrogen isotope ratios in human tissue are primarily indicative of the individual's trophic level: in other words, how much animal protein was consumed. Herbivores have lower $\delta^{15}N$ values than do carnivores. Among humans, vegans, who consume no animal protein, have lower $\delta^{15}N$ values than all other human omnivores. Ovo-lacto vegetarians, who consume animal protein other than meat, are not isotopically distinguishable from omnivores (Macko et al. 1999a; O'Connell and Hedges 1999).

Although high $\delta^{15}N$ values can discern marine contributions to the diet, this is complicated by the existence of nitrogen-fixing marine organisms in certain ecosystems (such as reefs and salt marshes), which have low $\delta^{15}N$ values. Better, sulfur isotopes can clearly discriminate marine and terrestrial components (Macko et al. 1999a; Macavoy et al. 1998).

USING HAIR AS THE TEST MATERIAL

In the search for information about the diets of past populations using stable-isotope analysis, human bone has been the material most often tested. Less frequently, hair has come under scrutiny, first from living people and animals (Webb et al. 1980; Jones et al. 1981; Nakamura et al. 1982), and more recently from mummies (White 1992; Macko et al. 1999a; Macko et al. 1999b). Any tissue protein could be used, but only bone collagen and hair keratin are durable enough to survive hundreds and even thousands of years without degradation and diagenesis, so that ancient materials can be tested. While bone has been the more common human remain to test, hair has great potential for the following reasons:

- It is ubiquitous. Human hair grows roughly 1 cm/month, and the scalp sheds about 100 hairs/day (Valkovic 1977). If archeologists were to look for hair (by using finer sieves and protection against contamination with modern hair), they could potentially find more hair than bone in some contexts.
- Because stable-isotope mass spectrometry is destructive to the sample, there is a reluctance to test human bone. Also, it is not at all practical to test living humans by bone sampling!
- With current stable-isotope mass spectrometry, even short fragments (2-4 cm) of hair can provide enough material to test. DNA testing and ^{14}C-dating can also be carried out on such small fragments, although each one of these tests is destructive (Bonnichsen et al. 2001).

- Hair is about 95% keratin (its protein fraction), while bone is only about 20% collagen (its protein fraction) (Taylor et al. 1995). Since the protein fraction provides the preferred material to test, hair requires no chemical purification, whereas collagen must be chemically extracted and purified (Ambrose 1993).
- In the case of sulfur testing, keratin has a much higher concentration of sulfur than does collagen (~5% vs. ~0.2%) (Robbins 1994).
- Hair is extremely durable. The hair shaft is encased in a cuticle, a hard protective covering. It is chemically resistant and biologically stable, being resistant to microbial attack (Lubec et al. 1987). Lubec et al. found no physical, chemical, or conformational changes in 3000-year-old mummy hair, just dehydration. Macko et al. (1999b) tested 5200-year-old mummy hair from the Ice Man and found it had an amino acid profile like modern hair. The quantities of amino acid had declined slightly in the old hair, but the relative proportions of the different amino acids had not changed. Buried hair appears not to undergo the diagenesis (including hydrolysis, decarboxylation, and deamination) that bone does (Macko et al. 1999a), at least in certain contexts.
- Because hair is a finished product when it emerges from the scalp, a segment of hair provides a "snapshot" view of diet at a particular point in time. In addition, because it is synthesized almost continuously (with static periods 11% of the time [O'Connell and Hedges 1999]), a hair strand also provides a chronological record of diet for the length of the hair. Since each centimeter of hair represents approximately a month of growth, it could be possible to resolve even seasonal changes in diet (such as practiced by hunter-gatherers). Bone, in contrast, is a structure undergoing continuous but slow turnover. It is estimated that the carbon in bone collagen represents an integration of the last 10-30 years of an adult's diet, and so provides a picture of long-term average consumption (van der Merwe 1982; Ambrose 1993).

In a study of modern Japanese diet that tested human hair as well as a variety of food products, Minagawa (1992) showed that the carbon and nitrogen isotope values of the hair agreed well with the food consumption pattern of the Japanese National Statistics Report. Numerous other studies have also found agreement between stable isotope values of modern hair and known dietary intake (Nakamura et al. 1982; Webb et al. 1980; Tokui et al. 2000).

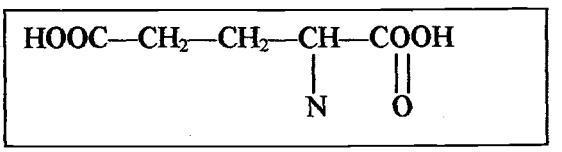
In reconstructing human diet using isotope information, it is important to know not only what isotopic compositions the potential food sources have, but what the fractionation factors are for the particular human tissue in question. Proteins, carbohydrates, and lipids have different fractionation factors. Even within proteins, different amino acids can have different fractionation factors. Hair keratin and bone collagen undergo similar fractionation of nitrogen and carbon isotopes, though some researchers report a difference for carbon fractionation.

$\delta^{15}N$ values in hair and bone are enriched (increased) 3 to 4‰ compared to dietary protein (Katzenberg 2000; Minagawa 1992). Wada et al. (1992) found the $\delta^{13}C$ of hair keratin to be more enriched than bone collagen by 3.5‰, but others report that bone collagen is more enriched than hair, with the fractionation for keratin being about +1‰ and the fractionation for collagen being about +5‰ (Katzenberg 2000). These differences probably reflect variables in environment, interpretation of diet, and testing. It is not important to pin these differences down unless one wants to directly compare hair and bone values, which is not advisable. In fact, any comparison between studies must focus on the relative results, and not the absolute numbers. What is important is that both bone and hair consistently track dietary intake through $\delta^{13}C$ and $\delta^{15}N$ values, as, in fact, does the whole organism (DeNiro and Epstein 1978, 1981). However, collagen may be more variable due to problems of diagenesis during burial and differences in methods of isolation of collagen from bone.

When considering the results of stable isotope studies of bone and hair, the most important difference to keep in mind is the time period represented by each material. Bone represents the long-term, cumulative diet, while hair represents a snapshot at the particular moment when the hair is grown.

DIETARY PROTEIN CONTRIBUTES TO TISSUE PROTEIN

The three major macronutrients are protein, carbohydrate, and lipid. Hair is 95% keratin, a protein. Proteins are composed of amino acids, which are made up of carbon, nitrogen, oxygen, and hydrogen (and, occasionally, sulfur). A typical amino acid in humans, and one of the most common ones in hair, is glutamate:



There is a question about the extent to which a specific dietary fraction (that is, protein, carbohydrate, or lipid) contributes to the same fraction in the tissue of the consumer. Researchers have noted that different tissues of the body fractionate the dietary carbon input differently, creating different $\delta^{13}C$ values in the different tissues (muscle, bone, hair, plasma) (DeNiro and Epstein 1978). Even the different fractions of a plant (protein, carbohydrate, lipid) have different $\delta^{13}C$ values (Tieszen 1991). Contrary to the idea that nutrients are broken down to small molecules and then reassembled, it appears that like dietary fractions—rather than "bulk" diet—contribute more to like tissue fractions in the animal (Klepinger and Mintel 1986; Lajtha and Michener 1994). Hair keratin is a protein (as is bone collagen), so one would expect most of its carbon to come from dietary protein.

Because keratin is a protein, it is made of a mix of essential and nonessential amino acids. The essential amino acids must come directly from ingested protein. The nonessential amino acids may be formed in part from other dietary fractions,

although their similarity to other ingested amino acids makes it likely that they are also formed in large part from other amino acids.

Schoeller et al. (1986) compared the carbon isotopic values of subjects' hair and C3and C4 plant tissue. They calculated the contribution of C3 and C4 foods to the diet of their subjects, and found the better match when using the $\delta^{13}C$ values from the plant protein fractions, rather than from the whole plant. "This comparison with dietary protein rather than total diet is more appropriate because hair is protein..."

This point is of relevance in my study, in which I cannot fully document the dietary components of the subjects. Since there are few sources of C4 foods in North America, it is significant to know, for instance, whether cane sugar and corn syrup would influence (that is, enrich) the $\delta^{13}C$ values of the individuals' hair. The answer is apparently not much, with one caveat.

The quantity and quality of dietary protein could affect the $\delta^{13}C$ value of tissue protein in the following ways. A low or low-quality protein diet could force the body to use more carbohydrate carbon input in the synthesis of nonessential amino acids, whereas a high-quality protein diet would supply more carbon from protein sources in the synthesis of amino acids (Klepinger and Mintel 1986; Tieszen 1991; Ambrose 1993).

On the other hand, the fractionation of nitrogen isotopes is similar throughout the animal body, because nitrogen occurs only in proteins, and not in carbohydrates or lipids (DeNiro and Epstein 1981). Therefore, the only metabolic processes in question are those for synthesizing (and breaking down) protein.

THE FINDINGS OF OTHER HAIR STUDIES

Hair isotope studies in different modern contexts have established the correspondence between dietary intake and hair isotope composition. Jones et al. (1981) showed that the $\delta^{13}C$ values of steer hair correspond to the $\delta^{13}C$ values of

their feed with a small enrichment (positive fractionation) factor, and that isotope values of the hair change when the diet is changed.

Studying living humans in Australia and New Zealand, Webb et al. (1980) compared two different populations and found their hair $\delta^{13}C$ values correlated with the C3:C4 levels of their livestock feed. Nakamura et al. (1982) analyzed the $\delta^{13}C$ values of foodstuffs and hair from Japan, the United States, and Germany, and found the hair and foodstuffs from each population to correspond, given a 1-2‰ enrichment factor. Nakamura et al. found that the isotope composition of hair begins to change within days of changing diets, but O'Connell and Hedges (1999) found that it can still take months for those values to equilibrate, due to the contributions of protein reservoirs in the body to new tissue synthesis.

In the 1990s, the studies of human hair began to include $\delta^{15}N$. Minagawa (1992) used a statistical method to evaluate the $\delta^{15}N$ and $\delta^{13}C$ values of Japanese human hair compared to the isotope values of an "average dietary pattern" based on food consumption statistics from the Ministry of Health and Welfare. Minagawa determined enrichment values from foodstuff to human hair for both $\delta^{15}N$ (4.3‰) and $\delta^{13}C$ (1.4-2.0‰). He also found no difference by age or sex of the person. This should preclude age and sex as variables that would intrinsically (that is, physiologically) create a difference in isotope fractionation.

Recent studies (O'Connell and Hedges 1999; Pflieger et al. 2000; Tokui et al. 2000) have confirmed the correlations between the $\delta^{15}N$ or $\delta^{13}C$ values of the diets and of the hair in populations around the world (China, England, U.S.). Not all studies show consistent correlations: Yoshinaga et al. in Papua New Guinea (1996) compared hair with dietary isotopic values, and found higher than expected isotope ratios three out of eight times. There are several variables other than diet known to affect isotope ratios, such as which part of the foodstuff (protein only or whole) was tested, and whether there was climate or dietary stress (as discussed earlier in this chapter under "Environmental Variability").

Several archeological studies have explored the diet of past people by testing the isotope composition of mummy hair. White (1992) analyzed two to four 2-cm segments of hair from each of 14 Sudanese Nubian mummies (dating to AD 350-1330) in order to look for seasonal patterns in $\delta^{13}C$ values over a 2- to 8-month period. (The Nubians farmed C3 plants in the winter and hardier C4 plants in the summer.) Although White found differences, they are not large, and the strands are too short to truly display seasonality.

Macko et al. (1999b) analyzed hair from the 5200-year-old Ice Man mummy that had been preserved in an Alpine glacier. Macko compared $\delta^{15}N$ and $\delta^{13}C$ values of the mummy hair to goat hair and a grass-like seed found with the Ice Man. Macko et al. concluded that the Ice Man was primarily vegetarian since his $\delta^{15}N$ value was enriched only 1.1‰ compared to the goat hair instead of the 3+‰ typical of a carnivore. However, there is clearly inadequate data to so limit the Ice Man's diet, given that only two samples of foodstuff were found with him. The Ice Man's $\delta^{13}C$ value was also too enriched compared to the grass seed (2.7‰ instead of ~1‰) to say that this grass seed (cereal grain?) was a major dietary source. Tests of other contemporaneous animals and plants would allow more solid conclusions.

Isotopic studies of mummy hair have also been used to show cultural adaptations via diet. Aufderheide et al. (1994) determined that a coastal population of northern Chilean mummies from around 1000 BCE was marine-adapted, based on the high $\delta^{34}S$ values of their hair.

LIFESTYLE AND DIET OF SIOUX AND BLACKFOOT PEOPLE IN THE NINETEENTH CENTURY

One would like to be able to extend the study of hair isotopic composition to hair fragments found in archeological contexts. Such hair would not be nearly as convenient as mummy hair in quantity and in characterization, but has the potential of being more common and, therefore, easier to acquire. As an intermediate step, I

am testing historic Native American hair from museum collections. The museum hairs test the ability of mass spectrometry to characterize the diet or the culture of unknown individuals through the isotopic analysis of their hair.

Most of the hair specimens are from the Lower Brule tribe of the Teton Sioux and from Blackfoot Indians of the Great Plains of the United States. The time period roughly spans the 19th and early 20th centuries, a period of great change and transition for these cultures. It was a period of increasing European-American influence and declining traditional subsistence, resulting in the Indians' limitation to reservation lands—in 1855 for the Blackfoot, and in 1876 for the Teton Sioux.

Traditional Diets

The western or Teton Sioux populations (known also as Lakota), located in and around South Dakota, and the Blackfoot populations, located in Montana and Alberta, were both Great Plains populations, though living at opposite sides of that landscape. Refer to Figure 2.1 (from DeMallie 2001a).

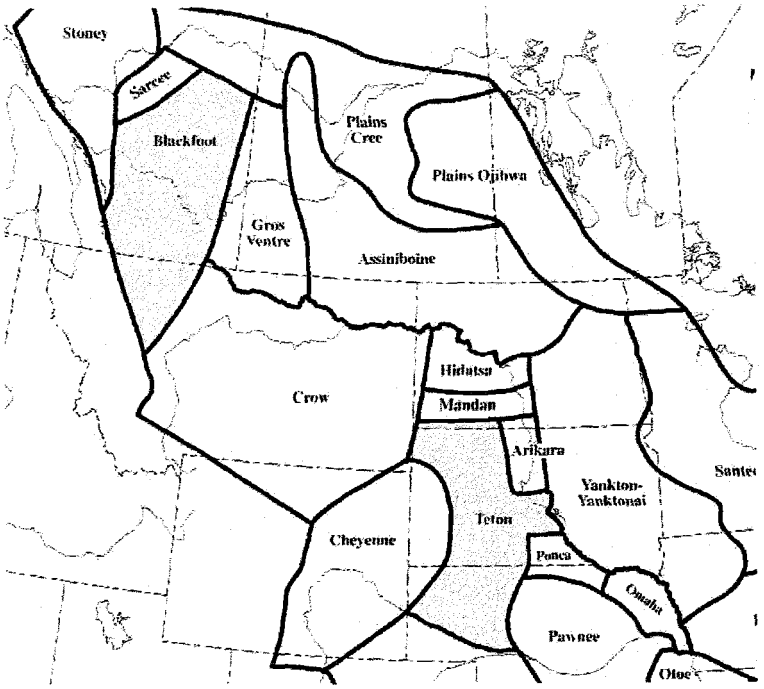


Figure 2.1. Blackfoot and Teton Territories, Great Plains, Early 19th Century

Although these groups were not cultural allies (they belong, for instance, to different linguistic groups), their physical environments were similar (prairie). This similarity, as well as various common cultural contacts, led to similar cultural adaptations by the 18th century. They were both hunting-and-gathering, horse-based cultures strongly dependent on bison and war. Ethnographic reports of the traditional Teton Sioux and Blackfoot populations report similar diets, including a heavy reliance on bison, the hunting of numerous small-to-medium mammals, little fish and fowl, and extensive gathering and preservation of roots (especially the prairie turnip) and berries (chokecherries, service berries, wild cherries, bullberries, and many others) (Wissler 1910; Hassrick 1975).

These populations moved seasonally. The Teton Sioux cycle in the early 1800s included spring along the Missouri River for trading and gathering fruits and vegetables. The Missouri River tribes, such as the Arikara and the Mandan, traded their farmed corn, beans, and squash to the Sioux. Fall and winters were spent

hunting and resting on the eastern edge of the Black Hills (Prince 1989). The Blackfoot moved to the prairies in the spring and summer, harvesting roots and berries, and moved to rivers and woods in the fall and winter for hunting (Dempsey 2001).

Two traditional differences in their diets were the Blackfoot use of camas and the Teton Sioux consumption of corn, beans, and squash. The camas bulb grew on the western edge of the Blackfoot range, in the eastern foothills of the Rocky Mountains. This dietary tradition perhaps resulted from Blackfoot contact with Nez Perce and Flathead tribes (Wissler 1910). Corn, on the other hand, came to the Teton Sioux through trade with the Omaha, Mandan, and Arikara, who grew it on the eastern Plains (Hassrick 1975). The corn component is significant here because corn is the only important C4-type protein source in any diet north of Mexico, and carbon isotope testing discriminates the consumption of C4 from C3 proteins.

In addition, the consumption of beans by the Teton groups could affect their nitrogen isotope profile, since legumes have lower $\delta^{15}N$ values than other plants do. Due to the importance of bison, both the Blackfoot and the Teton were very high consumers of meat (an animal protein, and therefore of high trophic level).

Reservation Diets

The Sioux uprisings of the 1862-1876 Indian Wars were settled by a series of treaties starting with the Fort Laramie Treaty of 1868, which created the Great Sioux Reservation of western South Dakota, and ending with the Treaty of 1876. The latter provided for subsistence rations to the Teton Sioux for an indefinite period, unique among Indian treaties. As late as 1900, the Commissioner of Indian Affairs figured that most Teton Sioux were still receiving about 70% of their original rations (Prince 1989). However, by 1889 they had also lost about half of their reservation lands, and were suffering from semi-starvation, epidemics, drought, crop and cattle failure, and delays in delivery of rations.

The Blackfoot signed their major treaty with the U.S. government in 1855, which ceded them a large reservation in Montana and annuities and grants for 10 years. Intermittent treaty amendments and executive orders through 1895 continuously decreased Blackfoot land holdings, but annuity payments for subsistence goods, including livestock, were renewed in 1895 for 10 years. By the late 1880s, most Blackfoot reserves had cattle ranching. Some also had farming. As late as 1921, the Board of Indian Commissioners reported monthly rations for elderly Blackfoot (Dempsey 2001).

The Indian populations on the reservations could no longer freely practice their traditional diets. They continued gathering local fruits and vegetables, while hunting opportunities were severely curtailed (DeMallie 2001b). Bison were essentially extinct by the 1880s (Mattison 1995).

According to government records, in 1892 the *weekly* rations supplied to the Lower Brule tribe of the Teton Sioux *per 100 persons* were: 10 pounds of bacon, 3 pounds of beans, 4 pounds of coffee, 7 pounds of sugar, 50 pounds of flour, and unspecified amounts of baking powder and salt. In addition, 3.25 million pounds of beef on the hoof were provided for the year (Schusky 1975). This latter figure is difficult to interpret in terms of actual meat received per person. In 1876, the Sioux agencies were promised the following daily ration: 1½ pounds beef or ½ pound bacon, ½ pound flour, and ½ pound corn per person; 4 pounds coffee, 8 pounds sugar, and 3 pounds beans per 100 persons (Mattison 1995).

The government rations for the Blackfoot were similar. The *daily* rations were reported by Ewers (1958) for the years 1880-1900 to be 1½ pounds meat and ½ pound flour per person, 1 pound bacon per 10 persons, 1 pound coffee per 10 persons; and 3 pounds beans, 8 pounds sugar, 1 pound baking soda, and one pound salt per 100 persons.

CHAPTER 3
MATERIALS AND METHODS

Hair samples were obtained from museum collections for 29 individuals and a bison. They were analyzed for their stable-isotope ratios of nitrogen and carbon. The hairs came from individuals who represent four past populations of Great Plains Native Americans, each with a broadly similar diet and environment, and all with similar yet distinct cultures. This provided me with comparisons across related culture groups and across time. To provide additional context and comparison, hairs from four modern urban individuals were also sampled.

These samples provide a case study to test the reliability, practicality, and usefulness of stable-isotope studies in obtaining dietary information for past populations from artifactual hair.

I studied the effects of culture (via diet), age, sex, and season on hair isotopic values. By studying old hairs, I am also exploring the applicability of stable-isotope methods to archeological studies. It was important to model the testing of hairs from past populations rather than current ones because past populations consumed very different diets and experienced seasonal variation in their diets. On the other hand, since these hairs come from museum collections and are likely less than 200 years old, they come from environments broadly similar to those existing today, and they have undergone no apparent degradation. (In fact, hair up to 5200 years old has been successfully tested by Macko et al. [1999a].)

SOURCES OF HAIR

Table 3.1, below, lists the source of each hair set, which is a sample of a particular population.

Table 3.1. Hair Sources

Population	Where Collected	When	Museum Collection*	*n*†
Lower Brule Sioux	Lower Brule Reservation, SD‡	1892	Boas, AMNH	13
Blackfoot	Flathead Reservation, MT‡	1892	Boas, AMNH	3
Blackfoot	Montana‡	1935	Trotter, Smithsonian	5
Sioux	Great Plains	early 19th C.	NMNH, Smithsonian	4
Blackfoot	Great Plains	early 19th C.	NMNH, Smithsonian	2
"Plains"	Great Plains	early 19th C.	NMNH, Smithsonian	2
Modern urban	Corvallis, OR‡	2001	none	4
bison	Great Plains	early 19th C.	NMNH, Smithsonian	1

* AMNH is the American Museum of Natural History, New York City. NMNH is the National Museum of Natural History, Washington, D.C.
† *n* is the number of individuals.
‡Collected by anthropologists from living individuals.

The Boas Collection

As chief assistant to the anthropology section of the 1892 World's Columbian Exposition in Chicago, Franz Boas, a professor of anthropology at Columbia University, 1899-1942, oversaw the ambitious collection of copious amounts of anthropometric data of North America's "vanishing people" (Jantz et al. 1992). He trained about 50 anthropologists who visited about 200 tribal groups throughout the United States and Canada and measured about 15,000 subjects. A small fraction of these anthropometric investigations included the collection of hair specimens. Neither Boas nor any of his contemporaries ever analyzed the hair. (In fact, Boas analyzed very little of the anthropometric data, leaving it as a treasure trove for future investigations. Many contemporary anthropologists have made use of these data; the entire June 1995 issue of *Human Biology* is dedicated to papers on the "Boas data set.")

The Boas hair collection comprises locks of cut hair curled into small envelopes marked with each person's name, age, sex, tribe, parentage, and location. The

envelopes have been kept in small paperboard storage boxes in the American Museum of Natural History, New York City. Although they have not been specially protected from (indoor) moisture and air, hair is quite durable, and the specimens appeared fully intact when viewed under a light microscope. Although the length of the hair locks varies, it is not known where the hairs were cut and if this followed a standard protocol. It would be useful to know if the hairs were cut at the scalp or elsewhere, but this is not documented. Nevertheless, this collection of hair is better documented than any other I found.

I received permission from the American Museum in 2001 to collect a few strands of hair from 16 different specimens, which I did in March. I stored each sample in a labeled ziplock bag.

The Trotter Collection

Mildred Trotter was a professor of physical anthropology at the Washington University School of Medicine, St. Louis, during the mid-20th century. She accumulated a large collection of hair samples from around the world, many of which were sent to her by other collectors. The Blackfoot hairs that I sampled were collected from living males by G.A. Matson, Montana State University, Missoula, in or near 1935. Other than their (male) names and parentage, no information is provided. The individuals are full blood Blackfoot and probably lived on a reservation.

Although Trotter published many comparative studies of hair morphology from different ethnic groups, she never published reports on these hair samples. The collection is now curated at the Smithsonian Institution, which granted permission for the removal and testing of individual strands in fall 2001.

The Collection from the National Museum of Natural History

The National Museum of Natural History at the Smithsonian Institution has a diverse collection of hair locks and scalps donated by a wide variety of private individuals. They were not collected professionally, for the most part, and the donor was often not the original collector. Many of the specimens were probably "trophy" pieces taken from dead individuals in battle, and therefore would date to the pre-reservation period, early to mid-19th century. The documentation is therefore rather sparse. I chose individuals whose cultural affiliation was ascribed to Plains groups ("Sioux", "Blackfoot", or "Plains"). More specific locations or tribes are not known.

The Smithsonian Institution granted permission for the removal and testing of individual strands in fall, 2001.

The Modern Hairs

The four modern hair specimens I tested are not meant to represent a particular population or diet. They were collected from four convenient individuals in Corvallis. These hairs were included merely to provide a rough sort of context of comparison with the historic hairs. Two of the modern individuals were omnivores and two were fish-eating, ovo-lacto-vegetarians (that is, they also ate eggs and dairy products).

The historic and modern hairs differ not just in time, but in environment, climate, and culture. There are too many variables to provide a controlled comparison, but such very different populations should look quite different isotopically due to dietary differences. The bison hair, also from the Smithsonian, was tested for the same reason. One bison's hair cannot say something about all bison; it merely provides a check as to whether the human omnivore hairs look isotopically different from the animal herbivore.

SPECIMEN PREPARATION

Two or more (if short) strands of hair from each individual were cleaned. The purpose of cleaning was to remove surface contaminants and sebum lipids. The individual hair strands were cleaned by soaking for 2 hr in a 2:1 mixture of reagent-grade methanol and chloroform, as per the procedure of O'Connell and Hedges (1999). Most researchers use organic solvents to clean hair of lipids (Katzenberg and Krouse 1989; Nakamura et al. 1982; Yoshinaga et al. 1996). Lipids are more depleted in ^{13}C than are proteins, so the presence of lipids could interfere with analyzing the hair itself (Ambrose 1993).

Following the soak, the strands were rinsed with deionized-distilled water, ultrasonically vibrated in closed vials of water for 5 mins, rinsed again, and air-dried for 2 hr. Finally, each hair was wiped clean with a few drops of reagent-grade ethanol on a Kimwipe, as recommended by Macko (personal communication, 2001). The cleaned strands for each individual were stored in separate, labeled ziplock bags.

Prior to cutting the hair into segments for testing, I determined the proximal and distal ends by visual examination under a microscope at 250× and 400× power. I then cut a 2- or 4-cm segment from the proximal end. In the case of testing entire strands, I cut contiguous segments numbered from proximal to distal ends, so that I would analyze one strand's segments in order from most recent to least recent segment. Each segment was stored in an individual, labeled ziplock bag.

In preparation for analysis, each cut segment was weighed on a Cahn C-31 electronic microbalance to a precision of ±0.0001 mg (0.1μg). The 2-cm segments weighed 100 to 150 μg, and longer segments were limited to 250 μg for testing. Each hair segment was cut into tiny pieces to fit inside a foil tin cup, which was then squeezed shut and rolled into a ball.

ANALYTICAL PROCEDURE

The foil-wrapped hair pellets were loaded into the carousel of a continuous-flow, stable-isotope-ratio mass spectrometer (Finnigan/MAT DeltaPlus-XL) coupled to an elemental analyzer (Carlo Erba NA 1500) via a Finnigan/MAT CONFLO-III interface. Each ball was combusted in the elemental analyzer to produce CO_2 and N_2 gases for isotope analysis. An inert carrier gas, helium, moved the CO_2 and N_2 into the mass spectrometer for analysis. The carrier gas served to dilute the experimental gases, and its flow rate and dilution factor could be adjusted to control the concentrations of CO_2 that were analyzed. A large specimen or one high in carbon (as organic compounds are) might need to be diluted, and this was accomplished in the CONFLO-III interface.

We ran two separate runs of the analytical instruments due to the number of specimens. The first run used the larger specimen (weight) size and included paired hair specimens (that is, two hair specimens from different strands of the same individual). The second run used the smaller specimen size and no duplicates, since the results of the first run indicated high sensitivity and good reproducibility.

The analysis determined the comparative abundance of the two main stable isotopes of carbon and of nitrogen, ^{13}C:^{12}C and ^{15}N:^{14}N, and then reported them relative to an internal reference gas, according to the following equation. The answers were expressed as ‰, or units "per mil" (parts per thousand). The notation for this is $\delta^{15}N$ or $\delta^{13}C$.

$$\delta = \{(R_{sample} - R_{standard}) / R_{standard}\} \times 1000 \text{ , in units ‰,}$$

where R is the ratio of the heavier isotope to the lighter isotope.

The final results were adjusted relative to the universal standards for carbon, PeeDee Belemnite, and for nitrogen, atmospheric N_2 in AIR, or Ambient Inhalable Reservoir (Richards et al. 2000). By definition, the PDB carbon and AIR nitrogen

standards have been assigned zero values (0‰) for $\delta^{13}C$ and $\delta^{15}N$, respectively (Katzenberg 2000).

PRECISION AND STANDARDS

The mass spectrometer measures the carbon and nitrogen isotope ratios (^{13}C:^{12}C and ^{15}N:^{14}N) in both the experimental specimens and in the internal reference gases (or "working standards") of CO_2 and N_2. For the calculation of the experimental $\delta^{13}C$ or $\delta^{15}N$ value according to the above equation, the mass spectrometer compares the isotope ratio in the sample to the isotope ratio in the internal reference gas. Although the reference gases are stable, other variables in the instrumentation can cause variability.

The isotope ratios of the reference gases are therefore calibrated against internationally referenced primary standards procured from the National Institute of Standards and Technology (NIST). The primary standards used were NIST 8541 (graphite) for carbon (by convention, $\delta^{13}C$=–15.90‰) and NIST 8548 (ammonium sulfate, $(NH_4)_2SO_4$) for nitrogen (by convention, $\delta^{15}N$=20.30‰). The NIST standards, in turn, have been calibrated to the universal standards, PeeDee Belemnite and AIR, whose δ values have been arbitrarily set to zero, thereby supplying the baselines for the δ values. The primary standards therefore serve to calibrate all results and provide a measure of how *accurate* the instrumentation is.

The secondary standard I used was acetanilide ($CH_3CONHC_6H_5$) for both carbon and nitrogen. Acetanilide has a measured value for $\delta^{13}C$ of –30.22‰ and for $\delta^{15}N$ of –0.87‰. Several samples of acetanilide accompanied each sample run. The sample weights of acetanilide were adjusted to provide carbon and nitrogen in roughly the same masses as in the experimental specimens, thereby providing a test of precision in the experimental range. The acetanilide standards were used to assess the *precision* of the system by providing a measure of replicability. That is, how close were the δ values for replicate acetanilide specimens? Based on the

standard deviations for 18 replicate acetanilide standards tested in weights ranging from 0.047 mg (4 μg N, 29 μg C) to 0.487 mg (51 μg N, 351 μg C), the experimental precision for $\delta^{15}N$ was ±0.27‰ (mean=–0.95‰) and the precision for $\delta^{13}C$ was ±0.22‰ (mean=–30.21‰).

The NIST standards were incorporated into the calculation for precision via the standard deviations from the mean of ten isotope values for NIST 8548 (ammonium sulfate) (containing 23-87 μg N) and of seven isotope values for NIST 8541 (graphite) (containing 36-250 μg C). The standard deviation of $\delta^{15}N$ for the tested samples of NIST 8548 was ±0.17‰ (mean=20.38). The standard deviation of $\delta^{13}C$ for the tested samples of NIST 8541 was ±0.10‰ (mean=–15.87). The average precision, taking the acetanilide (secondary) standard and the NIST (primary) standards into account, was ±0.22‰ for $\delta^{15}N$ and ±0.16‰ for $\delta^{13}C$.

STATISTICAL METHODS

The presentation of my data employs descriptive statistics (mean, median, sample standard deviation, range) with inferential statistics (correlation, regression, *t*-tests) in certain cases where inferences were drawn from the sample to the population (Kurtz 1999). I used the statistical and graphing functions of Microsoft Excel 97 SR-2, Microsoft Corporation, 1985-1997.

Sample Size

As shown in Table 3.2, all four Native American hair sample sizes were small, with the Lower Brule Sioux being the largest (n=13) and the Flathead Reservation Blackfoot being the smallest (n=3). I combined the Flathead group with the Montana group (n=5) for comparison with the reservation Lower Brule Sioux. The fourth group, the pre-reservation Plains sample (n=8) is actually composed of at least three different tribal groups (Sioux, Blackfoot, and "Plains"), but the small numbers and imprecise identification made it prudent to keep these individuals

together. However, I did break out the pre-reservation Sioux (n=4) for comparison with the reservation Sioux (Lower Brule).

Table 3.2. Sample Sizes

Population	Sample Size, *n*
Pre-reservation Plains Indians, 19th century	8
Lower Brule Sioux, Lower Brule Reservation, 1892	13
Blackfoot, Flathead Reservation, 1892	3
Blackfoot, Montana, reservation, 1935	5

The small sample sizes limit what conclusions may be drawn about the populations the samples are drawn from. Inferential statistics take the sample size into account in calculating the probabilities of finding differences between the mean values of two different groups. In practice, this means that with small samples it becomes very difficult to establish differences between populations. This can be done only if the sample values are extremely different.

Statistical inference from the sample to the population also assumes that the sample is a random one, "in which all members of the population have an equal and independent chance of being selected for the sample" (Kurtz 1999). This, unfortunately, is a criterion I could not meet. (In fact, it could never be met in archeological contexts, which must consider whatever sample is available.) While I used all specimens that were available in a specific sample of a population, the compilation of those original samples was opportunistic rather than random, although this is undocumented.

Descriptive Statistics

Sample means and standard deviations were calculated to characterize the nitrogen and carbon isotope values for all samples for two purposes: so the samples could be compared with each other, and so that precision (using a standard) could be judged.

Ranges for strand values (the difference between the segment with the highest value and the segment with the lowest) were calculated to show the maximum variability in a single hair from segment to segment.

The distributions of the nitrogen and carbon isotope data for the pre-reservation Plains, the Lower Brule Sioux, and the Blackfoot samples are shown in the next chapter, Results. Most of the distributions do not appear normal, but the small sample sizes make normality difficult.

When comparing the variability of carbon and nitrogen values within a sample, they cannot be normalized (such as by using the coefficient of variation), because stable isotope ratios are interval scale data, as opposed to ratio scale data. Interval scale data are expressed relative to an arbitrary zero point and can be positive as well as negative numbers. The variabilities of the carbon and nitrogen data should be compared directly through their standard deviations.

Inferential Statistics

I used linear regression and Student's *t*-test to make inferences about the larger population groups based on the population samples. The probability tests take sample size into account, preventing one from making a Type I error (that is, concluding that there is real difference between populations when there is not) (Kurtz 1999). Inferences are made from the sample to the entire population that it represents. For instance, how likely is it that the 13 specimens composing the Lower Brule sample represent the entire population of Lower Brule Reservation Sioux in 1892?

In each case, the null hypothesis being tested is that there will be no meaningful effect on isotope values due to the given variable. That is, gender and age would have no effect on isotope values, and cultural (population) differences also would have no effect. To disprove the null hypothesis, the probability value, P, must be less than 0.05. The value P reflects the likelihood (e.g. less than 5% if $P<0.05$) that

the differences between the sample results are due to chance, and would not be borne out in the population as a whole.

With linear regression, I tested the effect of age on isotope values and the effect of specimen size (hair weight) on isotope values. With the *t*-test, I calculated the probability that the null hypothesis was wrong regarding the effects of age, specimen size, gender, and culture group on isotope values. The specific cultural groups (population samples) compared were:

- Reservation Lower Brule Sioux and reservation Blackfoot.
- Pre-reservation Sioux and reservation Sioux.
- All pre-reservation Indians (Sioux, Blackfoot, "Plains") and all reservation Indians (Sioux, Blackfoot).

CHAPTER 4
RESULTS

I tested and compared four groups of Native American hairs from the Great Plains region from prior to and during the early reservation period. I wanted to establish whether hairs from the same individual are isotopically similar, whether hairs from different individuals but from the same population are isotopically similar, whether very small specimens can be reliably tested for isotope values, whether age and gender affect isotope values, how culture might affect isotope values, how generational time might affect isotope values, and what light all of this can shed on diet, given minimal supplemental information. The populations I have chosen are case studies for exploring the viability of this technique in using very small specimen weights to search for isotope signals that exceed background noise (that is, exceed the limits of instrument precision and the normal chemical variation produced by a living system). They are historic populations, and their old hair specimens serve as analogs for possible future investigations of ancient hair from archaeological sites.

CARBON:NITROGEN ATOMIC RATIOS

The carbon:nitrogen ratio of hair as determined by the elemental analyzer provides a rough check of the purity of the specimen and the completeness of its combustion. Excess lipids remaining on the hair, for example, would alter the C:N ratio because lipids and proteins have different C:N values.

Carbon:nitrogen atomic ratios were determined from the experimentally determined weight percentages of carbon and nitrogen provided by the elemental analyzer. Each weight percentage is divided by its atomic weight, and then compared:

$$\text{Atomic C:N} = [(\text{Wt\%C}) / 12.01\text{g/mol C}] \div [(\text{Wt\%N}) / 14.01\text{g/mol N}]$$

The C:N atomic ratios of all hair samples were in the range 3.42-4.09 (see Tables 1 to 4 in the Appendix for raw data values). The averages for the two experimental groups were 3.64±0.09 and 3.78±0.09. According to O'Connell and Hedges (1999), the theoretical C:N ratio of hair keratin is 3.4±0.5. While my averages are higher than that, they are quite consistent. I have no reason to discount any particular specimen.

As explained in chapter 3, Materials and Methods, all hairs were solvent-washed to remove lipids and debris. In addition, preliminary testing showed no effect of solvent-washing on C and N isotope values (see Table 5 in the Appendix; see also O'Connell and Hedges [1999]; Nakamura et al. [1982]), since the differences in isotope values for washed and unwashed hairs fell within the precision range of the instrumentation.

MINIMUM SPECIMEN WEIGHT FOR ISOTOPE TESTING

One of the objectives of this project is to establish a practical lower limit of specimen weight for stable-isotope analysis of hair by continuous-flow mass spectrometry. This project tested hair from museum samples which provided more than adequate specimen weights (such as a couple of strands). However, for applications in archeology, the smaller the minimum specimen weight, the better. Archeological excavations may find only very small hair specimens. In addition, the less hair needed for an isotope test, the more hair may be available for other tests.

Based on published information, stable isotope testing of hair has used hair specimens ranging from 1 g (Yoshinaga 1996) to 10 mg (Minagawa 1992; Macko et al. 1999a) to 600 μg (O'Connell and Hedges 1999). However, in discussions with geochemists using the latest generation of continuous flow, stable isotope spectrometry, a lower limit of 100-300 μg was recommended to obtain a sufficient quantity of nitrogen to test (personal communications from Macko 2000; Mix

2000; Rugh 2001). To find a reliable minimum hair weight, I ran a series of hair specimens in the range of 50-300 μg for two individuals (BF24 and BF57), testing for both C and N isotope ratios. The results for the hair are plotted in Figures 4.1 and 4.3. For comparison, the results for the standards are plotted in Figures 4.2 and 4.4. To be able to compare the different materials, the weights are plotted as mg of carbon or nitrogen, rather than of the whole specimen (hair weight).

Hair specimen weights as low as 100 μg (14 μg N, 43 μg C) yielded consistent isotope values (Figures 4.1 and 4.3). The precision (standard deviation) of the $\delta^{15}N$ measurements for hair samples from 100-250 μg was 0.11‰ for BF24 and 0.21‰ for BF57. These fall within the instrument's precision (0.27‰ for $\delta^{15}N$). However, when hair weights from 50-90 μg are included, the precision falls to 0.24‰ and 0.37‰, exceeding the instrument's range of precision. Using hair specimens smaller than 100 μg (14 μg N) is therefore undesirable, since it would provide less precision than the instrument is capable of providing. The precision of the $\delta^{13}C$ measurements for hair samples from 50-250 μg was 0.09‰ for BF24 and 0.13‰ for BF57, all well within the instrument's range of precision (0.22‰ for $\delta^{13}C$).

Acetanilide standards were tested in a range of specimen sizes from 47-487 μg acetanilide, containing from 4-51 μg N and 29-351 μg C (Figure 4.2 and 4.4). The standard deviation (precision) for the mean $\delta^{15}N$ value in the entire range was 0.27‰. If specimens smaller than 11 μg N are excluded (corresponding to about 100 μg of hair), the precision improves to ±0.22‰. The standard deviation for the mean $\delta^{13}C$ value in the entire range was 0.22‰. This improved slightly to 0.21‰ when specimens smaller than 40 μg C (corresponding to about 100 μg hair) were excluded.

I therefore decided to measure and test hair specimens weighing between 100-150 μg, containing 14-21 μg N and 43-64 μg C. (See Appendix Table 6 for the raw data.) There are roughly 50 μg/cm of hair, though this varies from individual to individual. For testing requirements, I defined a minimum weight (quantity) of hair.

I could then roughly translate this into hair length for easier measurement in the field and the laboratory. To obtain at least 100 μg hair, I cut 2 cm from a strand.

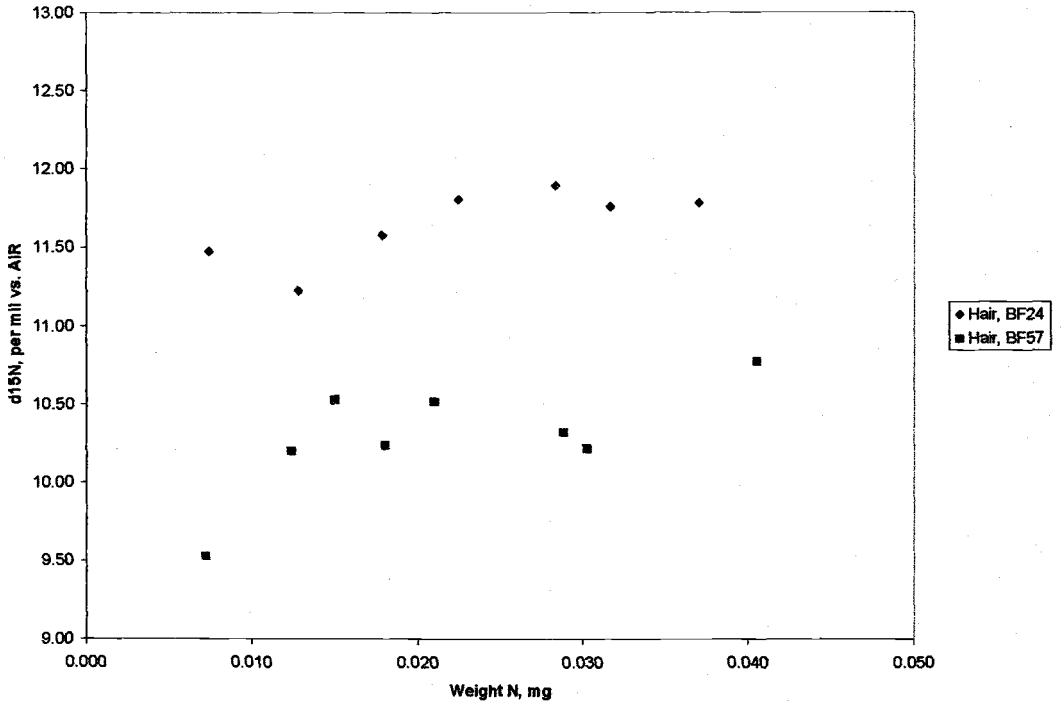


Figure 4.1. Effect of Nitrogen Weight on $\delta^{15}N$ Value in Hair

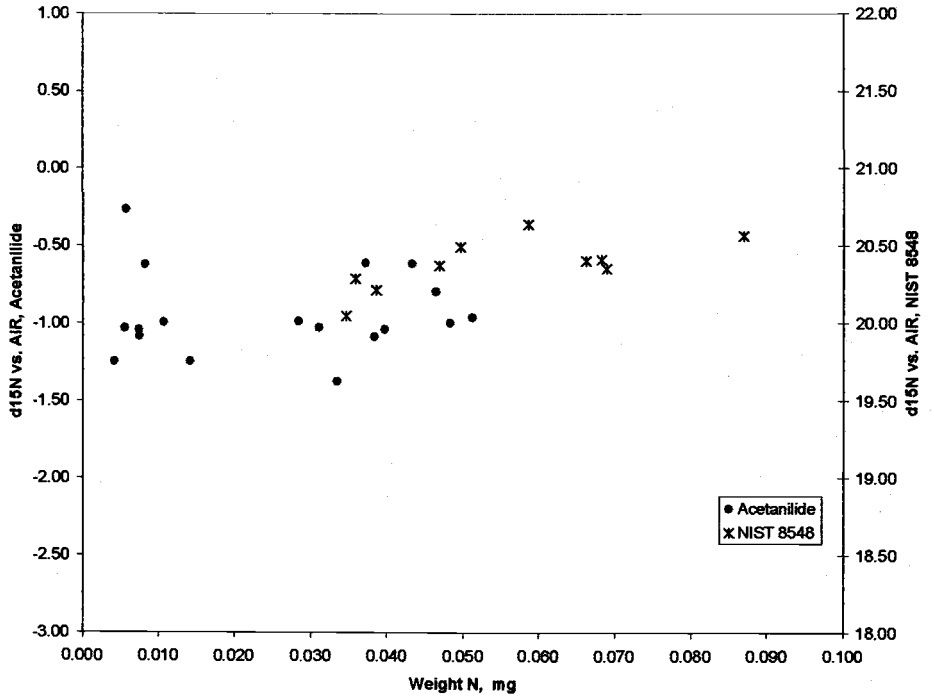


Figure 4.2. Effect of Nitrogen Weight on $\delta^{15}N$ Value in Standards

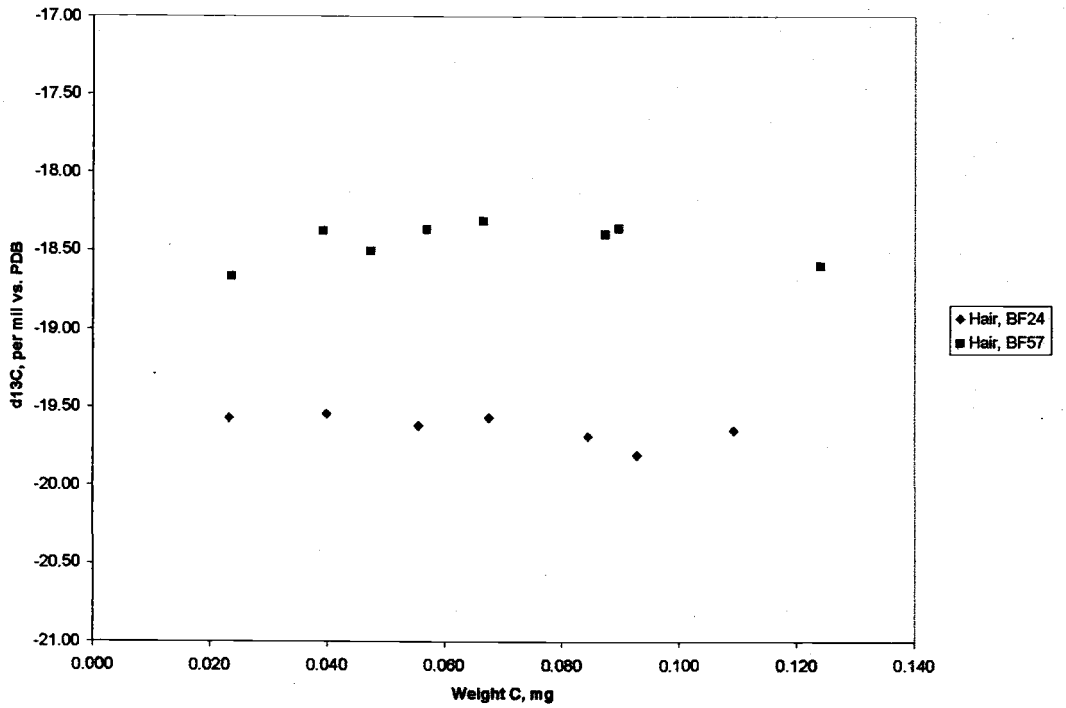


Figure 4.3. Effect of Carbon Weight on $\delta^{13}C$ Value in Hair

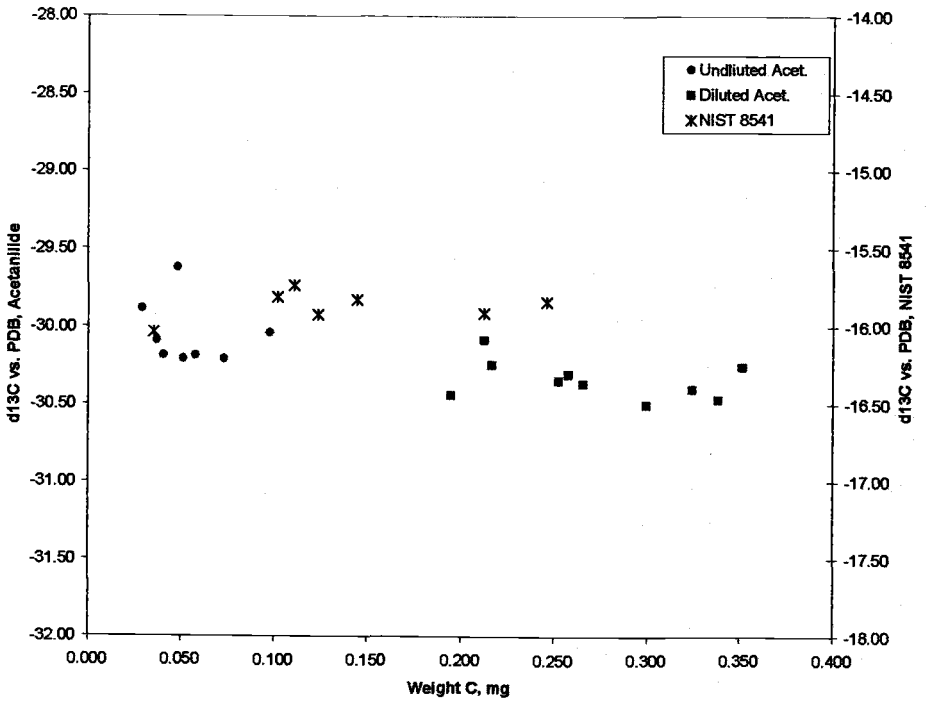


Figure 4.4. Effect of Carbon Weight on $\delta^{13}C$ Value in Standards

The smallest acetanilide samples were run without the usual diluter gas, due to the low mass of carbon available.

PRECISION AND ACCURACY

Reliability is affected by two processes: one is analytical (produced by the instrumentation), and one is biological (produced by the hair itself). How well does a hair represent an individual, and how well does an individual represent a population? Do different hairs from the same person provide consistent isotopic results? Do different parts of the same strand show measurable isotopic variability (due to diet or metabolism) that exceeds the analytical uncertainty (variability, precision)? Hypothesis: I would expect different strands from the same person to provide consistent results, and I would expect different segments from the same

strand to show variability (due to diet) that exceeds the analytical variability uncertainty.

Analytical Uncertainty (Precision)

The precision of the instrumentation was calculated as ±0.22‰ for $\delta^{15}N$ and 0.21‰ for $\delta^{13}C$, based on the standard deviations from the mean $\delta^{15}N$ and $\delta^{13}C$ values for 12 replicate tests of acetanilide containing ≥11 μg of N and 17 replicates containing ≥40 μg C. This quantity of nitrogen and carbon encompasses the minimum to be found in an experimental specimen of hair weighing 100-150 μg. Acetanilide standards accompanied each experimental run. (Refer to "Precision and Standards" in chapter 3, Materials and Methods.)

Variability Within an Individual

Based on the average difference in 15 sets of paired hairs (the most proximal segments from two strands from the same individual), the consistency of isotope values for different hairs from the same individual is estimated as 0.20‰ for $\delta^{15}N$ and 0.23‰ for $\delta^{13}C$. (The raw data are in Appendix Table 7.) This is a measure of how well an individual strand can represent the whole person. These variances are close to the limits of instrument precision (0.22‰ $\delta^{15}N$, 0.21‰ $\delta^{13}C$), and therefore the paired hair values are not significantly different.

Variability Within a Strand

The longest strands of hair I had available for testing were 24 cm long. With a minimum hair segment length of 2 cm (100-150 μg) for isotope testing, I could divide one strand into 12 distinct segments for testing. This provides two assessments:

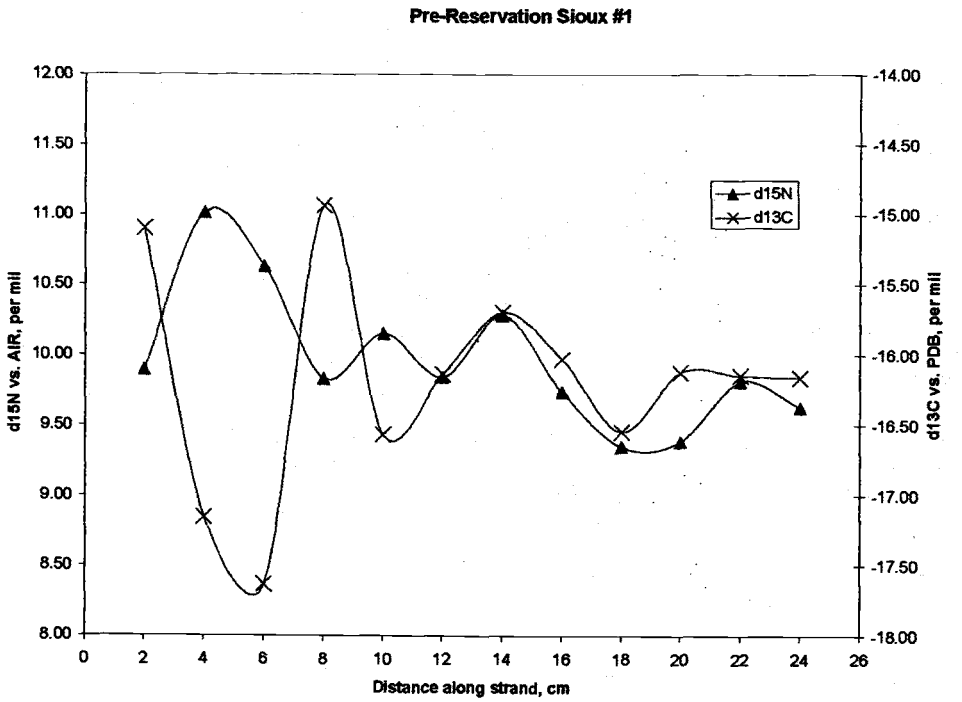
- A measure of isotopic variation within an individual over time (approximately 2 months per 2-cm segment), and therefore a measure of the maximal variation in one individual as compared to another individual from the same group. This variability could affect the isotopic variation (precision) of a population of individuals.
- A measure of the variability in diet of individuals *over time*, refined to approximately 2-month intervals.

As a pilot study, I tested six individuals (two each from three different groups) and one bison by dividing single strands of hair into sequential 2-cm segments. For the humans, each 2 cm represents about 2 months' growth. This scale is fine enough to yield evidence of dietary change or disruption. If the hair is long enough (over 12 cm), the change in isotopic values could possibly show seasonal or periodic changes in diet.

I selected the longest strands from two members of the pre-reservation Sioux population and two members of the Lower Brule Reservation Sioux population, divided them into 2-cm segments, and analyzed the isotope ratios for each segment in order from the proximal end to the distal end of the strand. For further comparison, I included two modern hairs and one bison hair from a museum collection. The human hairs were 20-24 cm long; therefore covering about a 24-month time period. The bison hair was 12 cm long, representing an unknown growth period. (The raw data are in Appendix Tables 9 and 10.)

The following seven graphs show the variations in $\delta^{15}N$ and $\delta^{13}C$ values for seven individuals (including a bison) with time, that is, along the length of a strand of hair. The graphs show that there were changes in diet over the 2-year time period for some individuals. These changes could be due to many reasons, including season. More data—both more strands and longer strands—would clarify this picture.

Pre-reservation Sioux #1 and Lower Brule Sioux #2 show the greatest range (difference between maximum and minimum values), other than the bison. These two plus Lower Brule Sioux #1 show possible periodic changes. However, those periods are not necessarily annual, nor are they the same from individual to individual. I specifically wanted to see whether the pre-reservation Sioux would differ from the reservation (Lower Brule) Sioux. This limited test cannot show a difference in isotope variability between earlier and later Sioux cultures, just between the individuals themselves.



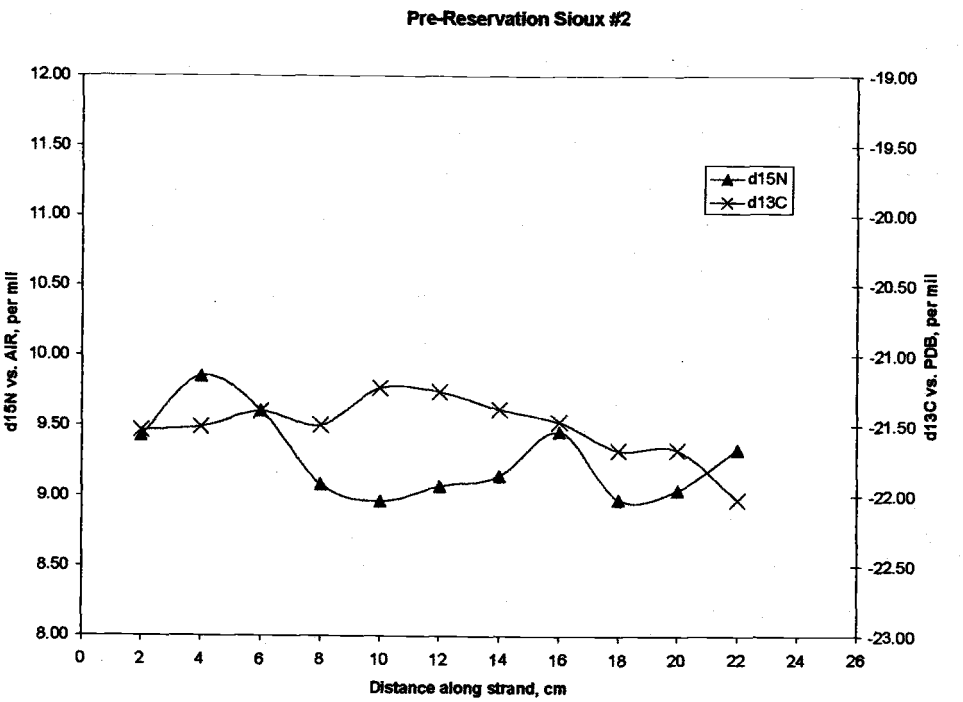


Figure 4.5. Isotope Variations Along a Strand: Two Pre-Reservation Sioux

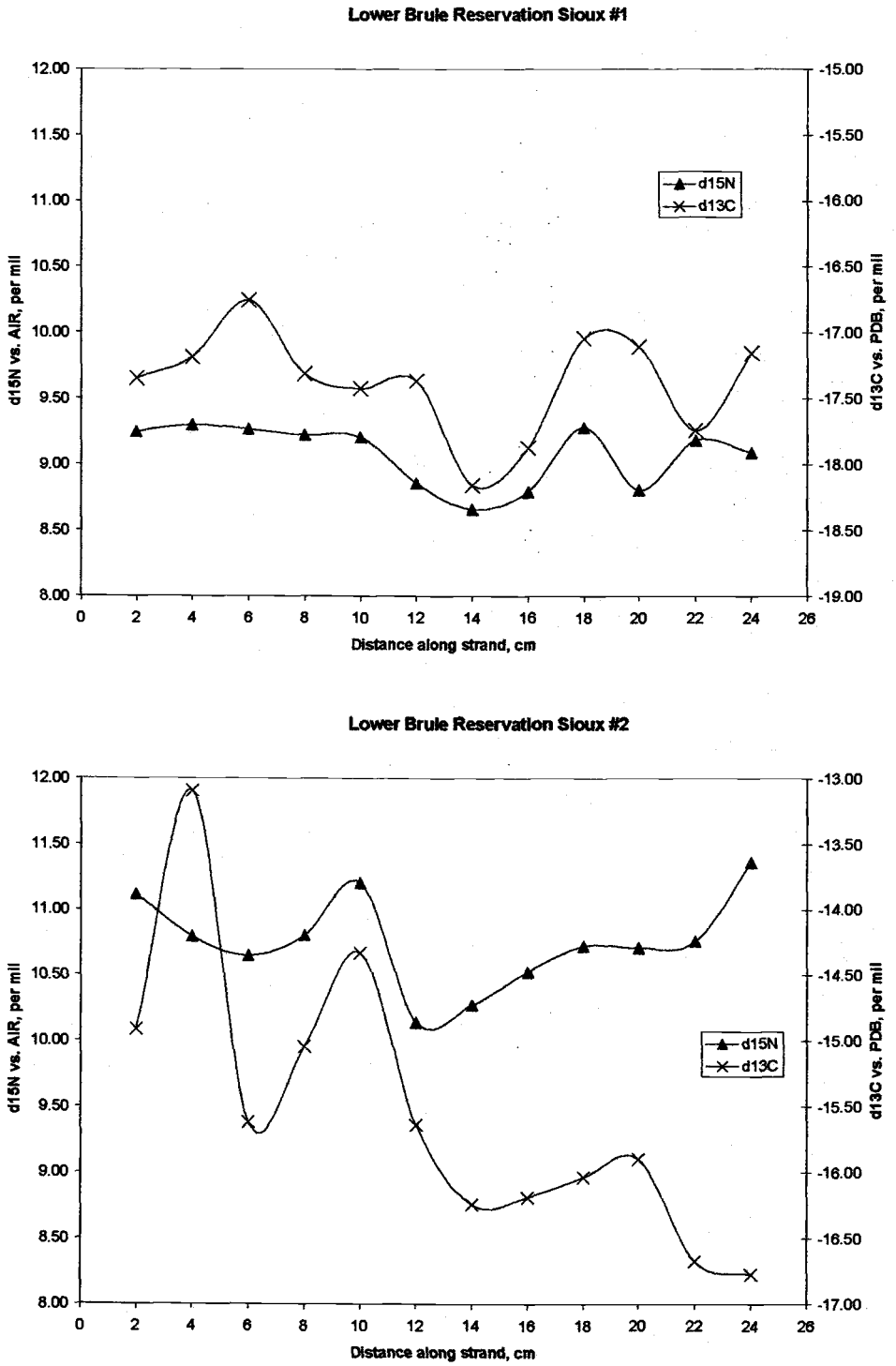
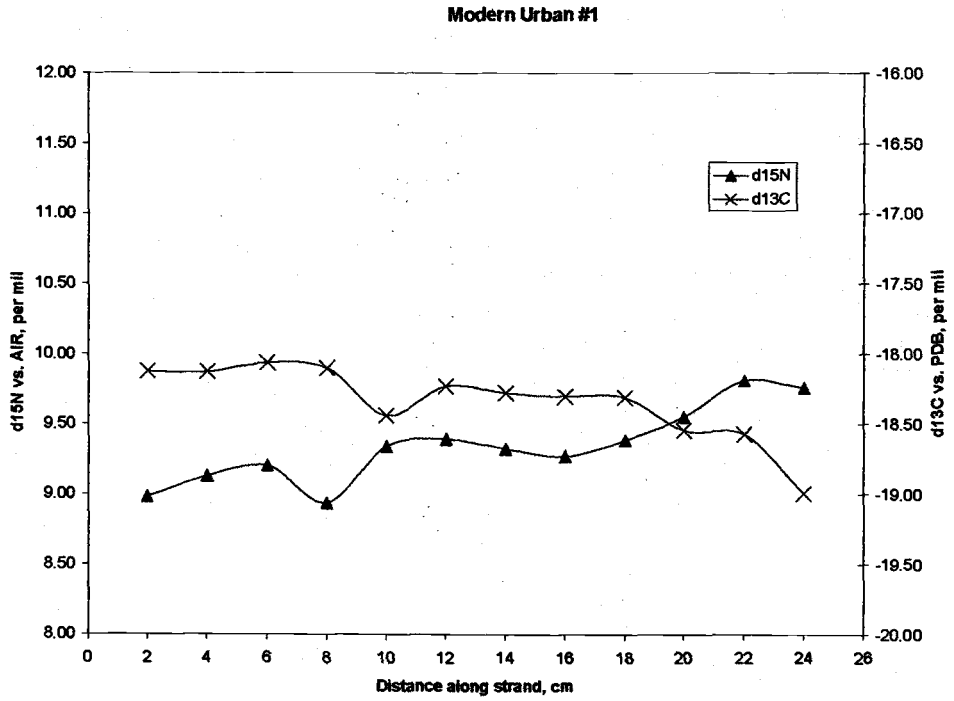


Figure 4.6. Isotope Variations Along a Strand: Two Reservation (Lower Brule) Sioux



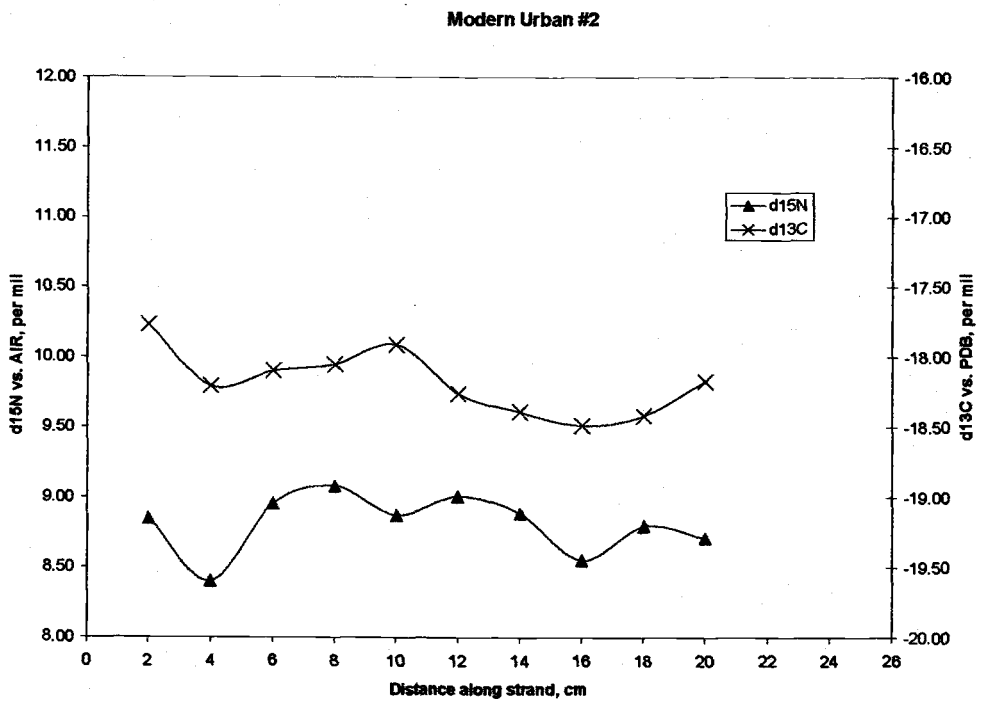


Figure 4.7. Isotope Variations Along a Strand: Two Modern Urbanites

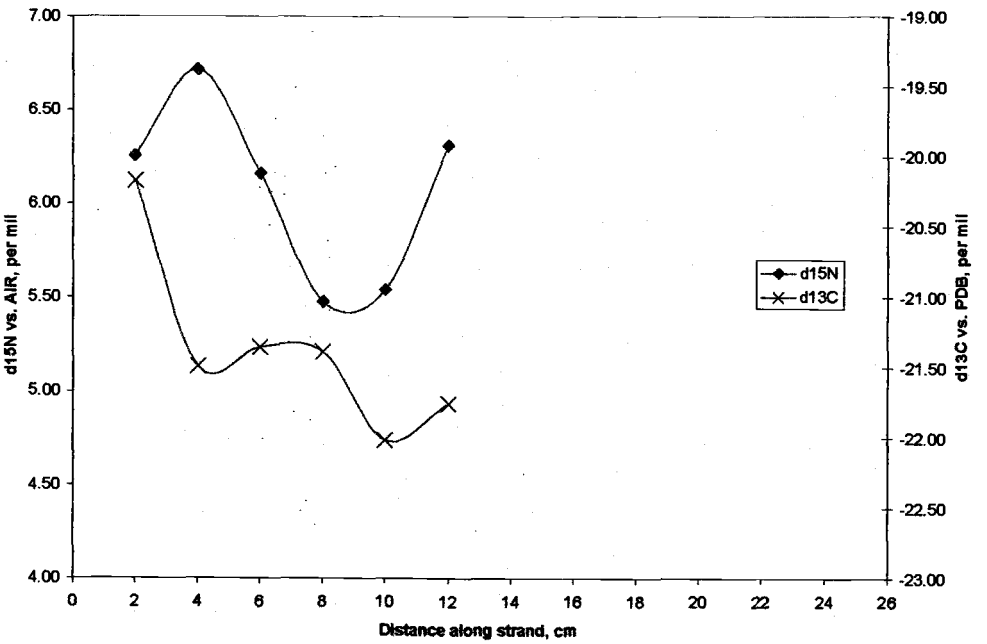


Figure 4.8. Isotope Variations Along a Strand: a Bison

Another way of considering the variation in a strand of hair (and therefore diet) is to look at the *range of isotope ratios* that occur in a strand, as in the following graph. The *range* is the difference between the maximum and minimum isotope ratios in the same strand. This comparison suggests that:

- Where the $\delta^{15}N$ and $\delta^{13}C$ ranges are different (four of seven cases), it is the $\delta^{13}C$ values that have the larger range (vary more).
- The differing ranges show that some individuals experience more isotope variability through time than others do. However, this could just reflect different time periods, or it could be a population effect.

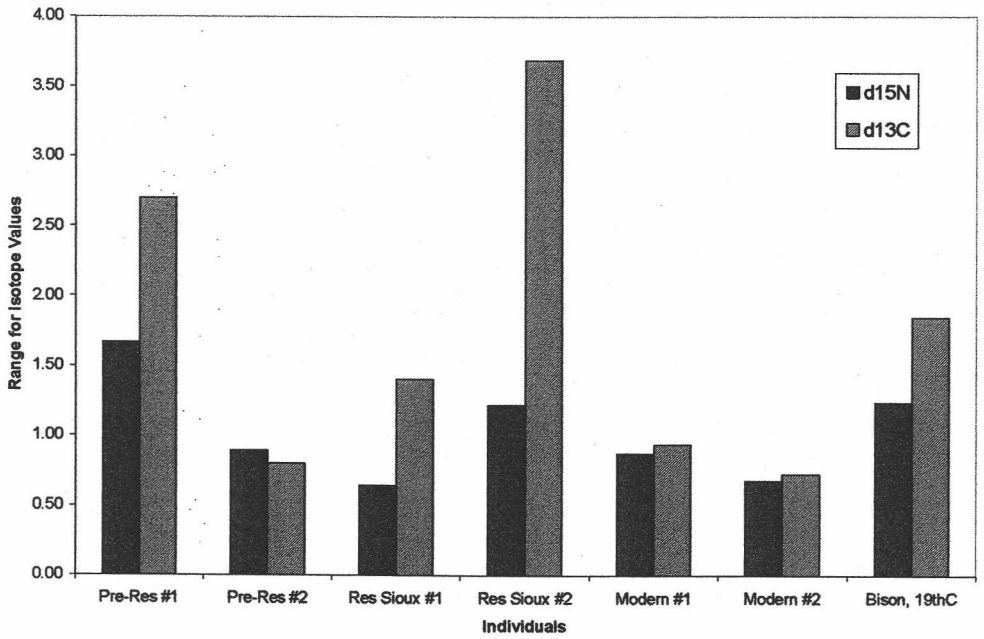


Figure 4.9. Range of Isotope Variation in a Strand

Do these variations represent meaningful seasonal change (as opposed to physiological fluctuations) in the historic hairs? The ranges in the historic strands are 0.64‰–1.67‰ for $\delta^{15}N$ and 0.80–3.68‰ for $\delta^{13}C$. The larger numbers could imply seasonal change in both $\delta^{15}N$ (meat intake) and $\delta^{13}C$ (plant sources), especially as these ranges are greater than what we see in the modern strands (representing a "supermarket diet" of minimal seasonality). To compare with other research, Christine White (1992) argues in her study of Nubian mummy hair strands (dated AD 350-1300) that a $\delta^{13}C$ range of 0.43‰–3.85‰ in individual strands supports seasonal C3-C4 crop rotation.

More directly, one can compare the standard deviations for the isotope values in each strand to the instrumental precision. According to the standard deviations in the following table, all historic individuals show an isotopic variability along the lengths of their hair that is meaningful because it exceeds the analytical uncertainty

(0.22‰ for $\delta^{15}N$, 0.21‰ for $\delta^{13}C$). In all cases, both isotope ratios varied above the background level, indicating a change in both the meat and plant sources of the diet. One modern urban dweller shows significant isotope variation over time, while the second one does not.

Table 4.1. Variability Along Strands (Standard Deviations)

Individual	Pre-Res Sioux 1	Pre-Res Sioux 2	L.B. Res Sioux 1	LB. Res Sioux 2	Modern Urban 1	Modern Urban 2	Bison
S.D. $\delta^{15}N$	0.49‰	0.29‰	0.23‰	0.35‰	0.27‰	0.21‰	0.48‰
S.D. $\delta^{13}C$	0.76‰	0.22‰	0.39‰	1.05‰	0.27‰	0.23‰	0.64‰

EFFECTS OF SEX AND AGE ON ISOTOPE VALUES

Either physiological differences or dietary differences could cause the carbon and nitrogen isotopic composition of hair to vary as a function of age or gender within the same population. Since the Boas collection of hair documents the age and sex of the individuals, I was able to investigate this question. I tested hairs only from Lower Brule Sioux from 1892 in order to minimize the effect of other cultural or environmental variables. This population sample is also the largest one available to me.

The effects of sex and age on human isotope values have been tested by other researchers for different populations, both in bone and in hair (Lovell et al. 1986; Nelson et al. 1986; Minagawa 1992; White 1992; Habicht-Mauche et al. 1994; Richards et al. 1998), usually with negative results.

Hypothesis: Based on the research of others, I did not expect a physiologically caused difference is isotope values. A difference based on dietary distinctions by age or sex is more likely, but unexpected based on ethnographic research. The effects of sex and age on dietary practices will vary from culture to culture, but small differences in diet are unlikely to produce measurable isotopic differences. This question of age and sex is fundamentally critical, since significant differences

based on age or sex would mean that hairs could not be compared isotopically without knowing the age and sex of their owners—almost impossible to know with archeological remains (although DNA testing of additional material can establish sex).

Sex as a Variable

The sample size is small, but the graphs and the statistics below show similar and overlapping values and, hence, no distinctions between the sexes. I ran a *t*-test comparing males and females, which did not find the means of the two groups to differ (for nitrogen: t=0.92, P=0.38, 11 df; for carbon: t=0.62, P=0.55, 11 df).

Table 4.2. Summary Isotope Values by Sex

Gender	*n*	Mean $\delta^{15}N \pm$ S.D.	$\delta^{15}N$ Range	Mean $\delta^{13}C \pm$ S.D.	$\delta^{13}C$ Range
Male	7	10.37 ± 0.56	1.37	-15.06 ± 1.04	3.47
Female	6	10.10 ± 0.52	1.50	-15.41 ± 1.01	3.16

The following figure plots the nitrogen and carbon isotope values for the Lower Brule Sioux men and women.

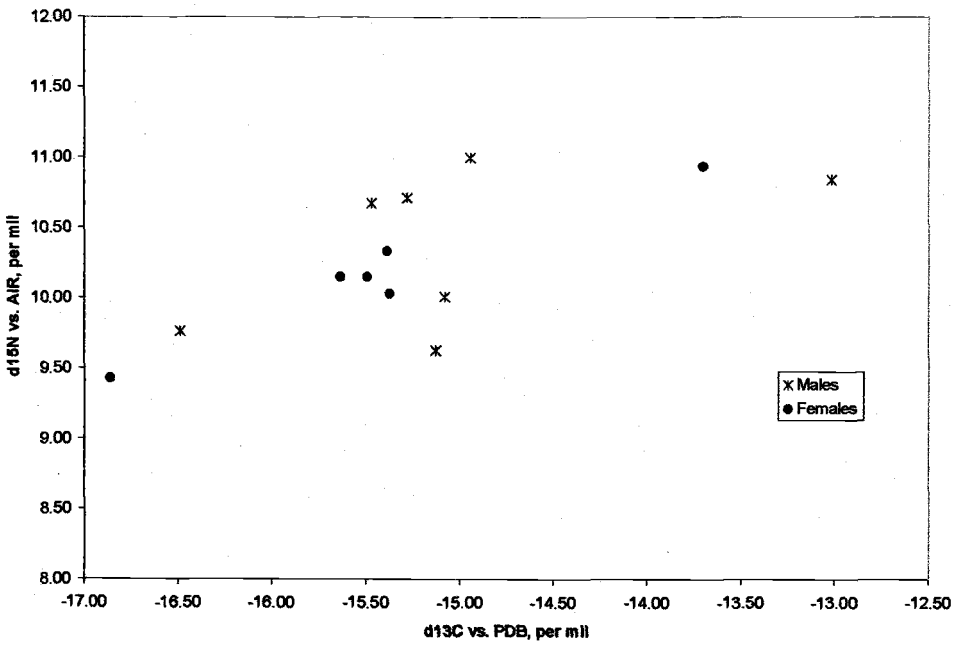


Figure 4.10. Isotope Values by Sex (Lower Brule)

By visual inspection, it appears that the female values might clump more than the male values. However, the *t*-tests do not indicate a signficant difference at α=0.05. Also, the standard deviations from the mean isotope values are similar for the two genders. It would be interesting to see the results in a much larger sample.

Age as a Variable

The age range in the Lower Brule sample is effectively adult, from 16 to 58 years. The regression statistics do not support a significant effect of age on either isotope value. The correlation coefficient represents the percentage of the resulting isotope value that is due to the age variable. The coefficient of determination represents the *variance* determined by the association of age and isotope value.

Table 4.3. Regression Statistics for the Effect of Age

Isotope Ratio	*n*	Mean ± S.D.	Corr. Coeff., *r*	Coeff. of Det., r^2	Slope, *b*	*t* Value (df=11)	Probability, *P*
$\delta^{15}N$	13	10.25‰ ± 0.54	0.46	0.21	0.02	1.70	0.12
$\delta^{13}C$	13	-15.22‰ ± 1.00	0.28	0.08	0.02	0.97	0.35

These results are consistent with other studies of C and N isotope values, which generally have also found no differences based on age. Exceptions were $\delta^{15}N$ values of breastfeeding infants (Katzenberg and Pfeiffer 1995) and $\delta^{13}C$ values of young children in a marine-adapted community, who apparently ate less fish (Nelson et al. 1986).

The following two graphs display the distribution of individual isotope ratios by age. The slopes (*b*) of both lines are very low (0.02), and the probabilities do not support isotopic difference based on age. However, the correlation coefficient for $\delta^{15}N$ (0.46) does indicate a moderate correlation. This could be resolved with a much larger sample size.

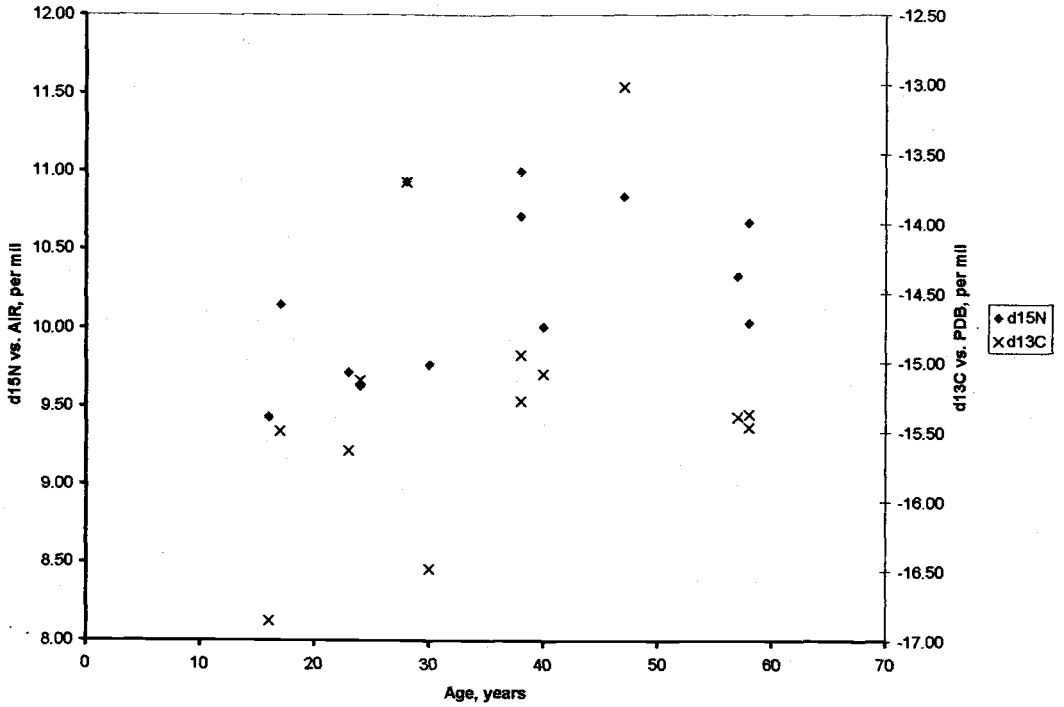


Figure 4.11. Isotope Values by Age (Lower Brule)

COMPARISONS OF POPULATION GROUPS' ISOTOPE VALUES

The hair samples I received from the museum collections came from three early reservation populations (one Sioux and two Blackfoot) and one mixed pre-reservation group (with Sioux, Blackfoot, and "Plains"). These samples represent different cultures and times, but in similar environments, with access to similar foods (animals as well as plants). For further context, I included several modern hairs and a Great Plains bison hair roughly contemporaneous with the pre-reservation group.

Distribution of Data Within Samples

The distributions of the nitrogen and carbon isotope data for the pre-reservation Plains, the Lower Brule, and the Blackfoot samples are shown in the following three figures. (The two Blackfoot samples are combined because of the small

sample sizes and the similarity of the results.) Most of the distributions do not appear normal, but the small sample sizes make determining normality difficult.

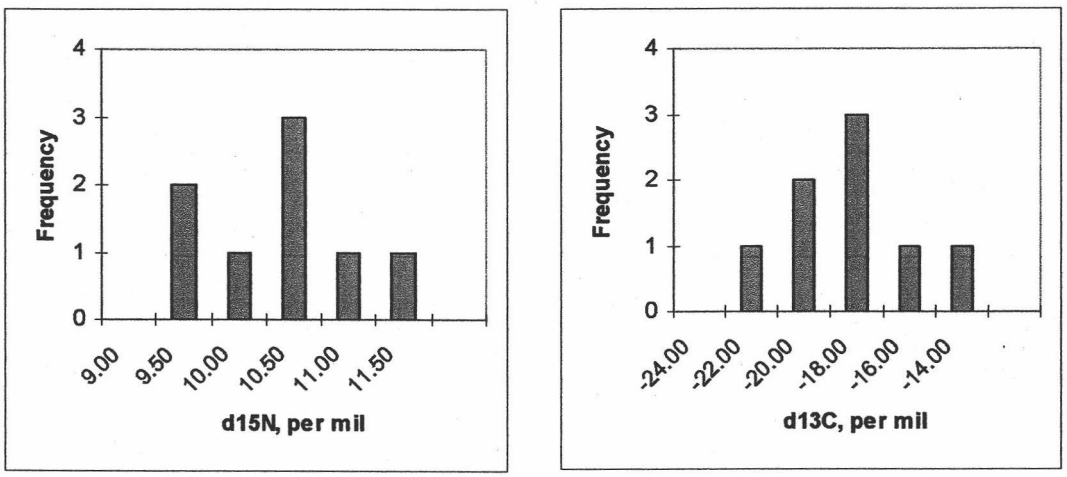


Figure 4.12. Distribution of Isotope Values in Pre-Reservation Sample

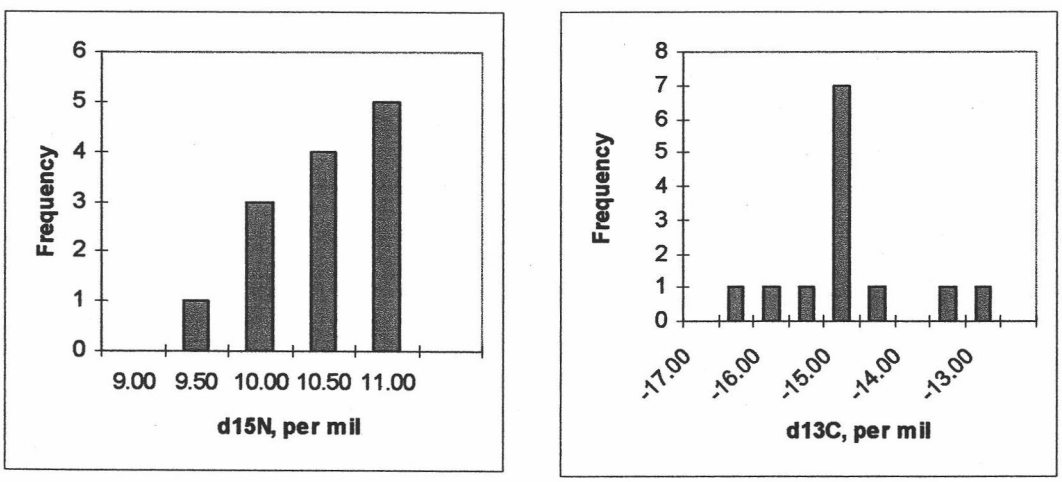


Figure 4.13. Distribution of Isotope Values in Lower Brule Sample

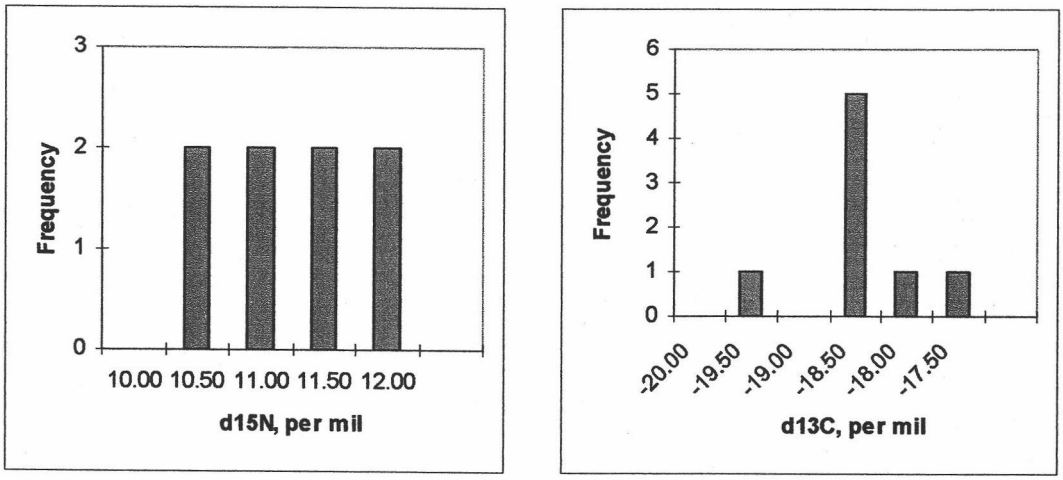


Figure 4.14. Distribution of Isotope Values in Blackfoot Sample

Nitrogen Isotope Data

The nitrogen isotope data by population are summarized below. Only the most proximal 2- or 4-cm hair segments from each strand were compared. The raw data appear in Appendix Table 8.

Table 4.4. $\delta^{15}N$ (vs. AIR, per mil) by Population

Population	*n**	Mean	Median	S.D.
Lower Brule Reservation Sioux, 1892	13	10.25	10.15	0.54
Flathead Reservation Blackfoot, 1892	3	11.01	10.75	0.68
Reservation Blackfoot, Montana, 1930s	5	11.10	11.23	0.48
Combined reservation groups	21	10.56	10.67	0.66
Pre-reservation Plains, 19th C.	8	10.14	10.18	0.70
Modern U.S. urban, 2001	4	8.71	8.92	0.68
Bison, 19th century, Plains	1	6.26		

**n* is the number of individuals.

By looking at the data, one can see that the modern $\delta^{15}N$ values are lower than the historic (Indian) groups, likely due to lower meat consumption. The bison, an herbivore, has a lower $\delta^{15}N$ value than all the humans, who are omnivores.

The differences among the Indian groups are not as great. The *t*-test shows no population difference between the two Blackfoot reservation groups (for $\delta^{15}N$, P=0.82; for $\delta^{13}C$, P=0.20). Because the two Blackfoot groups are similar yet small, I combined them for comparison with the Sioux. I used Student's *t*-tests to make the following comparisons:

- The reservation Blackfoot (both groups) and the reservation Sioux. This is a comparison of different cultures in different places but under similar circumstances.
- The pre-reservation group vs. all reservation groups (two Blackfoot, one Sioux). This is a comparison of cultures across time.

Table 4.5. *t*-Test Comparisons for δ15N Values

t-test pairs	Lower Brule Res. Sioux	FH+MT Res. Blackfoot	All Res. groups	All Pre-Res. Plains
Lower Brule Res. Sioux	--	t=3.441 P=0.003	--	t=-0.385 P=0.705
FH+MT Res. Blackfoot	t=3.441 P=0.003	--	--	t=-3.000 P=0.010
All Res. groups	--	--	--	t=-1.496 P=0.146
All Pre-Res. Plains	t=-0.385 P=0.705	t=-3.000 P=0.010	t=-1.496 P=0.146	--

Note that the small sample sizes make determining statistical significance especially difficult. The tests show that the Blackfoot samples tested had significantly higher $\delta^{15}N$ values than the Lower Brule Sioux, making it likely that the larger populations from which these samples are drawn would also be distinct,

since a *t*-test finds a significant difference between these two samples ($t = 3.44$, $P = 0.003$, 19 df).

The reservation Blackfoot sample also had significantly higher $\delta^{15}N$ values than did the pre-reservation Plains sample. However, since the Plains sample mixed several cultures, this difference may be a cultural one rather than a chronological one.

Carbon Isotope Data

The carbon data by population are summarized below. Only the most proximal 2- or 4-cm hair segments from each strand were compared. The raw data appear in Appendix Table 8.

Table 4.6. $\delta^{13}C$ (vs. PDB, per mil) by Population

Population	*n**	Mean	Median	S.D.
Lower Brule Reservation Sioux, 1892	13	-15.22	-15.37	1.00
Flathead Reservation Blackfoot, 1892	3	-19.00	-18.78	0.65
Reservation Blackfoot, Montana, 1930s	5	-18.49	-18.63	0.35
Combined reservation groups	21	-16.54	-15.63	1.91
Pre-reservation Plains, 19th C.	8	-19.10	-19.03	2.38
Modern U.S. urban, 2001	4	-18.21	-18.08	0.49
Bison, 19th century, Plains	1	-20.17		

**n* is the number of individuals.

Among the historic (Indian) groups, the carbon isotope values vary more from group to group than the nitrogen values do, yet they also diverge less sharply from the modern values than the nitrogen values do. In addition, the variance *within* a population (standard deviation) is higher for the $\delta^{13}C$ values than for the $\delta^{15}N$ values.

Table 4.7. *t*-Test Comparisons for δ13C Values

t-test pairs	Lower Brule Res. Sioux	FH+MT Res. Blackfoot	All Res. groups	All Pre-Res. Plains
Lower Brule Res. Sioux	--	t=-9.063 P=0.000	--	t=-5.239 P=0.000
FH+MT Res. Blackfoot	t=-9.063 P=0.000	--	--	t=-0.489 P=0.632
All Res. groups	--	--	--	*t*=-3.019 *P*=0.005
All Pre-Res. Plains	t=-5.239 P=0.000	t=-0.489 P=0.632	*t*=-3.019 *P*=0.005	--

Note that the small sample sizes make determining statistical significance especially difficult. The *t*-tests show that the Lower Brule Sioux have $\delta^{13}C$ values that are significantly higher (more enriched) than those for the reservation Blackfoot (t=–9.06, p=0.000, df=19), meaning that it is probable that the mean differences apply not just to the group tested, but to the larger cultural groups represented by these samples.

The Lower Brule were probably consuming a diet higher in C4 foods (corn or corn-fed meat) than the other groups, including the modern group. The low value of the modern group is surprising, since the modern North American diet is comparatively C4-rich due to our high consumption of corn, corn-fed livestock and poultry, and foods processed with corn products.

The reservation Lower Brule Sioux sample also had significantly higher $\delta^{13}C$ values than did the pre-reservation Plains sample. However, since the Plains sample mixed several cultures, this difference may be a cultural one rather than a chronological one.

The bison's low (–20.17‰) $\delta^{13}C$ value is consistent with a C3-grass-grazing herbivore. Tieszen (1991) reported modern bison feces from mid- to northern latitudes (South Dakota) having $\delta^{13}C$ values around –22 to –24‰. Bison from more southern latitudes, on the other hand, yield higher (more enriched) $\delta^{13}C$ values

reflecting a greater abundance of C4 grasses in the hotter, drier climates. Spielmann, et al. (1990) measured $\delta^{13}C$ values in old bison bone from Pecos Pueblo, New Mexico, and found the bison values to be as high as maize: –9.5‰. Clearly, bison values vary greatly with location, and the bison hair that I tested is from a more northern latitude.

Comparison of Groups Across Time

Does the isotopic composition of hair (and therefore diet) from people of the same cultural group vary across time? Determining that a change in diet has occurred over time in the same cultural group would provide an indication of significant social or physical change. Hypothesis: I would expect to see measurable isotopic differences resulting from dietary change when a population experiences a change in physical environment or a disruption or modification of societal structure or practice. The time period in question here spans the nineteenth century, a time of great social upheaval and physical dislocation for the Plains Indians.

I tested this hypothesis by comparing the hair isotope values of the reservation populations to the pre-reservation group. The reservation Blackfoot sample had significantly higher $\delta^{15}N$ values than did the pre-reservation Plains sample, while the Lower Brule Sioux sample had significantly higher $\delta^{13}C$ values than did the pre-reservation Plains sample. However, since the Plains sample mixed several cultures, these differences may be cultural rather than chronological.

Another source of variation for the pre-reservation group lies in the mode of hair collection. Whereas each reservation sample was collected at the same time and place within the same culture group, the pre-reservation samples came from different times and places. In addition, the pre-reservation group is internally highly variable, perhaps owing to a greater seasonality in diet among hunter-gatherers. This variability within the group makes it harder to show a significant difference between groups.

Comparison of Contemporaneous Cultural Groups

Does the isotopic composition of hair vary between different contemporaneous cultural groups occupying similar environments? Will the different dietary practices of two different but similar cultures produce a measurable difference in isotope composition? Hypothesis: different cultural groups that have different dietary practices will produce distinctive "profiles" of carbon and nitrogen isotope compositions of their hair. I tested this by comparing two cohesive cultural groups, the Lower Brule reservation Sioux of 1892 with the reservation Blackfoot of 1892-1935. I know something about the dietary traditions of these groups, as well as some of their food sources at the time.

This test is central to this study. While the other tests described so far establish the viability and reliability of isotope testing of hair, this hypothesis would establish whether isotope testing is useful and meaningful: can it distinguish dietary practices between groups, and can it distinguish different cultural groups based on their dietary practices? Do individuals form cohesive groups, isotopically speaking? I will develop population isotope profiles by plotting $\delta^{15}N$ vs. $\delta^{13}C$ for all individuals.

Population Profiles

By plotting $\delta^{13}C$ versus $\delta^{15}N$ for all individuals, we can create a carbon-nitrogen profile for each population. Figure 4.15 shows at a glance that the Lower Brule Sioux, the Blackfoot, and the modern Americans form distinctive isotope/dietary profiles. The combined pre-reservation group, on the other hand, does not form a characteristic picture, although it does include the three lowest human $\delta^{13}C$ values.

The modern urban subject with the lowest $\delta^{15}N$ and lowest $\delta^{13}C$ values was one of the two ovo-lacto-vegetarians. Ovo-lacto-vegetarians (as opposed to the vegans, who consume no form of animal protein) do not generally produce isotope profiles

that are different from those of omnivores (O'Connell and Hedges 1999). Perhaps this ovo-lacto-vegetarian consumed less of the animal proteins than the other vegetarian (as well as the omnivores) did.

Note that the theoretical trophic shift is 0-1‰ for $\delta^{13}C$ and 3-4‰ for $\delta^{15}N$. This puts the isotope profile for the theoretical, combined food sources at a point about 1‰ to the left and 4‰ below each population's profile.

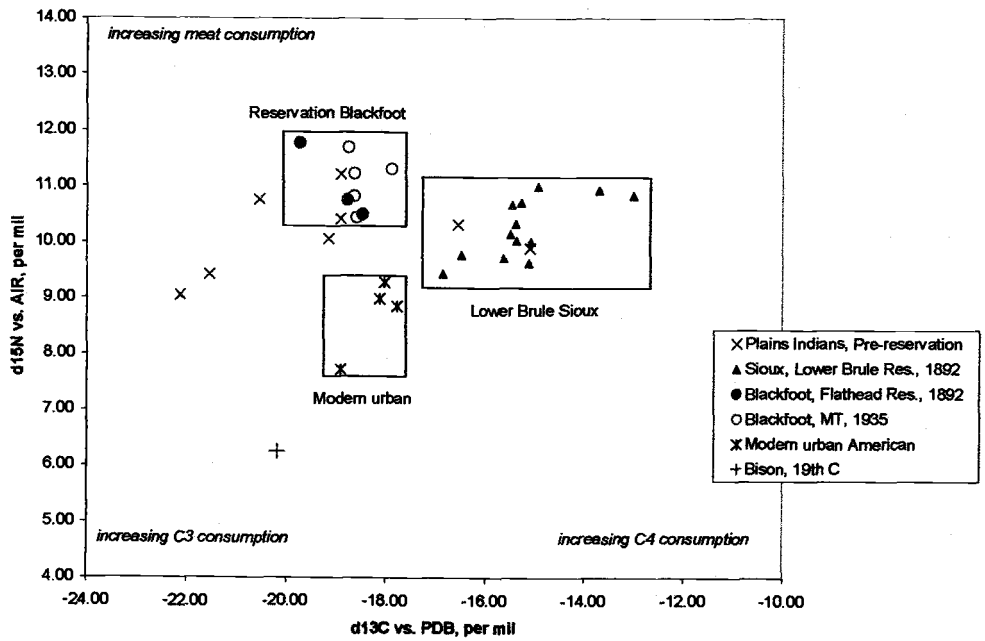


Figure 4.15. Population Profiles for $\delta^{15}N$ and $\delta^{13}C$

Correlating Nitrogen and Carbon Isotope Ratios

Figure 4.15, above, integrates both nitrogen and carbon data into a profile that is more informative than one isotope alone. Ambrose (1993) suggests further that correlating two isotopes can provide additional insight into diet. I looked for correlations between $\delta^{15}N$ and $\delta^{13}C$ values in the Lower Brule Sioux sample, the

combined Blackfoot sample, the pre-reservation sample, and a combined reservation sample (Lower Brule plus Blackfoot).

Only one sample showed a correlation between $\delta^{15}N$ and $\delta^{13}C$ values that was greater than what would be expected by chance: the Lower Brule sample (r=0.707, p=0.007). In other words, among the Lower Brule, it appears that as one isotope ratio became more enriched, so did the other. Why would this be true for only the one population sample? This might be related to the Lower Brule having the highest (most enriched) $\delta^{13}C$ values. If the meat eaten by the Lower Brule was corn-fed to a much greater extent than the meat consumed by the other Indian groups, then the Lower Brule $\delta^{13}C$ values would increase with their δ15N values (since corn has a high $\delta^{13}C$ value and meat has a high $\delta^{15}N$ value).

CHAPTER 5
DISCUSSION

MINIMUM HAIR WEIGHT FOR TESTING

Tests of different weights of both human hair and acetanilide standard indicate that material containing as little as 14 μg of nitrogen and 43 μg of carbon can be reliably analyzed for stable isotope ratios. This translates to about 100 μg of hair, which is a segment of scalp hair about 2 cm long (although hair varies in its diameter from person to person).

VARIATION

In order to decide if a variation in isotope ratios is meaningful in terms of diet (that is, in representing a real difference in food intake), we must first account for sources of variation that are *not* meaningful. The instrumentation provides one such source of variation, as discussed in chapter 4 under "Precision and Accuracy". The physiology of living systems provides another source of variability, both within an individual and between individuals. It was reported by DeNiro and Epstein (1978) that the isotope compositions of hair were more variable than those of collagen. This is because bone represents one large pool of interacting material, whereas any segment of hair represents a snapshot in time. This produces a variability along the strand length reflecting truly different values at different times, not merely imprecision in replicate measurement. Is the variability of $\delta^{15}N$ and $\delta^{13}C$ within a single strand (that is, different segments from the same strand) greater than analytical precision?

The table below compares the variability along a hair strand to the precision of the instrumentation (as established by the variability in the measurement of the standards, especially acetanilide). One modern hair shows no significant variation in either isotope, while one modern hair, one pre-reservation Sioux hair, and one

Lower Brule Sioux hair show significant variation in one isotope. The hair from one pre-reservation Sioux, one Lower Brule reservation Sioux (except one $\delta^{15}N$ value), and the bison show variability of both isotope values above that of the "background noise" (precision). Hence, strands that show isotope variability greater than instrument variability are truly showing a change, possibly seasonal, in isotope composition over time.

Table 5.1. Using Standard Deviations to Compare Isotope Variability in a Hair to Isotope Variability in Standards

Sample	*n**	$\delta^{15}N$ S.D.	$\delta^{13}C$ S.D.
Hairs			
Pre-Res. Sioux #1	12	0.49	0.76
Pre-Res. Sioux #2	11	0.29	0.22
LB Res. Sioux #1	12	0.23	0.39
LB Res. Sioux #2	12	0.35	1.05
Modern #1	12	0.27	0.27
Modern #2	10	0.21	0.23
Bison	6	0.48	0.64
Standards			
Acetanilide (4-51 µg N, 29-351 µg C)	18	0.27	0.22
Acetanilide (11-51 µg N)	12	0.22	
Acetanilide (40-351 µg C)	17		0.21
NIST 8548 (36-87 µg N)	10	0.17	
NIST 8541 (36-249 µg C)	7		0.10

*The number of segments tested in a single strand of hair, or the number of samples of a standard tested.

What is a Meaningful Variation in Isotope Ratios?

Statistical tests offer a measure of statistical meaningfulness, but we still must show that variations we see in isotope values have a *biological* meaning. That is, do the

differences between individuals or between groups transcend a *background* level of biological variability? There are several ways to consider this question.

- For a group of laboratory animals fed a controlled, homogenous diet, what level of variability occurs in its $\delta^{15}N$ and $\delta^{13}C$ values?
- For a traditional human population subsisting on the same diet, what level of variability occurs in its $\delta^{15}N$ and $\delta^{13}C$ values? Is the variability between individuals greater than the variability within an individual?
- Is any variability attributable to the metabolic differences of sex, age, and/or health status?

Nelson et al. (1986) realized that the need to understand the sources of variability underlay the ability to draw dietary conclusions from isotope values. They studied bone $\delta^{13}C$ values from two species of wild herbivores and 13 prehistoric Canadian human sites. Within all groups, they found $\delta^{13}C$ values that varied by no more than 0.5‰ about the mean. Consequently, they concluded that one can infer dietary differences if $\delta^{13}C$ values differ by >>0.5‰. These baseline figures are for bone, which will vary less than hair due to the integration of values over time.

Lovell et al. (1986) specifically addressed the question of ascertaining what level of $\delta^{13}C$ variation is attributable to inherent physiological differences (including sex and age), as opposed to a dietary difference. In a study of 50 prehistoric Canadian bison hunters of both sexes and a wide range of ages, they found a "surprisingly small variation" in the bone collagen values for $\delta^{13}C$; specifically, a standard deviation of 0.3‰. They concluded that, "Variations larger than this observed in other populations must therefore be due to real dietary differences." Note that this level of variation is larger than our level of instrument precision.

Other studies of traditional, subsistence populations have found higher variations (standard deviations) in isotope ratios, but none lower. So a variation of over 0.5‰ for $\delta^{13}C$ appears to be a good benchmark. Values for $\delta^{15}N$ have not been studied as

much. In some studies, $\delta^{15}N$ values have varied more than $\delta^{13}C$ values in the same population, with standard deviations ranging from 0.5‰ to 1.3‰ (Yoshinaga et al. 1996; Spielmann et al. 1990; Habicht-Mauche et al., 1994; Richards et al. 1998).

Variability Within Populations

The variability in isotope ratios within a hair strand appears no greater than the variability manifested between individuals of the same population, as measured by standard deviations. Therefore, the isotopic variability *within* the same individual is no greater than the variability *between* individuals.

The four different museum populations of hair do not show the same degree of variation in their isotope ratios. Not surprisingly, the greatest variation appears in the most loosely defined group, the pre-reservation Plains.

Table 5.2. Variability of Isotope Ratios Within Populations

Population	*n**	$\delta^{15}N$ S.D.	$\delta^{15}N$ Range	$\delta^{13}C$ S.D.	$\delta^{13}C$ Range
Lower Brule Reservation Sioux, 1892	13	0.54	1.57	1.00	3.84
Flathead Reservation Blackfoot, 1892	3	0.68	1.28	0.65	0.31
Reservation Blackfoot, Montana, 1930s	5	0.48	1.26	0.35	0.86
Pre-reservation Plains, 19th century	8	0.70	2.17	2.38	7.04
Modern U.S. urban, 2001	4	0.68	1.56	0.49	1.14

**n* is the number of individuals.

To conclude that individuals *within a group* were eating differently, the standard deviation would need to exceed the standard deviations for values *within individuals'* strands of hair.

Comparing the Variabilities of δN and δC Values

Within their groups, some population groups had greater variability in $\delta^{15}N$ values, while others had greater variability of $\delta^{13}C$ values. However, when comparing the population groups to each other, the carbon isotope ratios differ much more than the nitrogen values do (Table 5.2).

The comparative variability of $\delta^{15}N$ and $\delta^{13}C$ depends on the population. Many studies of prior and current populations around the world have found $\delta^{15}N$ values that vary more than $\delta^{13}C$ values (Spielmann et al. 1990; Richards and Hedges 1998; Habicht-Mauche et al. 1994). Some studies have found similar variabilities of nitrogen and carbon within a population (Minagawa 1992). Others have found carbon's variability exceeding nitrogen's (Yoshinaga et al. 1996; O'Connell and Hedges 1999).

CHRONOLOGICAL CHANGE IN ISOTOPE COMPOSITION

Hair is almost continuously synthesized, aside from periods of quiescence, yet static once it leaves the scalp. Hence a hair strand, unlike the continuously remodeled bone, provides a chronological record of protein synthesis and the raw materials (diet) used in that synthesis. There is presumably a lag time between the intake of nutrients and their effect (or "signature") on end-products such as hair. This lag time is an issue only if one wants to establish absolute time, such as the season of death of a mummy. For my purposes, it is enough to see hair isotope values (and, therefore, diet) change with relative time, without being able to tie those changes to specific times.

Figures 5.1 and 5.2 illustrate the ranges of variations in isotope ratios in individual hairs over the course of 1 to 2 years. The variation is different from individual to individual.

- The modern urban strands exhibit no meaningful isotope change over time, since their variabilities do not exceed the analytical uncertainty (see Table 5.1).
- In all four of the historic individuals, there are isotope variabilities that can represent a change in diet, since they exceed the analytical uncertainty (see Table 5.1).
- In general, $\delta^{13}C$ values fluctuate more than $\delta^{15}N$ values do, indicating that the dietary plant sources changed more with the season or time than did the amount (or kind) of meat consumed.
- The variation manifested in the bison's isotope signature could reflect the effect of seasonal water stress on 1) the growth of C4 grasses, and 2) the fractionation of nitrogen isotopes (Ambrose 1991).

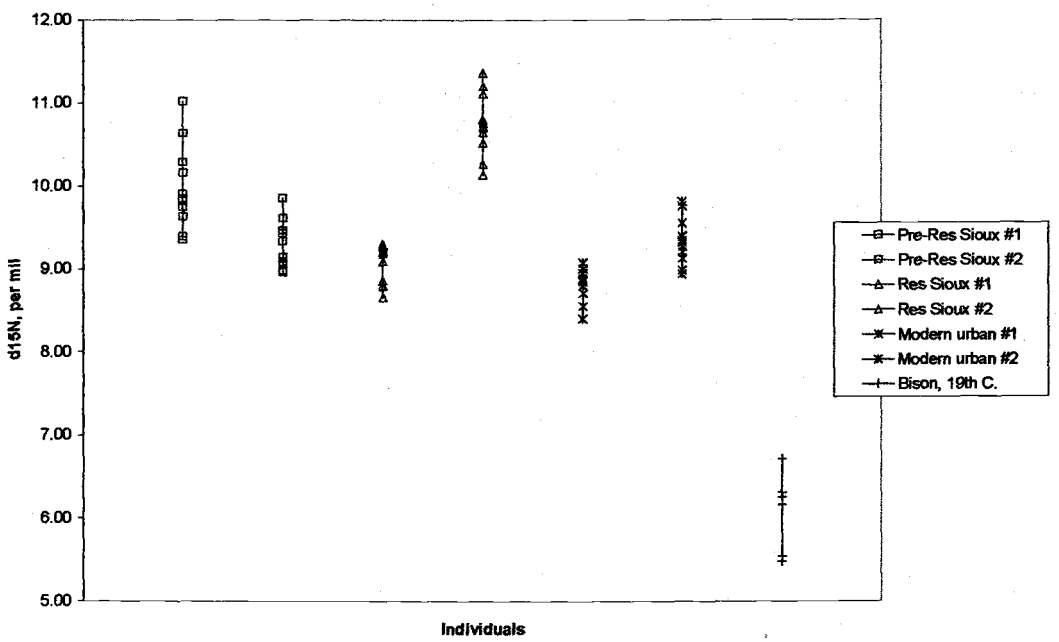


Figure 5.1. Range of $\delta^{15}N$ Values Along a Strand

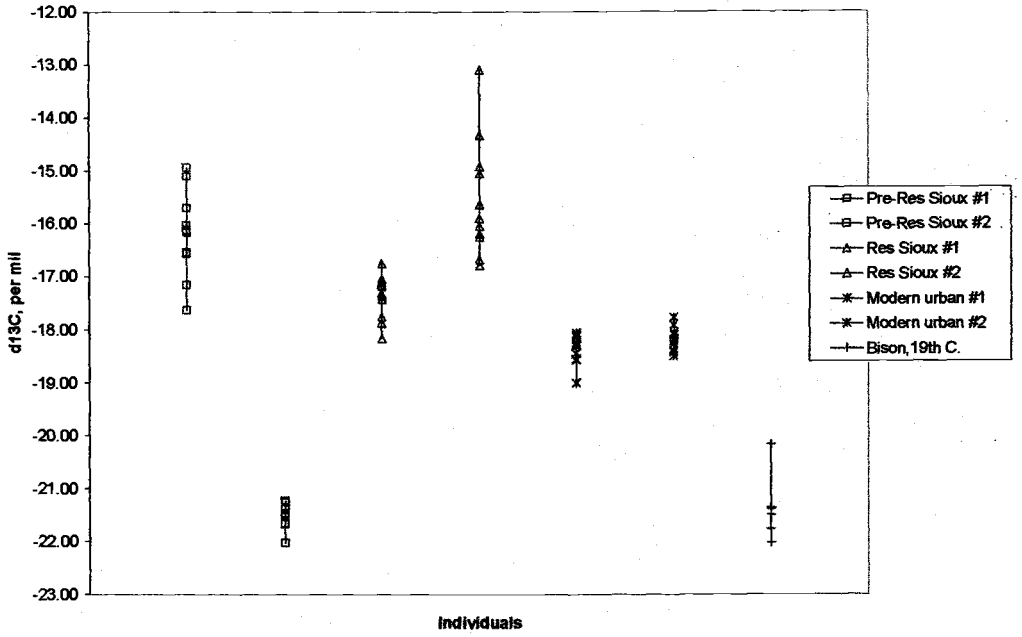


Figure 5.2. Range of $\delta^{13}C$ Values Along a Strand

Although more work with more specimens needs to be done in this area, this technique represents a valuable new method to assess seasonality of diet in past cultures. Dietary seasonality is an important adaptation to the environment, and entails both technological and social accommodations.

EFFECTS OF SEX AND AGE ON ISOTOPE COMPOSITION

There was no demonstrated effect of sex on nitrogen or carbon isotope ratios among the Lower Brule Sioux (hairs collected 1892). This supports the conclusions of other researchers that there is no gender-based physiological difference in the way nitrogen and carbon isotopes are metabolized (Lovell et al. 1986; Minagawa 1992). Consequently, if one were to find a significant difference in isotope ratios by sex, it would in fact reflect a dietary difference. As in most other studies, however, there was no such difference here.

There was also no demonstrated effect of age on nitrogen and carbon isotope ratios among the Lower Brule Sioux, which is again consistent with the research of others, such as Lovell et al. (1986) with ancient bone collagen and Minagawa (1992) with modern hair. However, my sample size was rather small (n=13), so it would bear investigating whether the modest trend toward a positive correlation between age and $\delta^{15}N$ shown in Figure 4.11 (r=0.45, P=0.12) would strengthen or weaken given a larger sample size. Such a trend in this population could be explained if older individuals claimed or obtained more meat from rations, purchase, or hunting.

PROFILING CULTURES BY ISOTOPE COMPOSITION

The Plains Indians groups whose hair I studied were chosen for their similar environments, similar yet distinct cultures, and different time periods. Yet isotopic compositions of hair did vary with culture and diet, with the strength of association dependent on the homogeneity of the cultural group. Therefore, Blackfoot reservation groups from two different periods formed a cohesive group in the dietary isotope profile, quite distinct from their Lower Brule Sioux reservation cohorts across the Plains. The pre-reservation group was too heterogeneous to provide a cohesive profile, however. Its mean values (± one standard deviation) overlapped both the Blackfoot (both $\delta^{13}C$ and $\delta^{15}N$) and the Lower Brule Sioux ($\delta^{15}N$ only), as shown in Figure 5.3, below.

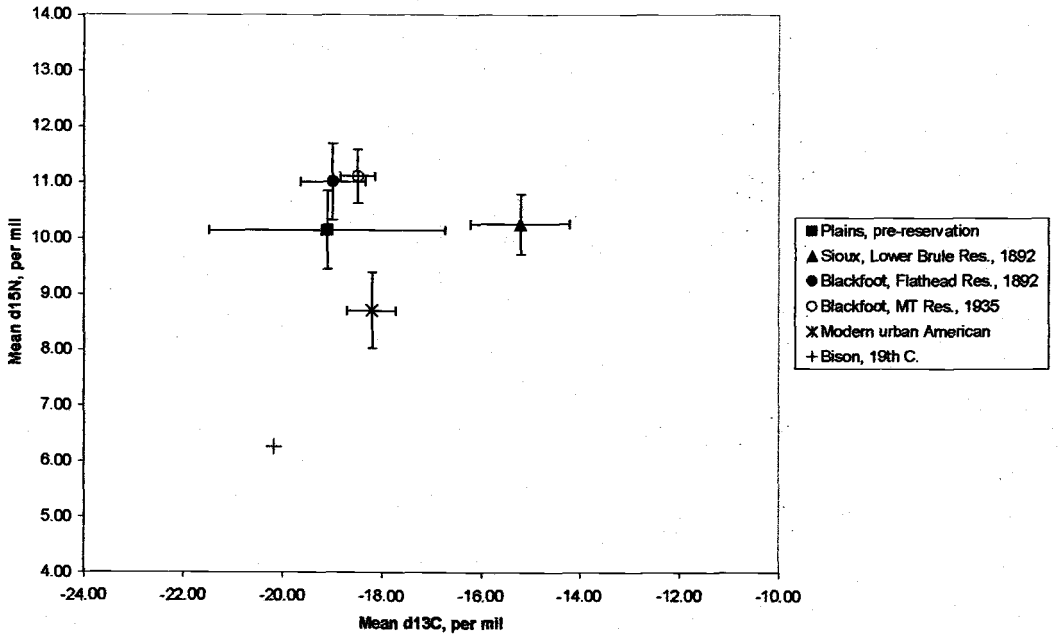


Figure 5.3. Dietary Isotope Profiles

Implications for Dietary Differences

For the purposes of this study, and the different isotopic profiles I found for the Blackfoot and the Lower Brule Teton Sioux, it is germane to explore what their dietary differences could be. As discussed in chapter 2, Background and Theory, two traditional differences in diet were the Blackfoot use of camas (a C3 plant food) and the Teton Sioux consumption of corn (C4), beans (C3), and squash (C3). Corn is the only important C4-type protein source in this area, and carbon isotope testing discriminates C4 and C3 proteins. Nitrogen isotope testing can discriminate beans from other plant sources, since legumes have lower $\delta^{15}N$ values than other plants do.

Otherwise, the diets of these two populations were similar. They ate the same kinds of prairie parsnip and berries, as well as very high quantities of meat. Animal protein produces very enriched (high) $\delta^{15}N$ values.

The Indian populations on the reservations did not have access to the same foods they had eaten before their relocations. They received government rations in some form until the early 1920s, and supplemented this to varying degrees with hunting and gathering local resources and with farming and ranching. Therefore, one would expect noticeable changes in diet before and after the establishment of reservations (1855 for the Blackfoot, 1876 for the Teton Sioux). While the Indians clearly experienced increased poverty, disease, and mortality once they were restricted to reservations, the nitrogen and carbon isotope signals in my samples do not significantly change between the pre-reservation and the reservation samples. There may be several reasons for this:

- The make-up of my pre-reservation sample is very heterogeneous and poorly documented. To isolate the effect of dietary change with time, other variables, such as culture, must be held constant. Yet my pre-reservation sample consisted of hairs from individuals from different Plains cultures at different times and places. Apparently, just being Plains Indians did not give them enough dietary commonality to provide a comparison to the Blackfoot or Lower Brule Sioux cultures.
- The great heterogeneity (based on high standard deviations of isotope values) of the pre-reservation group could also be due to a much greater variability in pre-reservation times in each individual's diet over time and season.
- Both the Blackfoot and the Teton Sioux peoples continued their high-meat diet on the reservation. The meat source changed from bison to beef (and, to a lesser extent, pork), but it remained high. The prescribed government meat rations were liberal, especially for the Teton Sioux. In addition, the Blackfoot were moderately successful in taking up ranching (Dempsey 2001).
- The Teton Sioux's traditionally higher consumption of corn and beans could have continued into the reservation period. Government rations for the Teton in

1869 and 1876 included corn and beans (as well as beef, pork, flour, coffee, and sugar) (Mattison 1995).

How the Nitrogen Profiles Differ

The reservation Blackfoot have a significantly higher $\delta^{15}N$ than do the reservation Lower Brule Sioux, according to Student's *t*-test ($P<0.005$). The difference between the Blackfoot and the Lower Brule Sioux is surprising, given their similar and contemporaneous situations: traditionally high meat-eaters subsisting on high-meat government rations. Broadly speaking, the meat rations look similar. Ewers (1958) cites government rations for the Blackfoot at the end of the 19th century (1880-1900) that included 1½ pounds of meat per person per day; Prince (1995) cites government rations for each Teton Sioux as prescribed by the Treaty of 1876 to include 1½ pounds of beef or ½ pound of bacon per person per day (although what was received was not necessarily what was mandated).

With meat rations so similar, why would the Blackfoot have more enriched $\delta^{15}N$ values? There is no way to know for sure, but these differences are real. There are several considerations:

- Perhaps the Blackfoot got more meat than from their rations by supplemental hunting or cattle ranching. Note that the later reservation Blackfoot group (from the 1930s) had equally high $\delta^{15}N$ values. By this time, government rations would not have played a role (as they ended in the early 1920s), but perhaps cattle ranching did. While both the Blackfoot and the Teton Sioux tried their hands at ranching, and with greater success than farming, the Blackfoot might have done better than the Lower Brule (Dempsey 2001; Schusky 1977).
- Perhaps the Lower Brule received smaller meat rations than the Blackfoot. Prince (1989) found that, while some Teton Sioux reservations continued to receive high beef rations in the late 19th century, the Lower Brule Sioux received much less than others, perhaps due to their smaller reservation.

- If the Lower Brule Sioux consumed more (nitrogen-fixing) beans than the Blackfoot did, this would lower their $\delta^{15}N$ values. Fertilizer use further lowers the $\delta^{15}N$ of food crops.
- Different sources of meat (bison, beef, horse, rabbit) can have different $\delta^{15}N$ values, thereby affecting the consumer's $\delta^{15}N$ (Ambrose 1991; Pate 1998). Cattle driven north from the southern states may have had different $\delta^{15}N$ values than locally raised cattle, due to both climate and feed.

The relatively high meat consumption of all the Indian groups is supported by their enriched (high) $\delta^{15}N$ values, particularly when compared to the four modern urban individuals (Figure 5.3). Although the modern group includes two ovo-lacto-vegetarians, they consume other forms of animal protein besides meat (that is, eggs and dairy products), and this enriches their $\delta^{15}N$ levels as much as meat does. Note also the very low $\delta^{15}N$ value of the bison, a strict herbivore.

How the Carbon Profiles Differ

Again, the biggest difference is between the reservation Blackfoot and reservation Lower Brule Teton Sioux, and not between the pre-reservation and reservation groups (Figure 5.3). The reservation Lower Brule Sioux have a significantly more enriched $\delta^{13}C$ level than do the reservation Blackfoot, according to Student's *t*-test ($p<0.005$). In the context of North America (where corn is the primary C4 plant component of the diet), this tells us that the Lower Brule were consuming either more corn or more corn-fed livestock, or both, than the Blackfoot—and more than some modern, urban Americans, as well. This is despite the fact that modern diets incorporate much hidden corn into the diet from non-protein sources (e.g., corn syrup in processed foods). I conclude that non-protein components of corn do not contribute much to the synthesis of protein (at least of hair keratin) in its consumers, and therefore do not contribute much to the isotope signature of hair.

That the Lower Brule have a higher C4 signal (that is, more enriched $\delta^{13}C$) than the Blackfoot may have to do with the Teton Sioux's traditional inclusion of corn in the diet, unlike the Blackfoot (Hassrick 1975). Corn also appears in the government rations for the Teton Sioux (Mattison 1995). In addition, livestock from the eastern Plains might have been fed more corn than livestock from the western Plains and plateau regions. The $\delta^{13}C$ values of meats influence the consumer's isotope composition as much as the $\delta^{13}C$ values of plant foods do. As documented in chapter 4 under "Correlating Nitrogen and Carbon Isotope Ratios," the $\delta^{13}C$ and $\delta^{15}N$ values of the Lower Brule correlate highly, which could mean that the meat they ate had high $\delta^{13}C$ values. This would result in both the $\delta^{15}N$ and the $\delta^{13}C$ values increasing with increasing meat consumption.

Note also that the bison hair had a very low $\delta^{13}C$ value. Apparently, the bison grazed on more C3 than C4 grasses and sedges, probably at a northerly latitude.

CHAPTER 6
CONCLUSION

This study supports the conclusion that stable-isotope analysis for carbon and nitrogen can be reliably employed to analyze hair fragments as small as 100-150 μg (about 2 cm) for dietary information. This technique, therefore, is suitable for consideration in archeological excavations, which are more likely to find hair fragments than whole strands (if hair is searched for at all).

For the quantities of nitrogen and carbon present in the hair specimens, the analytical precision (uncertainty) of the mass spectrometer was 0.22‰ for nitrogen isotope ratios and 0.21‰ for carbon isotope ratios. This was good (low) enough to allow the discrimination of real isotopic differences between experimental samples. That is, the variation in isotope values between individuals, between populations, and even between different segments on the same hair strand exceeded the instrument-caused variation (background) from specimen to specimen.

This study establishes the ability to isotopically analyze hair segments as small as 2 cm. It is the first study to analyze a single strand of hair for information about an individual's chronological variability of diet, and it was able to assess chronological variability for a longer hair length (and, therefore, covering a longer time period) and for more isotopes than previous studies. (White [1992] analyzed mummy hair up to 8 cm long for carbon isotope values only, and did so by cutting 15 strands into 2-cm segments.) While this test did show chronological variability within some individuals that exceeded the level of background variation, it is not clear that these changes were seasonal (cyclical). This is a phenomenon that should be further explored using more strands and strands from different cultures.

Analyses of hair isotope composition by sex and age showed no significant differences, which allowed me to consider all hairs from one population as "equals"

when making comparisons to other populations or comparisons over time. This also means that, among the Lower Brule Sioux, men and women and younger and older adults ate similar diets. This is consistent with most other isotope studies that have considered differences based on sex or age of adults.

This study also showed that different cultures—even similar ones—can produce distinctive isotopic profiles (by plotting $\delta^{15}N$ vs. $\delta^{13}C$) that encompass all individuals from the same population yet separate individuals from different populations. Since diet directly affects isotopic composition, one can deduce that the distinctive isotope profiles are based on diet.

This study was not able to show that the isotope profile for the same culture changed over a time of known cultural change (during the transition to reservation life). However, I did not have appropriate samples to test (that is, samples from the same cultural group separated by time), and tried to approximate a chronological test by using poorly documented hair from the same general area.

This study compared individuals and populations, but did not attempt to reconstruct diets, which would be difficult with a complex diet, and would have required contemporaneous food materials for testing. Stable isotope analysis cannot pinpoint specific food sources, only broad classes of food—and only if their isotope ratios do not overlap. By comparing the isotope values from the Lower Brule reservation Sioux, the reservation Blackfoot, and four modern Americans, I can deduce that these Native American groups ate diets heavy in animal protein, that the Blackfoot ate more meat than the Lower Brule, and that the Lower Brule ate more corn and/or corn-fed meat than the Blackfoot. The differences of isotope profiles, and hence diet, appeared despite similarities of environment (Great Plains) and circumstance (reservation life). Apparently, cultural differences produced distinctive dietary patterns, despite certain cultural similarities (such as a preference for meat). The limited ethnographic information on the diets of these populations neither supports

nor contradicts such differences. Consequently, the isotope data provide a new source of information about diet.

The variability and range of isotope ratios within an individual bear closer scrutiny. Analyzing even longer strands of hair (longer than 24 cm) cut into 2-cm segments (each segment representing about 2 months' growth) could better document seasonal dietary changes. There is also a need to extensively test the potential variability in an individual, such as by testing numerous hair specimens from all over the head and all along long strands. This could help define the bounds of variability, especially in a person with a seasonal diet. Not only can isotope compositions vary along the length of a strand, but not all hairs represent the same time period, even when the root is included. Not all follicles produce growth at the same time, since about 11% of them at any given time are in a quiescent phase (O'Connell and Hedges 1999).

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APPENDIX

APPENDIX

Table 1. Raw Data from Run E011029, 29 Oct 2001

Population	ID	Wt., mg	δ15N	Wt%N	δ13C	Wt%C	AtomicC:N
Standard	Acetanilide	0.3012	-1.38	11.09	-30.25	71.90	
Standard	Acetanilide	0.3780	-1.04	10.51	-30.36	70.29	
FHBlackfoot	BF24	0.2522	11.79	14.67	-19.65	43.32	3.44
FHBlackfoot	BF24A	0.2184	11.76	14.48	-19.81	42.50	3.42
FHBlackfoot	BF57	0.2786	10.77	14.53	-18.59	44.49	3.57
FHBlackfoot	BF57A	0.2105	10.22	14.36	-18.36	42.58	3.46
FHBlackfoot	BF59	0.2217	10.68	14.70	-18.89	44.38	3.52
FHBlackfoot	BF59A	0.2141	10.83	14.81	-18.67	43.70	3.44
LBSioux	LB243.1	0.2066	11.04	14.28	-14.85	42.71	3.49
LBSioux	LB243.1A	0.2297	10.96	14.46	-15.04	43.55	3.51
LBSioux	LB262.1	0.2407	9.37	14.32	-16.80	44.31	3.61
LBSioux	LB262.1A	0.2434	9.49	14.37	-16.92	44.06	3.58
LBSioux	LB262.2	0.2194	9.22	14.08	-17.16	43.63	3.62
LBSioux	LB262.2A	0.2596	8.99	13.97	-17.07	43.92	3.67
LBSioux	LB262.3	0.2045	8.62	13.88	-17.71	43.74	3.68
LBSioux	LB262.3A	0.2397	8.87	13.83	-17.70	43.65	3.68
LBSioux	LB262.4	0.2558	9.46	13.93	-16.95	44.14	3.70
LBSioux	LB262.4A	0.2653	9.46	14.05	-17.05	44.44	3.69
LBSioux	LB262.5	0.2051	9.48	13.92	-16.58	44.42	3.72
LBSioux	LB262.5A	0.2113	9.36	13.96	-16.70	43.59	3.64
LBSioux	LB262.6	0.2122	9.72	13.59	-16.62	43.69	3.75
LBSioux	LB262.6A	0.2003	9.48	13.61	-16.73	43.39	3.72
LBSioux	LB262.7	0.2392	9.33	13.65	-16.69	43.57	3.72
LBSioux	LB262.7A	0.2384	9.20	13.80	-16.62	43.81	3.70
LBSioux	LB262.8	0.2038	9.18	13.97	-15.98	43.58	3.64
LBSioux	LB262.8A	0.2533	9.42	14.04	-16.09	43.64	3.63
LBSioux	LB262.9	0.2112	9.37	13.63	-16.41	43.48	3.72
LBSioux	LB262.9A	0.2566	9.37	13.72	-16.62	43.88	3.73
LBSioux	LB262.10	0.2234	9.47	13.75	-17.84	43.78	3.72
LBSioux	LB262.10A	0.2235	9.67	13.72	-17.30	43.66	3.71
LBSioux	LB264.1	0.2135	10.12	13.93	-14.76	43.80	3.67
LBSioux	LB264.1A	0.2465	9.90	13.93	-15.40	44.00	3.68
LBSioux	LB265.1	0.2115	10.79	14.21	-15.13	43.26	3.55
LBSioux	LB265.1A	0.2259	10.64	14.03	-15.43	43.34	3.60
LBSioux	LB288.1	0.2174	10.14	14.13	-15.60	43.60	3.60
LBSioux	LB288.1A	0.2412	10.52	14.23	-15.18	43.77	3.59
Standard	Acetanilide	0.4471	-0.62	9.67	-30.50	67.01	
Standard	Acetanilide	0.3674	-0.61	10.17	-30.31	70.53	
Standard	NIST 8548	0.2951	20.64	19.83			
Standard	NIST 8548	0.1643	20.04	21.08			
Standard	NIST 8541	0.1447			-15.83	101.32	
Standard	NIST 8541	0.2461			-15.84	101.34	

Table 2. Raw Data from Run E011025, 25 Oct 2001

Pop'n	ID	Wt., mg	δ15N	Wt%N	δ13C	Wt%C	AtomicC:N
Standard	Acetanilide	0.4873	-0.96	10.50	-30.26	72.12	
Standard	Acetanilide	0.2991	-1.03	10.39	-30.29	71.08	
LBSioux	LB289.1	0.2174	10.07	15.42	-15.53	47.67	3.61
LBSioux	LB289.1A	0.2127	10.00	14.51	-15.22	43.86	3.53
LBSioux	LB289.3	0.2174	9.71	13.95	-15.06	44.04	3.68
LBSioux	LB289.3A	0.1833	10.18	14.11	-15.28	43.71	3.61
LBSioux	LB289.4A	0.1971	9.85	14.08	-14.98	44.43	3.68
LBSioux	LB289.5	0.2077	9.56	13.87	-14.35	44.27	3.72
LBSioux	LB289.5A	0.2097	9.50	13.88	-14.74	43.76	3.68
LBSioux	LB296.1	0.2473	10.18	14.40	-15.56	45.31	3.67
LBSioux	LB296.1A	0.2239	10.12	14.54	-15.42	45.26	3.63
LBSioux	LB297.1	0.2733	9.86	14.53	-15.79	45.11	3.62
LBSioux	LB297.1A	0.2392	9.57	13.89	-15.47	42.81	3.59
LBSioux	LB297.2	0.2470	9.74	14.18			
LBSioux	LB297.2A	0.2092	9.90	14.55	-15.45	45.35	3.64
LBSioux	LB297.3	0.2300	10.09	14.29	-15.39	45.23	3.69
LBSioux	LB297.3A	0.2023	9.72	14.97	-14.65	46.53	3.63
LBSioux	LB297.4	0.2403	9.50	14.14	-14.95	45.09	3.72
LBSioux	LB297.4A	0.2344	9.11	14.33	-13.82	44.70	3.64
LBSioux	LB297.5	0.2167	9.51	14.11	-14.76	44.77	3.70
LBSioux	LB297.5A	0.2029	9.10	14.04	-13.84	44.67	3.71
LBSioux	LB297.6A	0.2042	9.21	13.42	-15.00	42.65	3.71
LBSioux	LB299.1	0.2063	9.81	14.23	-16.57	45.63	3.74
LBSioux	LB299.1A	0.2481	9.72	14.37	-16.40	44.93	3.65
LBSioux	LB301.1	0.2105	10.67	14.44	-15.47	44.58	3.60
LBSioux	LB326.1	0.2224	10.67	14.27	-13.02	44.52	3.64
LBSioux	LB326.1A	0.2288	11.01	14.19	-13.01	44.64	3.67
LBSioux	LB326.2	0.1952	10.49	14.31	-14.86	44.57	3.63
LBSioux	LB326.2A	0.2388	11.38	14.33	-15.75	49.94	4.07
LBSioux	LB326.3	0.2304	10.44	14.35	-16.05	45.00	3.66
LBSioux	LB326.3A	0.2620	10.51	14.44	-15.12	45.08	3.64
LBSioux	LB326.4A	0.2464	10.93	14.40			
LBSioux	LB326.5	0.1938	11.05	14.33	-16.71	44.79	3.65
LBSioux	LB326.5A	0.2117	10.72	14.25	-15.93	44.91	3.68
LBSioux	LB326.6	0.2000	10.23	14.42	-15.21	44.81	3.63
LBSioux	LB326.6A	0.1969	10.12	14.41	-15.37	44.88	3.63
LBSioux	LB326.7	0.2208	10.30	14.40	-15.09	45.41	3.68
LBSioux	LB342.1	0.2092	9.71	15.85	-15.32	49.12	3.61
LBSioux	LB342.1A	0.2064	9.55	14.42	-14.94	44.97	3.64
LBSioux	LB402.1	0.2449	10.82	14.37	-13.46	44.58	3.62
LBSioux	LB402.1A	0.2488	11.05	14.48	-13.94	44.65	3.60
Standard	Acetanilide	0.2780	-0.99	10.22	-30.44	70.06	
Standard	NIST 8548	0.4151	20.56	20.93			
Standard	NIST 8548	0.1627	20.28	22.02			
Standard	NIST 8541	0.1236			-15.93	108.54	
Standard	NIST 8541	0.2124			-15.91	101.35	

Table 3. Raw Data from Run E020204, 04 Feb. 2002

Pop'n	ID	Wt., mg	δ15N	Wt%N	δ13C	Wt%C	AtomicC:N
Standard	Acetanilide	0.0591	-1.05	12.44	-29.62	81.89	
Standard	Acetanilide	0.1030	-1.00	10.31	-30.21	70.89	
Standard	Acetanilide	0.0468	-1.25	8.94	-29.89	67.76	
Blackfoot	MT1	0.1277	10.44	14.37	-18.58	48.45	3.93
Blackfoot	MT2	0.1526	10.83	14.20	-18.64	45.42	3.73
Blackfoot	MT3	0.1123	11.23	13.56	-18.63	44.26	3.81
Blackfoot	MT4	0.1325	11.70	13.95	-18.74	44.42	3.72
Blackfoot	MT5	0.1393	11.31	14.32	-17.88	44.37	3.61
Blackfoot	E008421	0.1232	10.31	13.10	-16.55	42.16	3.76
preResSioux	E008844.1	0.1170	9.90	13.65	-15.10	43.04	3.68
preResSioux	E008844.2	0.1082	11.02	13.61	-17.15	42.77	3.66
preResSioux	E008844.3	0.1188	10.63	13.28	-17.63	43.41	3.81
preResSioux	E008844.4	0.1198	9.84	13.39	-14.94	43.18	3.76
preResSioux	E008844.5	0.1241	10.16	13.24	-16.56	43.84	3.86
preResSioux	E008844.6	0.1244	9.84	13.53	-16.13	43.71	3.77
preResSioux	E008844.7	0.1286	10.28	13.45	-15.70	43.71	3.79
preResSioux	E008844.8	0.1236	9.74	13.37	-16.03	43.24	3.77
preResSioux	E008844.9	0.1234	9.35	13.39	-16.55	43.06	3.75
preResSioux	E008844.10	0.1384	9.39	13.45	-16.12	43.76	3.80
preResSioux	E008844.11	0.1488	9.81	13.54	-16.15	43.64	3.76
preResSioux	E008844.12	0.1571	9.63	13.60	-16.16	43.89	3.77
preResSioux	E021678	0.1010	10.77	13.43	-20.54	44.22	3.84
preResSioux	E153543.1	0.1014	9.43	13.18	-21.53	43.03	3.81
preResSioux	E153543.2	0.1272	9.85	13.77	-21.51	43.86	3.72
preResSioux	E153543.3	0.1231	9.61	13.93	-21.40	44.74	3.75
preResSioux	E153543.4	0.1209	9.08	13.31	-21.50	43.65	3.82
preResSioux	E153543.5	0.1100	8.96	13.85	-21.23	45.28	3.82
preResSioux	E153543.6	0.1140	9.07	13.14	-21.25	43.25	3.84
preResSioux	E153543.7	0.1496	9.14	13.57	-21.38	43.91	3.78
preResSioux	E153543.8	0.1557	9.46	13.33	-21.47	43.53	3.81
preResSioux	E153543.9	0.1504	8.98	13.41	-21.67	43.96	3.82
preResSioux	E153543.10	0.1573	9.05	13.71	-21.67	45.07	3.84
preResSioux	E153543.11	0.1633	9.33	13.24	-22.03	44.27	3.90
preResSioux	E154350	0.1189	11.22	13.46	-18.90	44.10	3.82
Blackfoot	E200977	0.1254	9.05	12.99	-22.14	42.92	3.85
Plains	E358388	0.1433	10.06	13.29	-19.15	43.51	3.82
Plains	E358743	0.1225	10.42	13.75	-18.91	44.39	3.76
modern	NHN.8	0.1292	8.55	13.55	-18.49	45.53	3.92
modern	NHN.9	0.1450	8.80	13.83	-18.42	45.50	3.84
modern	NHN.10	0.1321	8.71	14.15	-18.17	44.05	3.63
bison	ET10754.1	0.1201	6.26	14.28	-20.17	44.58	3.64
bison	ET10754.2	0.1521	6.72	14.21	-21.49	44.41	3.65
bison	ET10754.3	0.1466	6.16	13.87	-21.35	43.80	3.68
bison	ET10754.4	0.1200	5.48	13.60	-21.39	42.69	3.66
bison	ET10754.5	0.1433	5.54	14.02	-22.02	43.82	3.65
bison	ET10754.6	0.1426	6.31	13.80	-21.75	43.05	3.64
Standard	Acetanilide	0.0580	-0.27	9.79	-30.19	69.85	
Standard	NIST 8548	0.3202	20.41	21.31			
Standard	NIST 8548	0.1878	20.21	20.55			
Standard	NIST 8541	0.0295			-16.27	74.20	
Standard	NIST 8541	0.0352			-16.04	103.18	

Table 4. Raw Data from Run E020206, 06 Feb 2002

Pop'n	ID	Wt., mg	δ15N	Wt%N	δ13C	Wt%C	AtomicC:N
Standard	Acetanilide	0.1380	-1.25	10.28	-30.04	70.71	
Standard	Acetanilide	0.0496	-1.04	11.13	-30.09	74.43	
Standard	Acetanilide	0.0698	-1.09	10.69	-30.21	73.22	
LBSioux	LB262.1	0.1480	9.25	13.73	-17.34	43.57	3.70
LBSioux	LB262.2	0.1080	9.30	13.86	-17.18	44.31	3.73
LBSioux	LB262.3	0.1170	9.27	14.27	-16.75	46.05	3.76
LBSioux	LB262.4	0.1390	9.22	13.66	-17.31	44.14	3.77
LBSioux	LB262.5	0.1550	9.21	13.69	-17.42	44.62	3.80
LBSioux	LB262.6	0.1310	8.86	13.51	-17.36	44.94	3.88
LBSioux	LB262.7	0.1390	8.66	13.51	-18.16	44.52	3.84
LBSioux	LB262.8	0.1350	8.79	13.38	-17.87	44.10	3.84
LBSioux	LB262.9	0.1500	9.28	13.80	-17.04	45.10	3.81
LBSioux	LB262.10	0.1450	8.81	13.60	-17.10	44.35	3.80
LBSioux	LB262.11	0.1310	9.18	13.76	-17.75	48.25	4.09
LBSioux	LB262.12	0.1460	9.09	13.44	-17.15	43.98	3.82
LBSioux	LB326.1	0.1030	11.12	13.75	-14.91	45.26	3.84
LBSioux	LB326.2	0.1350	10.80	13.25	-13.09	44.17	3.89
LBSioux	LB326.3	0.1080	10.65	13.43	-15.62	44.57	3.87
LBSioux	LB326.4	0.1460	10.81	13.74	-15.05	44.85	3.81
LBSioux	LB326.5	0.1510	11.21	13.73	-14.33	44.98	3.82
LBSioux	LB326.6	0.1510	10.14	13.40	-15.64	44.26	3.85
LBSioux	LB326.7	0.1480	10.27	13.91	-16.25	45.49	3.82
LBSioux	LB326.8	0.1500	10.53	13.67	-16.19	44.62	3.81
LBSioux	LB326.9	0.1490	10.72	13.74	-16.04	44.59	3.79
LBSioux	LB326.10	0.1540	10.71	13.76	-15.90	44.30	3.76
LBSioux	LB326.11	0.1410	10.76	13.74	-16.67	44.10	3.74
LBSioux	LB326.12	0.1530	11.36	13.83	-16.78	44.56	3.76
modern	DMR.1	0.0900	8.98	13.77	-18.12	43.61	3.70
modern	DMR.2	0.1320	9.13	13.90	-18.13	43.73	3.67
modern	DMR.3	0.1560	9.20	14.00	-18.06	43.41	3.62
modern	DMR.4	0.1560	8.94	13.64	-18.10	41.96	3.59
modern	DMR.5	0.1070	9.34	13.95	-18.44	44.57	3.73
modern	DMR.6	0.1340	9.39	14.00	-18.23	43.68	3.64
modern	DMR.7	0.1540	9.33	14.06	-18.28	44.28	3.67
modern	DMR.8	0.1210	9.27	14.00	-18.30	43.75	3.65
modern	DMR.9	0.1220	9.39	13.89	-18.31	43.64	3.66
modern	DMR.10	0.1440	9.55	13.78	-18.55	43.83	3.71
modern	DMR.11	0.1230	9.81	13.38	-18.57	43.99	3.83
modern	DMR.12	0.0950	9.76	13.60	-19.00	44.25	3.80
modern	NHN.1	0.1430	8.85	14.67	-17.77	44.15	3.51
modern	NHN.2	0.1020	8.40	13.99	-18.21	44.94	3.75
modern	NHN.3	0.1080	8.95	13.82	-18.10	44.38	3.74
modern	NHN.4	0.1050	9.08	13.86	-18.05	44.93	3.78
modern	NHN.5	0.1520	8.88	13.81	-17.91	44.95	3.80
modern	NHN.6	0.1270	9.01	14.12	-18.26	46.29	3.82
modern	NHN.7	0.1350	8.89	13.65	-18.39	45.23	3.87
Standard	Acetanilide	0.1350	-0.62	9.38	-30.19	65.99	
Standard	NIST 8548	0.4172	20.35	16.51			
Standard	NIST 8548	0.2321	20.49	21.37			

Table 5. Effect of Solvent-Washing on Isoptope Values. Run #EA0823, 23 Aug 2001

Individual	DC			CM			BF		
Treatment	Wt, mg	δ13C	δ15N	Wt, mg	δ13C	δ15N	Wt, mg	δ13C	δ15N
cleaned1	0.261	-18.94	7.82	0.162	-17.94	9.32	0.292	-19.88	11.40
cleaned2	0.148	-18.64	7.53	0.232	-18.01	9.30	0.287	-20.10	11.44
cleaned3	0.231	-19.15	7.82	0.170	-18.13	9.23	0.160	-19.97	11.41
Avg		-18.91	7.72		-18.03	9.28		-19.98	11.42
Std Dev		0.26	0.17		0.10	0.05		0.11	0.02
uncleaned1	0.168	-19.02	7.68	0.236	-18.29	9.42	0.258	-20.16	11.45
uncleaned2	0.154	-18.76	7.72	0.230	-18.19	9.26	0.256	-19.96	11.42
uncleaned3				0.146	-18.05	9.27	0.215	-19.96	11.34
uncleaned4							0.160	-19.81	11.43
Avg		-18.89	7.70		-18.18	9.32		-19.97	11.41
Std Dev		0.13	0.02		0.10	0.07		0.12	0.04

Table 6. Specimen Weights and Isotope Values. Run #011102, 02 Nov 2001

Individual	µg	δ15N vs AIR	δ13C vs PDB
BF24	252	11.79	-19.65
	218	11.76	-19.81
	195	11.90	-19.69
	154	11.81	-19.57
	124	11.58	-19.62
	90	11.23	-19.54
	52	11.47	-19.57
BF57	289	10.77	-18.59
	210	10.22	-18.36
	198	10.32	-18.39
	145	10.52	-18.31
	126	10.24	-18.36
	106	10.53	-18.50
	87	10.20	-18.37
	50	9.53	-18.66
Acetanilide	356	-1.09	-30.35
	479	-1.00	-30.47
	453	-0.80	-30.40
NIST 8548	226	20.37	
	304	20.40	
NIST 8541	102		-15.82
	110		-15.74

Table 7. Comparing Paired Hair Specimens (Data from Tables 1 and 2)

Individual	Wt., mg	δ15N	Difference	δ13C	Difference
BF24	0.2522	11.79	0.03	-19.65	0.16
"	0.2184	11.76		-19.81	
BF57	0.2786	10.77	0.56	-18.59	0.24
"	0.2105	10.22		-18.36	
BF59	0.2217	10.68	0.16	-18.89	0.22
"	0.2141	10.83		-18.67	
LB243.1	0.2066	11.04	0.08	-14.85	0.20
"	0.2297	10.96		-15.04	
LB262.1	0.2407	9.37	0.13	-16.80	0.12
"	0.2434	9.49		-16.92	
LB264.1	0.2135	10.12	0.21	-14.76	0.65
"	0.2465	9.90		-15.40	
LB265.1	0.2115	10.79	0.14	-15.13	0.29
"	0.2259	10.64		-15.43	
LB288.1	0.2174	10.14	0.39	-15.60	0.42
"	0.2412	10.52		-15.18	
LB289.1	0.2174	10.07	0.07	-15.53	0.31
"	0.2127	10.00		-15.22	
LB296.1	0.2473	10.18	0.06	-15.56	0.14
"	0.2239	10.12		-15.42	
LB297.1	0.2733	9.86	0.29	-15.79	0.32
"	0.2392	9.57		-15.47	
LB299.1	0.2063	9.81	0.09	-16.57	0.17
"	0.2481	9.72		-16.40	
LB326.1	0.2224	10.67	0.34	-13.02	0.01
"	0.2258	11.01		-13.01	
LB342.1	0.2092	9.71	0.17	-15.32	0.11
"	0.2064	9.55		-14.94	
LB402.1	0.2449	10.82	0.23	-13.46	0.04
"	0.2488	11.05		-13.94	
Average			0.20		0.23

Table 8. Proximal Hair Segment Isotope Values for All Individuals (from Tables 1 to 4)

Population	Date	Collection*	Individual	δ15N	δ13C
Pre-reservation					
Blackfoot	19th C.	NMNH, S.I.	E008421	10.31	-16.55
Blackfoot	19th C.	NMNH, S.I.	E200977	9.05	-22.14
Sioux	19th C.	NMNH, S.I.	E008844.1	9.90	-15.10
Sioux	19th C.	NMNH, S.I.	E021678	10.77	-20.54
Sioux	19th C.	NMNH, S.I.	E153543.1	9.43	-21.53
Sioux	19th C.	NMNH, S.I.	E154350	11.22	-18.90
Plains	19th C.	NMNH, S.I.	E358388	10.06	-19.15
Plains	19th C.	NMNH, S.I.	E358743	10.42	-18.91
Reservation					
Blackfoot, Montana	1935	M.T., S.I.	MT1	10.44	-18.58
Blackfoot, Montana	1935	M.T., S.I.	MT2	10.83	-18.64
Blackfoot, Montana	1935	M.T., S.I.	MT3	11.23	-18.63
Blackfoot, Montana	1935	M.T., S.I.	MT4	11.70	-18.74
Blackfoot, Montana	1935	M.T., S.I.	MT5	11.31	-17.88
Blackfoot, Flathead Res.	1892	Boas, AMNH	BF24	11.77	-19.73
Blackfoot, Flathead Res.	1892	Boas, AMNH	BF57	10.50	-18.48
Blackfoot, Flathead Res.	1892	Boas, AMNH	BF59	10.75	-18.78
Sioux, Lower Brule Res.	1892	Boas, AMNH	LB243	11.00	-14.94
Sioux, Lower Brule Res.	1892	Boas, AMNH	LB262	9.43	-16.86
Sioux, Lower Brule Res.	1892	Boas, AMNH	LB264	10.01	-15.08
Sioux, Lower Brule Res.	1892	Boas, AMNH	LB265	10.71	-15.28
Sioux, Lower Brule Res.	1892	Boas, AMNH	LB288	10.33	-15.39
Sioux, Lower Brule Res.	1892	Boas, AMNH	LB289	10.03	-15.37
Sioux, Lower Brule Res.	1892	Boas, AMNH	LB296	10.15	-15.49
Sioux, Lower Brule Res.	1892	Boas, AMNH	LB297	9.72	-15.63
Sioux, Lower Brule Res.	1892	Boas, AMNH	LB299	9.76	-16.49
Sioux, Lower Brule Res.	1892	Boas, AMNH	LB301	10.67	-15.47
Sioux, Lower Brule Res.	1892	Boas, AMNH	LB326	10.84	-13.02
Sioux, Lower Brule Res.	1892	Boas, AMNH	LB342	9.63	-15.13
Sioux, Lower Brule Res.	1892	Boas, AMNH	LB402	10.94	-13.70
Other					
bison	19th C.	NMNH, S.I.	ET10754	6.26	-20.17
urban U.S.	2001	personal	DMR	8.98	-18.12
urban U.S.	2001	personal	NHN	8.85	-17.77
urban U.S.	2001	personal	DC	7.72	-18.91
urban U.S.	2001	personal	CM	9.28	-18.03

Note: Where replicates were run, the average is given. Weights given in Tables 1-4.
* NMNH is the National Museum of Natural History, Washington, D.C.; M.T. is the Mildred Trotter collection; S.I. is the Smithsonian Institution; AMNH is the American Museum of Natural History, New York City.

Table 9. $\delta^{15}N$ Values in Segments of a Single Strand (from Tables 3 and 4)

Indiv	2 cm	4 cm	6 cm	8 cm	10 cm	12 cm	14 cm	16 cm	18 cm	20 cm	22 cm	24 cm
EE008844	9.90	11.02	10.63	9.84	10.16	9.84	10.28	9.74	9.35	9.39	9.81	9.63
E153543	9.43	9.85	9.61	9.08	8.96	9.07	9.14	9.46	8.98	9.05	9.33	
LB262	9.25	9.30	9.27	9.22	9.21	8.86	8.66	8.79	9.28	8.81	9.18	9.09
LB326	11.12	10.80	10.65	10.81	11.21	10.14	10.27	10.53	10.72	10.71	10.76	11.36
DMR	8.98	9.13	9.20	8.94	9.34	9.39	9.33	9.27	9.39	9.55	9.81	9.76
NHN	8.85	8.40	8.95	9.08	8.88	9.01	8.89	8.55	8.80	8.71		
ET10754	6.26	6.72	6.16	5.48	5.54	6.31						

Note: Two-cm segments measured from proximal end to distal end of strand.
Note: "Pre" means pre-reservation Sioux, 19th century; "Res" means Lower Brule Reservation Sioux, 1892; "Mod" means modern urban American, 2001.

Table 10. $\delta^{13}C$ Values in Segments of a Single Strand (from Tables 3 and 4)

Indiv	2 cm	4 cm	6 cm	8 cm	10 cm	12 cm	14 cm	16 cm	18 cm	20 cm	22 cm	24 cm
EE008844	-15.10	-17.15	-17.63	-14.94	-16.56	-16.13	-15.70	-16.03	-16.55	-16.12	-16.15	-16.16
E153543	-21.53	-21.51	-21.40	-21.50	-21.23	-21.25	-21.38	-21.47	-21.67	-21.67	-22.03	
LB262	-17.34	-17.18	-16.75	-17.31	-17.42	-17.36	-18.16	-17.87	-17.04	-17.10	-17.75	-17.15
LB326	-14.91	-13.09	-15.62	-15.05	-14.33	-15.64	-16.25	-16.19	-16.04	-15.90	-16.67	-16.78
DMR	-18.12	-18.13	-18.06	-18.10	-18.44	-18.23	-18.28	-18.30	-18.31	-18.55	-18.57	-19.00
NHN	-17.77	-18.21	-18.10	-18.05	-17.91	-18.26	-18.39	-18.49	-18.42	-18.17		
ET10754	-20.17	-21.49	-21.35	-21.39	-22.02	-21.75						

Note: Two-cm segments measured from proximal end to distal end of strand.
Note: "Pre" means pre-reservation Sioux, 19th century; "Res" means Lower Brule Reservation Sioux, 1892; "Mod" means modern urban American, 2001.