University of Nevada, Reno

Stable Carbon and Nitrogen Isotope Analysis: A Comparison of Modern Calculus, Hair and Fingernail

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Arts in
Anthropology

by

Lindsay A. E. Dorio

Dr. G. Richard Scott/Thesis Advisor

May, 2012

University of Nevada, Reno
Statewide • Worldwide

THE GRADUATE SCHOOL

We recommend that the thesis
prepared under our supervision by

LINDSAY A. E. DORIO

entitled

Stable Carbon And Nitrogen Isotope Analysis: A Comparison Of Modern Calculus, Hair And Fingernail

be accepted in partial fulfillment of the
requirements for the degree of

MASTER OF ARTS

G. Richard Scott, Ph. D., Advisor

Geoffrey Smith, Ph. D., Committee Member

Simon Poulson, Ph. D., Graduate School Representative

Marsha H. Read, Ph. D., Dean, Graduate School

May, 2012

Abstract

Stable carbon and nitrogen isotope analysis has been used to reflect dietary signatures in humans and animals. For ancient human remains, bone collagen and bone apatite are the traditional biomaterials used to estimate stable isotope ratios. For modern populations, hair and fingernail are used toward this end. Recent work indicates that dental calculus from ancient remains may be another viable biomaterial for stable isotope analysis. Because its collection is technically non-destructive, the use of dental calculus for stable isotope analysis could have benefits in cases where destructive analysis is prohibited. To help establish the utility of calculus as an isotope proxy, the present research analyzed modern calculus, along with the established biomaterials of hair and fingernail, to determine the extent to which they yield comparable isotope ratios. The analysis shows there is a strong and significant correlation between the stable carbon isotope ratios of modern calculus, hair and fingernails. In contrast, there is no correlation for stable nitrogen isotope ratios between calculus and either hair or nail. Based on the high weight percentages of carbon and nitrogen in some calculus samples, these findings may be complicated by components in plaque and saliva.

Acknowledgements

I would like to express thanks and appreciation to my advisory committee: Dr. G. Richard Scott, Dr. Simon Poulson, and Dr. Geoffrey Smith. Special thanks to for the opportunity to do this stable isotope research and for all of your advice, patience and time. Special thanks to Dr. Poulson for allowing me access to the Stable Isotope Laboratory of the Mineral Science Department at the University of Nevada, Reno.

Table of Contents

List of Tables

List of Figures

1.0 INTRODUCTION

1.1 Related Studies

Stable isotope analysis has been successfully used in paleodietary reconstructions from fossil hominids to modern humans (Schoeninger, 2010; Buchardt et al., 2007; Ambrose, 1986). Carbon isotope ratios were the first to be applied with success, and they are still of great use when evaluating prehistoric diets (van der Merwe and Vogel, 1978; Drucker and Bocherens, 2004). For example, stable carbon isotope ratios are used to evaluate the spread of agricultural staples, such as maize, from tropical to temperate regions (Vogel and van der Merwe, 1977; Schwarcz and Schoeninger 1991; Tafuri et al., 2009). In addition to carbon isotope ratios, nitrogen isotope ratios are used to infer the amounts of animal and marine resources in prehistoric human diets (Schoeninger, 1995; Kusaka et al., 2010).

In conducting studies on human remains, the biomaterials most often used for isotope analysis are bone collagen, bone apatite and/or dental enamel (Yesner et al., 2003; Ambrose, 1990). When mummified remains are available for study, muscle, hair and fingernails can also be analyzed (Aufderheide et al., 1994; White and Schwarcz, 1997). For living humans, hair and nail samples are the most readily available biomaterials for isotope analysis and can be obtained with minimal inconvenience to the test subjects (O‘Connell et al., 2001). For analyzing isotopes in ancient human remains, destructive procedures are usually necessary for analysis, especially when the extraction of collagen from bone is involved (DeNiro and Schoeniger; 1983).

A potential biomaterial for isotope analysis yet to be fully explored in detail is human dental calculus. Although calculus is made up primarily of calcium-phosphate

minerals, 20% of calculus is organic in nature (Lieverse, 1999). A pilot study assessing carbon and nitrogen isotopes in the calculus of a medieval Spanish sample yielded promising results that fall closely in line with collagen based values of European samples (Scott and Poulson, 2012). The potential benefit of dental calculus is that collection is a non-destructive procedure. It is neither part of the bone nor tooth but an add-on that accumulates during the life of an individual. This paper surveys the development and applications of stable isotope analysis in anthropology and reviews the potential role of dental calculus in stable isotope testing.

1.2 Objectives

The objective of this research is to test the applicability of dental calculus as an isotope proxy by comparing stable carbon and nitrogen isotope ratios derived from dental calculus to those from hair and fingernails of the same individuals sampled in a living American population. While this research uses calculus from living subjects, the potential use of dental calculus is only practical on ancient human remains where hair and nail samples are not available. One question underlying this research is why study dental calculus when there are already well established proxies for isotope research, notably bone collagen, apatite, hair, muscle, and fingernails. The reason is that dental calculus does not require any destruction to skeletal or dental tissues. This is a major concern to many museum curators, who often preclude destructive research on specimens under their care.

2.0 STABLE CARBON AND NITROGEN ISOTOPE ANALYSIS

2.1 Origin of Isotope Studies

Stable isotope analysis was first developed and applied in geological studies in the 1930s. In an early publication, Nier and Gulbransen (1939) examined the C^{12}/C^{13} ratios in igneous rocks, limestone, select plants, and a few other sources. Analysis was done through mass spectroscopy. The outcome of this research suggested that natural ratios varied in nature with some consistency, opening the door to further research (Nier and Gulbransen, 1939).

After C^{12}/C^{13} variation was demonstrated in natural sources, the next objective was to estimate these ratios for different kinds of samples. The C^{12}/C^{13} ratio was only used in the publication of Nier and Gulbransen (1939). From the 1950s on, the ratio used was C^{13}/C^{12}. Craig (1953) published an article on C^{13}/C^{12} values from hundreds of samples from different geological sources. While most of the early work on isotopes dealt with geological C^{13}/C^{12} variations, some samples were taken from marine invertebrates and marine and terrestrial plants for comparative purposes. Surprisingly, terrestrial plants displayed no correlation to the geologically derived stable carbon ratios. This led to questions as to how the C^{13}/C^{12} values were formed in plant sources (Craig, 1953, 1957; Nier and Gulbransen, 1939).

In the 1950s, many samples were analyzed for C^{13}/C^{12}, but a few problems with stable carbon isotope analysis had to be addressed. With multiple researchers collecting samples and deriving isotope ratios, a uniform set of standards was needed to establish the comparability and reproducibility of results (Craig, 1957; Tykot, 2006). Nier and Gulbransen (1939) set their stable carbon isotope ratio standards relative to the value of

carbon in air. In 1955, the National Bureau of Standards used carbon from limestone deposits in Solnhofen, Bavaria to serve as a common standard. At the University of Chicago, the PDB standard was established, and this was broadly adopted by 1957. The PDB standard was from *Belemnitella americana*, a Cretaceous belemnite from the Peedee formation in South Carolina (Craig, 1957; Mohler, 1955). No material is currently available from the original source of PDB, so VPDB (Vienna Peedee belemnite) was substituted at the National Bureau of Standards laboratories to ensure comparative reliability. VPDB is very similar to the previous PBD standard (Tykot, 2006). The PDB standard was still used into the 2000s when available (Prowse et al., 2005; Clayton et al., 2006), but, by 2010, this material had become scarce. Now, VPDB has largely replaced PDB as the carbon stable isotope standard (Thompson et al., 2010; Williams et al., 2011).

The equation for the C^{13}/C^{12} ratios is:

$$\delta^{13}C‰ = [\{(^{13}C/^{12}C_{\ sample})/(^{13}C/^{12}C_{\ standard})\}-1] \times 1000$$

$\delta^{13}C$ is the common notation for the amounts of ^{13}C relative to the standard. The VPDB standard for ^{13}C is 0‰. Because of naturally high ^{13}C levels in the VPDB standard, most biological sources produce negative values for $^{13}C/^{12}C$ (Schoeninger, 1995; Tykot, 2006).

Although stable nitrogen isotope ratios were sampled in the 1950s along with those of other elements, most research continued to focus on the isotope ratios $^{13}C/^{12}C$ and $^{18}O/^{16}O$ (Nier and Gulbransen, 1939; Craig, 1953, 1957; Mohler, 1955). Because of the geological purposes of isotope analysis, stable nitrogen isotope analysis did not become prominent until the 1980s when $^{15}N/^{14}N$ ratios were shown to reflect trophic levels (Tykot, 2006). The stable nitrogen isotope standard was easier to establish than the carbon standard. The standard for $\delta^{15}N$ is set relative to the amount of atmospheric N_2. Similar

to the carbon standard, the standard value for ^{15}N is set at 0‰ (Adams, 2000; Mohler, 1955).

The ^{15}N values are expressed similarly to the ^{13}C values. The standard equation for determining ^{15}N values is:

$$\delta^{15}N‰ = [\{(^{15}N/^{14}N_{\text{sample}})/(^{15}N/^{14}N_{\text{standard}})\}-1] \times 1000$$

In contrast to the carbon standard, the atmospheric standard has relatively low levels of ^{15}N, so most natural substances are enriched in ^{15}N and are expressed as positive values (Ambrose, 1991).

While stable carbon isotope ratios were first explored in the late 1930s, it was not until the late 1970s that they were used to detect dietary signatures in humans (Vogel and van der Merwe, 1977). Nitrogen was not applied to human paleodietary studies until the 1980s (DeNiro and Schoeninger, 1983). The use of carbon ratios in anthropology came after many decades of research on the levels of ^{13}C found in nature. It also came after the discovery of the different photosynthetic pathways that determine these levels.

2.2 Photosynthetic Pathways - C3, C4 and CAM Plants

A better understanding of photosynthetic pathways opened the door for stable carbon isotope analysis in anthropology. In the 1950s, stable carbon isotope ratios showed that neither geological location nor soil type had an impact on ratios (Craig, 1953, 1957). The carbon in plants comes from CO_2 in air. Calvin and Benson provided the details of photosynthesis, including the path carbon takes during the process (Schoeninger, 2005; Benson and Calvin, 1950). Researchers then discovered three different photosynthetic pathways: CAM, C_3, and C_4. Hatch and Slack (1966, 1967) explored the differences of the C_4 and C_3 pathways. The CAM (crassulacean acid metabolism)

pathway was explored earlier, but it was the C_4 and C_3 pathways that proved the most useful for carbon stable isotope analysis on humans (Tykot, 2006; Schoeninger 2005).

For stable isotope ratios, C_3 and C_4 plants have photosynthetic processes that allow different levels of ^{13}C enrichment. The difference between C_3 and C_4 plants is found in the enzymes used to fix CO_2 from the atmosphere. C_3 and C_4 plants differ because the environmental conditions of temperate and tropical areas favor different photosynthetic pathways. Tropical C_4 plants are efficient in conditions of high amounts of sunlight, high temperatures, droughts, and environments with low amounts of CO_2. To be efficient in these conditions, C_4 plants use of the enzyme phosphoenol pyruvate carboxylase. The C_4 pathway is a derived form that thrives in hot or arid conditions. In contrast, C_3 plants use the enzyme ribulose bisphosphate carboxylase. C_3 plants have a photosynthetic process that selects for the lighter ^{12}C which is faster and easier to process than ^{13}C (DeNiro, 1987; Ehleringer et al., 1991, 1997; Ehleringer and Björkman, 1997; Tykot, 2006).

CAM plants are mainly desert succulents and planktonic species. Their pathways often depend on environmental conditions. The CAM pathway varies in stable carbon isotope ratios because CAM plants can use the enzymes of either C_3 or C_4 photosynthesis. Although other enzymes are used for photosynthesis, these are rare and generally insignificant as dietary sources (Schoeninger, 1995; DeNiro, 1987; Tykot, 2006).

The vast majority of plants (85%) have the ancestral C_3 photosynthetic pathway. Since this photosynthetic pathway allows plant enzymes to have an even greater selection for ^{12}C than C_4 plants, their $^{13}C/^{12}C$ ratios are significantly lower than those of C_4 plants. In the atmosphere, the values for ^{13}C are around -8‰ (Schoeninger, 2005). On average, the $\delta^{13}C$ for C_3 plants is -27.1‰, with a range from about -33‰ to -22‰. The average $\delta^{13}C$

for C_4 plants is -13.1‰, with a range from approximately -16‰ to -9‰ (DeNiro, 1987; Bell, 2001).

While atmospheric CO_2 levels have changed through the centuries, these changes have only had a small impact on the $\delta^{13}C$ found in plants. The Industrial Revolution marked a change in $\delta^{13}C$ of probably no more than 1‰. If this is taken into account, plants prior to the Industrial Revolution would be expected to have $\delta^{13}C$ values approximately 1‰ more positive than the same plants today. While this change is worth noting, the space between the C_3 and C_4 ranges is much greater than this change. Even when comparing modern to prehistoric $\delta^{13}C$ levels, the C_3 and C_4 ranges are very distinct making the atmospheric shift negligible for most research (DeNiro, 1987).

2.3 Carbon and Nitrogen Isotopes and Animal Diet

The study of stable carbon isotope ratios in animals started in the early 1960s. Initial focus was on marine animals, and it soon became apparent that their ^{13}C values fell within the range of available plant ^{13}C values with some relatively consistent enrichment. In 1978, a study of stable carbon isotope ratios from animals of known diets yielded a positive correlation between ^{13}C values in animals and ^{13}C values in components of their diets (DeNiro and Epstein, 1978).

DeNiro and Epstein (1978) provided insights into the details of stable carbon isotope analysis. They found that tissue types in a single animal varied from one another in isotope ratios. In early studies on animals, many samples were taken that have not been tested in humans, such as specific organ tissues. It was shown that animal tissues on average were significantly enriched in ^{13}C values compared to dietary sources through a process called fractionation. Fractionation is the difference in stable isotope ratios

between an organism and the foods it consumes (DeNiro and Epstein, 1978). This occurs because metabolic reactions process the lighter and heavier isotopes at different rates. This process also accounts for isotopic differences between different types of tissues from the same organism (Roy et al., 2005).

Stable nitrogen isotope ratios as dietary indicators were first explored by DeNiro and Epstein (1981). While some work had been done on marine levels of ^{15}N, this was the first study on terrestrial animals with known diets. This study showed a direct correlation between the ^{15}N levels of diet and the ^{15}N levels in animals with trophic level enrichment. This trophic level enrichment means that ^{15}N levels in carnivores is greater than that of herbivores and ^{15}N levels of herbivores is greater than that of plants (DeNiro and Epstein, 1981).

For leguminous plants, there is no difference in $\delta^{15}N$ values between prehistoric and present day plants because legumes fix their ^{15}N to the atmospheric levels and atmospheric $\delta^{15}N$ has not changed within human history. There is, however, a change in present day non-legumes which do not fix nitrogen and take some ^{15}N from the soil. This change is due to the modern use of fertilizers in agriculture. In prehistoric times, non-leguminous C_3 plants would have an expected $\delta^{15}N$ of around 9‰. While the use of fertilizers has increased the overlap for $\delta^{15}N$ values of legumes and non-legumes, leguminous plants still give lower $\delta^{15}N$ values. With modern day chemical fertilizers, leguminous $\delta^{15}N$ values are about 2‰ to 4‰ more negative than non-leguminous C_3 plants (Ambrose, 1991; DeNiro, 1987).

Studies of ^{13}C values and ^{15}N values from animals triggered the beginning of stable isotope studies on human remains. While assessing the exact diet of animals and humans

outside of confinement is not possible, early tests showed the possible isotopic distinctions between animals that had significantly different diets. While ^{13}C values are useful for determining dietary amounts of different plant sources, ^{15}N values provide information concerning the amount of animal consumption in the diet (Grocke, 1997).

^{15}N values are not always ideal for determining plant consumption due to the effect of nitrogen levels in different soils. The enrichment of ^{15}N can also be related to an organisms' inability to get enough water. This is seen in arid environments where carnivores are enriched by 5‰ to 6‰ compared to herbivores. With trophic levels, ^{15}N values show enrichment of between 1‰ and 6‰ moving higher from one trophic level to another (Ambrose, 1991; Grocke, 1997).

^{13}C values from plant and animal food sources range between -7‰ and -35‰ depending on the photosynthetic pathways of the plants in the diet. There is some trophic level effect in ^{13}C values, but it is too small for reliable detection. Trophic level shifts are best detected through ^{15}N values (Ambrose, 1991; Ambrose, 1986; Grocke, 1997). Examples of the $\delta^{13}C$ and $\delta^{15}N$ averages of selected animals and plants are listed in Table 1 and represented in Fig. 1.

Table 1. $\delta^{13}C$ and $\delta^{15}N$ ranges for natural dietary sources**.**

Plants and Animals	Average δ13C Range	Average δ15N Range	Source
C3 Plants	-35 to -21	3 to 8	Roy et al., 2005; Ambrose and DeNiro, 1986; Scheoninger, 1995
C4 Plants	-16 to -8	2 to 8	Ambrose and DeNiro, 1986; Gröcke 1997; Tykot 2006
CAM Plants	-28 to -10	4 to 8	Gröcke, 1997; Ambrose and DeNiro, 1986
Legumes (Nitrogen fixing Plants)	-36 to -22	-3 to 7	Ambrose, 1991; Ambrose and DeNiro, 1986
Freshwater fish	-22.5 to -20.5	11.25 to 12.5	Drucker and Bocherens, 2004
Marine mammals	-15.5 to -12.7	12.2 to 18.8	Naito et al., 2010
C3 herbivores	-22.6 to -18	5.4 to 7.2	Herrscher and Le Bras-Goude, 2010
C3 omnivores	-20.0 to -18.8	4.8 to 8.4	Herrscher and Le Bras-Goude, 2010
C3 carnivores	-20.2 to -18.8	7.8 to 9.4	Herrscher and Le Bras-Goude, 2010
Millet	-12 to -10	3 to 4	Tafuri et al., 2009
Maize	-13.5 to -9.5	1.25 to 2.6	Tykot, 2006
Deer	-23.0 to -20.7	2 to 4.1	Naito et al., 2010
Fox	-19.9 to -19.1	6.5 to 7.8	Naito et al., 2010
Kelp	-14	7	Scheoninger, 1995
C4 grass	-12	3	Scheoninger, 1995
Tree leaves	-26	3	Scheoninger, 1995
Salmon	-16.1	12.1	Drucker and Bocherens, 2004

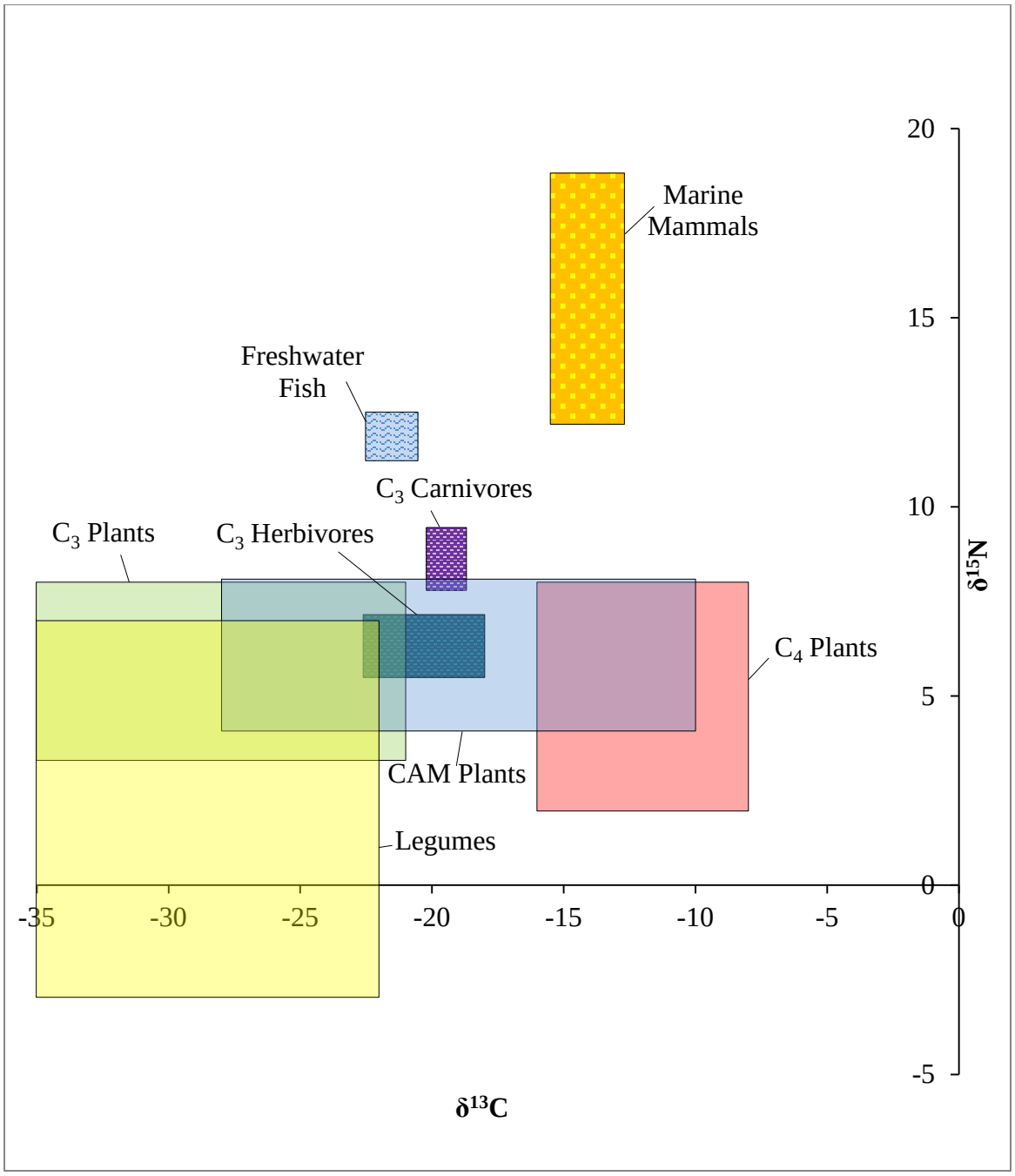

Fig. 1. Stable isotopic ranges for common dietary sources (values, ranges and sources specified in Table 1).

2.4 Stable Carbon and Nitrogen Isotope Ratios from Human Samples

Once the correlation between the stable isotope ratios of animals and the foods they consumed was established, attention was quickly drawn to human applications. The first form of stable isotope testing benefiting anthropology was ^{13}C analysis. At an anthropological conference in 1973, Van der Merwe proposed that diets with different amounts of C_3 and C_4 plants would cause different ^{13}C levels in humans. In 1976, human remains were first analyzed for ^{13}C. This was done on an individual from a South African site dating to roughly 1270 AD. The remains had ^{13}C levels that reflected a C_4 dietary signature. The purpose of that isotope analysis was to determine if sorghums and millets were present, but it was soon noted that almost all grasses in the region were C_4 plants. A C_4 signature was expected for both hunter-gatherer and agricultural populations. While this did not clearly indicate the presence of agricultural domesticates, it supported Van der Merwe's suggestion that human remains would reflect the ^{13}C of the available plant resources (Rightmire and Van der Merwe, 1976; Van der Merwe, 1982).

One benefit of stable carbon isotope testing is its sensitivity in tracking the spread of domesticated C_4 plants. For the first time, isotopic signatures of ^{13}C from human remains helped researchers detect the spread of maize (C_4) agriculture from the warm climates of Mesoamerica into the far reaches of northeastern North America (Van der Merwe and Vogel, 1977). $^{13}C/^{12}C$ was analyzed from bone collagen taken from pre- and post-maize horticultural sites. The pre-maize horticultural human remains gave ^{13}C values ranging from -19.8‰ to -21.3‰, while the post-maize horticultural human remains gave ^{13}C values between -13.5‰ and -16.6‰. This was the first publication that showed a contrast in carbon isotopic signatures between pre-maize and post-maize samples (Van

der Merwe, 1977). These values showed ^{13}C enrichment of bone collagen. They also gave ^{13}C amounts for C_3 and C_4 diets that have been reproduced and confirmed by subsequent studies (Larsen et al., 1992; Katzenberg et al., 1993; Herrscher and Le Bras-Goude, 2010; Van der Merwe and Vogel, 1978; Van der Merwe, 1992; Matson and Chisholm, 1991).

While DeNiro and Epstein (1981) demonstrated the potential of ^{13}C analysis to track the spread of agriculture, there were problems with their dietary reconstructions. Fractionation rates of bone collagen were based on the rates in mice. The ^{13}C amounts assigned to CAM plants were relatively fixed and researchers assumed there were no climatic shifts over the 7000 year period of site occupation. However, DeNiro and Epstein (1981) still successfully demonstrated an isotopic shift from mixed plant and animals resources to near complete dependence on domesticated plants, especially maize (DeNiro, 1987; DeNiro and Epstein, 1981; Farnsworth et al., 1985; Tykot and Staller, 2002).

Used together, stable carbon and nitrogen isotope ratios distinguish a wide range of human diets (Tykot, 2006). Bone collagen was first analyzed for both $\delta^{13}C$ and $\delta^{15}N$ from skeletal remains recovered at the site of Tehuacan, one of the earliest centers of maize domestication in Mesoamerica. This study estimated $\delta^{13}C$ and $\delta^{15}N$ values from bone collagen, and noted the difference in values through different phases of site occupation. Importantly, stable isotope values could be compared to dietary reconstructions based on archaeological data (DeNiro and Epstein, 1981). The ^{13}C values of the bone collagen reflected a shift from mixed C_3 and C_4/CAM food sources to nearly complete C_4 dependence between about 6000 BC and 4500 BC. The shift towards more positive ^{13}C

was mirrored by a shift towards more negative ^{15}N, which is typically the case when an agricultural domesticate becomes a staple. For the site of Tehuacan, stable isotopic evidence generally supported the archaeological finding of increased reliance on maize through time (DeNiro, 1987; DeNiro and Epstein, 1981; Farnsworth et al., 1985; Tykot and Staller, 2002).

While the use of $^{13}C/^{12}C$ was first shown to detect the spread of maize, it has since been used to track the spread of other C_4 plants, including millet, sugarcane, and sorghum (Tafuri et al, 2009; Tykot, 2006). When discriminating between C_4 and C_3 agriculture, $^{13}C/^{12}C$ ratios are effective alone. This is because $^{13}C/^{12}C$ ratios differ significantly while $^{15}N/^{14}N$ ratios are quite similar. Since $\delta^{13}C$ values for C_4 plants are typically between -16‰ and -8‰ and $\delta^{13}C$ values for C_3 plants range between -35‰ and -21‰, the spread of C_4 agriculture is seen as a sudden jump in ^{13}C enrichment even in a diet that is a mixture of C_3 and C_4 sources (Ambrose and DeNiro, 1986; Gröcke, 1997; Roy et al., 2005; Schoeninger, 2006; Schoeninger, 2009; Schoeninger 2010).

Recently, stable carbon and nitrogen isotope ratios have been applied together with greater sophistication. The benefit of using $\delta^{13}C$ and $\delta^{15}N$ concurrently became common in the 1990s and continues to this day. Fluctuation of both carbon and nitrogen values within a population can vary by about 1‰. The early studies on humans sampled bone collagen on a relatively small scale (DeNiro and Schoeninger, 1983; DeNiro and Epstein, 1981). Expanding from this base, Ambrose and DeNiro (1986) began sampling bone collagen from historic and prehistoric sites in Kenya and South Africa for stable isotope analysis. Such studies led to a greater understanding of how these values vary in paleodietary studies and the inferences that can be made from patterns of variation

(Ambrose, 1990; Ambrose and DeNiro, 1986; Schulting et al., 2008; Slovak and Paytan, 2009; Reitsema et al., 2010).

Studies started showing clusters for both stable carbon and nitrogen isotopes from populations with different diets. These studies refined what was seen in the work of van der Merwe and Vogel (1978) where human collagen showed consistent ^{13}C enrichment compared to diet. This is because the metabolic reactions in animals and humans utilize the heavier and lighter carbon isotopes differently resulting in different ^{13}C fractionation in varied tissues. For human bone collagen, fractionation causes approximately 5‰ enrichment in ^{13}C compared to dietary levels (Gil et al, 2011; Schoeller, 1999; Tykot, 2006).

Bone collagen is both a useful and problematic sampling choice for stable isotope analysis. Collagen quantities are typically low and great effort has to be expended to ensure the purest collagen samples for testing. This is especially true for ancient remains. On the plus side, bone collagen is a reliable biomaterial for stable isotopes if prepared properly. On the negative side, ^{13}C and ^{15}N values vary depending on the bones they are sampled from, since some bones regenerate at different rates within the body (e.g., collagen from dentine and ribs). Dentine is exceptionally slow to remodel, while bones regenerate in a matter of years. Even then, they can regenerate at different rates from each other. In certain cases, such as examining childhood development or where little mobility and diet change is expected, this can meet the objective (Ambrose, 1990; Fuller et al., 2003; Walker and DeNiro, 1986; Choy et al., 2010; Dipras and Tocheri, 2007; Jay et al., 2008). However, when the issue is reconstructing the diets of two or more disparate groups, differential regeneration can cause problems.

While collagen was the primary biomaterial for stable isotope analysis in the 1980s and early 1990s, other sample sources have also been utilized. Dental enamel, bone apatite, hair, nail and muscle tissue (from mummies) have been used successfully as isotope proxies (Aufderheide et al., 1994; Ambrose et al., 1997; Wright and Schwarcz, 1998; O'Connell et al., 2001; O'Connell and Hedges, 1999).

Bone apatite, a bone mineral which forms from the CO_2 in plasma, is used in situations similar to that of bone collagen with some provisos. Bone collagen is enriched in ^{13}C by about 5‰ over diet. By contrast, bone apatite is enriched in ^{13}C by about 6.5‰ to 7‰ compared to collagen and 11.5‰ to 12‰ compared to diet. In addition to fractionation issues for carbon isotopes, bone apatite is not the favored choice for isotopic research because it cannot be used for ^{15}N analysis (Schwarcz and Schoeninger, 1991; Ambrose et al, 1997; Tykot, 2006).

Bone apatite is sometimes used in conjunction with bone collagen because ^{13}C enrichment in bone apatite differs by trophic level. While an herbivore has approximately 8‰ bone apatite ^{13}C enrichment compared to bone collagen, a carnivore has approximately 4‰ bone apatite ^{13}C enrichment over bone collagen. This is often only seen in grazers and their predators (Schwarcz and Schoeninger, 1991). When examining protein sources in terms of C_3, C_4 or mixed protein sources, the use of collagen and apatite together has shown to be promising. This also includes marine protein sources (Kellner and Schoeninger, 2007).

The ^{15}N enrichment of bone collagen by around 3-4‰ is a direct reflection of trophic level effect. This enrichment is typically around 3-4‰, but conditions can broaden the range. Under drought conditions, $\delta^{15}N$ can be enriched by an extra 2-4‰.

Under a variety of conditions, trophic level enrichment varies from 1-6‰. Such conditions are not common though, so 3-4‰ enrichment is more typical. It is this particular aspect of ^{15}N testing that is useful in dietary analysis. Enrichment also varies relative to sample source. While bone collagen is about 4-5‰ higher than dietary input, the greatest enrichment is in muscle and blood which give values 6-8‰ greater than diet (Ambrose, 1986; Ambrose, 1991; Atahan et al., 2011; White and Schwarcz, 1994).

Dental enamel is a biomaterial that is not often used to obtain general dietary information. However, because the enamel on permanent teeth forms during childhood, it has the unique characteristic of reflecting childhood diet. Since mineralized materials cannot be used for ^{15}N testing, the use of dental enamel for weaning practices is limited to ^{13}C testing, sometimes augmented by other isotopic tests such as ^{18}O. A key element of using enamel in isotope studies is that different teeth form at variable times. The first molar forms between birth and 3 years. Premolar dental enamel forms between 2 and 6 years. These two teeth give insights into whether weaning occurs between these time periods. The downside is that it is limited to the 1‰ trophic level increase seen in ^{13}C. The great benefit to studies using dental enamel is that it is maintained throughout adulthood. An individual's dental enamel always reflects childhood dietary intake (Wright and Schwarcz, 1998).

When using bone collagen to detect weaning, only the remains of children can be used since bone regenerates entirely within a few years (Schurr and Powell, 2005; Clayton et al., 2006). Of the stable isotopes, nitrogen is best for detecting weaning ages, yet carbon and oxygen stable isotope testing can also be effective. The ^{15}N level in a breastfed child is one trophic level greater than the mother. This enrichment diminishes

rapidly after weaning (Williams et al., 2005; Wright and Schwarcz, 1998).

Because hair and fingernails are pure protein (keratin), they are an ideal biomaterial for stable isotope studies in modern populations because sampling is minimally invasive (Minagawa, 1992; Yoshinaga et al, 1996; Macko et al, 1999). To standardize values relative to bone collagen, O'Connell et al. (2001) took hair and nail samples from people who were undergoing knee or hip replacement surgeries so that the hair and nail values could be compared to bone collagen samples from the same individual. Hair and nails have generally similar stable isotope ratios. Hair samples were enriched in ^{13}C by an average of 0.21‰ compared to nails. On average, nail samples were enriched in ^{15}N by 0.65‰ over hair. These differences are slight, and neither differed significantly from ratios obtained from bone collagen. Hair was determined to be 0.5‰ less enriched in ^{13}C and 1‰ less enriched in ^{15}N than bone collagen (O'Connell et al., 2001). After 2001, stable isotope testing from hair and nail samples has been commonly used on modern populations as well as human remains where hair is still available (Roy et al., 2005; Nardoto et al., 2006; Thompson et al., 2010; Williams et al 2011). The $\delta^{13}C$ and $\delta^{15}N$ enrichment averages for common sample biomaterials are shown in Table 2.

Table 2. $\delta^{13}C$ and $\delta^{15}N$ enrichment over diet in human samples.

Sample Type	**$\delta^{13}C$ Enrichment**	**$\delta^{15}N$ Enrichment**
Bone Collagen	4-5‰	4-5‰
Muscle	4.5-5.5‰	6-8‰
Hair	7.5-8.5‰	3-4‰
Nails	7.3-8.3‰	3.5-4.5‰
Tooth Enamel	14-15‰	
Bone Apatite	11.5-12‰	

Buchardt et al., 2007; White and Schwarcz, 1994; O'Connell et al., 2001; Tykot, 2006; Cerling et al., 1997; Ambrose et al., 1997; Ambrose, 1986

The studies of stable isotopes in human populations show certain trends for different human diets. A list of some human populations with different diets and the location of these populations is provided in Table 3. All examples are listed in terms of their collagen ^{13}C and ^{15}N values. This information is also represented in Fig. 2.

Table 3. $\delta^{13}C$ and $\delta^{15}N$ collagen averages for human populations and diets.

	Area and Dietary Context	Average Collagen $\delta^{13}C$ Values	Average Collagen $\delta^{15}N$ Values	Source
C_3 ENVIRONMENT	Swanport, Lower Murray, Australia (Temperate/Terrestrial C_3 Hunter & Gatherers (without marine resources))	-20.0 ± 0.8	10.1 ± 1.1	Pate et al., 2002
	10,400-9200 cal BC cemetery, Ukraine (Temperate/Terrestrial C^3 Hunter & Gatherers (with freshwater marine resources))	-22.2 ± 0.2	12.8 ± 0.6	Lillie et al., 2011
	5000-4000 BP Jomon: Funamoto, Japan (Marine/C_3 Terrestrial Hunter-Gatherers)	-16.0 ± 0.9	13.8 ± 1.1	Kusaka et al., 2010
	Roonka, Upper Murray, Australia (Semi-Arid/Terrestrial C_3 Hunter-Gatherers)	-20.1 ± 1.2	13.4 ± 1.2	Pate et al., 2002
	4500-3500 cal BC Northwest Mediterranean, Garonne, France (C_3 Pastoralism (with freshwater marine resources))	-20.6 ± 0.3	9.8 ± 0.9	Herrscher and Le Bras-Goude, 2010
	4500-3500 cal BC Northwest Mediterranean, Languedoc, France (C_3 Agricultural)	-19.3 ± 0.5	8.3 ± 1.0	Herrscher and Le Bras-Goude, 2010
C_4 ENVIRONMENT	1200 -1300 AD, Pecos Pueblo, New Mexico (C_4 Agricultural)	-7.5 ± 0.3	9.1 ± 0.7	Coltrain et al., 2007
	1150 - 1300 AD, Georgia Bight (C_4 Agricultural/Marine)	-13.0 ± 2.0	10.7 ± 1.1	Larsen et al., 1992
	Historic Pokot Site, Kenya (Pastoralism (with C_4 Agriculture))	-10.4 ± 1.0	14.2 ± 1.1	Ambrose SH, 1986
MARINE	1150 AD to 1804 AD, Channel Islands of southern California (Marine/Coastal/Terrestrial)	-13.9 ± 0.5	16.8 ± 0.9	Walker and DeNiro, 1986
	1500-500 BP Teirra del Fuego Atlantic Coastal Adult remains, Caleta Falsa site (Marine/Coastal)	-12.2 ± 0.9	17.3 ± 1.9	Yesner et al., 2003
	Sadlermiut: Kamarvik, Eastern Arctic (Arctic Marine)	-14.3 ± 0.4	18.2 ± 1.5	Coltrain et al., 2004

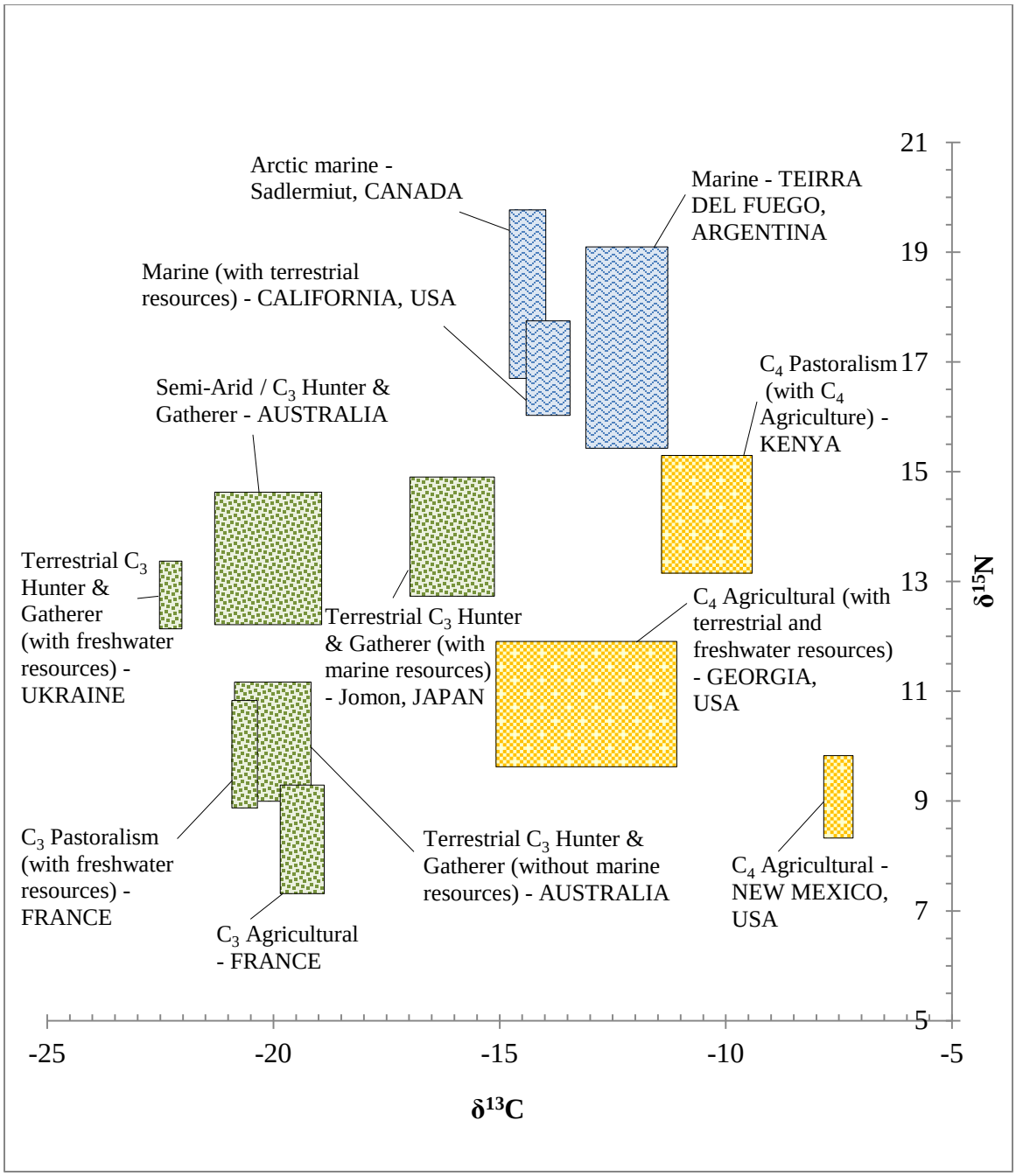

Fig. 2. Stable isotope ranges for specific dietary groups around the world (values, ranges and sources specified in Table 3).

C_3 Environment

C_4 Environment

Marine

3.0 DENTAL CALCULUS

3.1 Calculus Formation

Plaque is a biofilm on teeth that reflects the interaction of oral microbiota, ingested food, and saliva. If plaque is not removed by brushing or flossing, it mineralizes within a few days. In modern humans, this mineralized plaque, or dental calculus, adheres to the tooth while the external surface of the crown is covered by a layer of non-mineralized plaque containing a myriad of microorganisms. Subgingival calculus forms along the surface of the tooth below the gum-line. Supragingival calculus forms along the surface of the tooth above the gum-line (Lieverse, 1999; Chistersson et al., 1992). In ancient human skeletons, plaque degrades so the dental calculus that remains is entirely mineralized.

The pellicle is an organic layer that covers the tooth. The formation of plaque begins with the colonization of the pellicle by bacteria. Plaque is formed from various oral bacteria as well as salivary and gingival fluid. The bacteria responsible for plaque formation are fueled by proteins, glycoproteins, amino acids, and peptides from saliva or gingival crevice fluid (Lieverse, 1999).

Dental plaque is approximately 80% water and contains between 200 and 300 species of bacteria. Of the solid portion, approximately half is protein and bacteria. These proteins, which are a component of the saliva, are little affected by diet. There are also carbohydrates and lipids in plaque which make up about 25% of the solid portion. Carbohydrates are influenced by diet in terms of both amounts and types of carbohydrates. Similarly, polysaccharide levels in plaque are affected by diet. Certain immunoglobulins have also been detected in plaque. Only about 5-10% of the solid portion of plaque is

inorganic. The inorganic constituents are mainly calcium, phosphate and some fluoride taken from the saliva (Rajendran and Sivapathasundharam, 2009; Loesche, 1996).

In contrast to plaque, dental calculus is mostly comprised of inorganic materials, dominated by calcium and phosphate minerals. The organic component of calculus is typically between 15 and 20%. Organic constituents are a mix of amino acids, proteins, peptides, carbohydrates, lipids and glycoproteins (Lieverse, 1999).

The major inorganic minerals of calculus are calcium, phosphorous and magnesium. Of the inorganic portion of calculus, 39 % is calcium, 19% is phosphorous, and 0.8% magnesium. Other minerals found in trace amounts include sodium, zinc, strontium, bromine, copper, manganese, tungsten, gold, aluminum, silicon, iron, and fluorine. The rest of the inorganic portion is mainly oxygen and hydrogen. Calcium phosphate is the major compound in calculus making up about 76% of the compounds present. This is followed by about 3% calcium carbonate (Abraham et al., 2005; Hinrichs JE, 2006).

The mineralization of plaque to dental calculus goes through mineral phases: hydroxyapatite, whitlockite, octacalcium phosphate and brushite. Since these phases vary is mineral composition, components have been studied to detect differences between the newer and older layers. These studies show that while there are local differences in minerals, mineral composition does not significantly change over time (Tsuda and Arends, 1993). Minerals are supplied primarily by the saliva (Slomiany et al., 1983). Lipids are thought to be of great importance in the mineralization of plaque. The source of these lipids is largely attributed to bacteria. Mineralization occurs among the bacteria as crystals form on and within bacterial structures (Slomiany et al., 1983; Lieverse, 1999).

Supragingival and subgingival calculus are similar in appearance, but they differ in composition. The greatest difference is in the inorganic materials where subgingival calculus has greater amounts of calcium, magnesium, strontium, fluoride and sodium. This difference is attributed to the variation in the fluid environments below and above the gumline, or gingiva (Lieverse, 1999).

The organic materials in dental calculus are more complicated to evaluate than the inorganic constituents. Although much is known about the mineralization process where plaque is transformed to calculus, questions still remain, including the role of specific microorganisms, oral pH, and oral fluids. There is no consensus on how these factors act and interact to form dental calculus. Moreover, dietary factors also contribute to plaque and calculus development, such as high carbohydrate quantities (Slomiany et al., 1983; Lieverse, 1999), adding another layer of complexity to the aetiology of calculus.

3.2 Calculus in Anthropological Research

In earlier anthropological studies of dental calculus, focus was mainly on quantity and composition. It was usually examined in contexts relating to periodontal disease. The amount of dental calculus was used to gauge the level of carbohydrate consumption in particular populations. Since carbohydrates contribute to the process of calculus formation, the shift in calculus amounts between different periods of time was thought to indicate a shift in the percentage of carbohydrates in a diet. This was usually examined in conjunction with the number of carious lesions per individual, an oral pathology also linked to high carbohydrate diets (Christersson et al., 1992; Evans, 1973).

Dental calculus has been recently examined for the presence of food residuals, including phytoliths and starch granules. Research on these residuals starts with the

removal of the dental calculus, followed by chemical processing to remove the mineral components. Once a precipitate is isolated, either light microscopy or SEM is used to detect and classify phytoliths and starch granules. Starch is problematic since only some granules can be identified down to the genus level. This is due to the similarity in the appearance of granules across a broad range of species. Phytoliths are opal silicates that help form the structure of plant cell walls. They are difficult to find because of their size, and they are also difficult to identify because most plants have many different types of phytoliths. Despite these limitations, the methods for assessing starch grains and phytoliths in dental calculus have become more sophisticated and the results are proving to be very informative (Fox et al., 1996; Henry and Piperno, 2008; Hardy et al., 2009; Wesolowski et al, 2010; Blatt et al., 2011; Henry et al., 2011). Because calculus can preserve well in the archaeological record (due to its highly mineralized content), it has been successfully examined for starch globules and phytoliths that date as far back as 46,000 years ago (Henry et al., 2011).

Despite increased attention on calculus during the past decade, it was not used as a biomaterial for stable carbon and nitrogen isotope analysis until the study of Scott and Poulson (2012). Their research examined $\delta^{13}C$ and $\delta^{15}N$ in calculus samples from Spain and Alaska. $\delta^{13}C$ values for Spain registered within the range of -17.4‰ to -24.1‰, while the one Alaskan Inuit $\delta^{13}C$ was at -19.5‰. The Spanish sample average for $\delta^{13}C$ values was -21.2‰, a bit lower than the Inuit although there was overlap. Compared to the human dietary clusters in Fig. 2, the medieval Spanish sample compares favorably to European populations who subsisted on mixed resources in a C_3 environment (Scott and Poulson, 2012; Lillie et al., 2003; Herrscher and Le Bras-Goude, 2010).

The medieval Spanish calculus had significantly lower $\delta^{15}N$ than the single Alaskan Inuit sample (Scott and Poulson, 2012). The average $\delta^{15}N$ value for the medieval Spanish was 11.8‰ with a range from 9.4‰ to 15.1‰. For this group, there was no overlap with the Alaskan Inuit sample which had a $\delta^{15}N$ value of 17.5‰. Similar to the $\delta^{13}C$ values, the $\delta^{15}N$ values of the calculus samples are very similar to the $\delta^{15}N$ values from populations subsisting on similar diets (Scott and Poulson, 2012; Coltrain et al., 2004; Herrscher and Le Bras-Goude, 2010). The Spanish compare favorably to European collagen-based isotope values reflecting the consumption of C_3 grasses and the animals that consume C_3 grasses (for both ^{13}C and ^{15}N), while the single Inuit values fall within the range of prehistoric and modern Inuit who consume primarily protein and fat and are near the top of the trophic pyramid for living human populations.

The research by Scott and Poulson (2012) prompted the current research. Their results are consistent with collagen-based values for comparable populations despite the low weight percent values of carbon and nitrogen in dental calculus. There is the potential that soil and the surrounding environment after death might affect the $\delta^{13}C$ and $\delta^{15}N$ of calculus samples. While this is seen in ancient plant specimens, bone collagen and apatite appear to trap the original stable isotope ratios and protect them from contamination (DeNiro, 1987). Although that issue cannot be resolved in this study, the goal here is to move this research forward by testing three biomaterials in living individuals – calculus, hair, fingernails – to determine if calculus in modern populations has isotopic signatures that show agreement with the signatures derived from substances made up entirely of organic molecules (i.e., keratin).

4.0 MATERIALS AND METHODS

Calculus was collected by a dental hygienist from 33 different subjects along with hair and nail samples. Following IRB (Institutional Review Board) protocols, the sex, age and dietary information on these individuals was known only to the dental hygienist. Samples were provided to the researchers labeled as individuals 1-33. The goal of the hygienist was to collect as much calculus as possible from each individual without concern for area of the mouth that produced the calculus.

Because of potential organic contaminants, nail and hair samples were cleaned using a mixture of 2:1 chloroform:methanol for 3 minutes and allowed to air dry. Once dry, they were cleaned again using a solution of 1:30 water:Fisher Versa-Clean. They were then rinsed 3 times in 100 ml jars of deionized water and dried for at least 24 hours in a drying oven.

While the method for cleaning hair and nail was comparable to that employed by other researchers (Thompson et al., 2010, Paritte and Kelly, 2009), the protocol for cleaning calculus for stable isotope testing had no precedent. Some samples were dried and rinsed with deionized water. These samples turned the deionized water milky in appearance, especially the smallest samples. To avoid losing sample materials, samples were dried for a second time in a drying oven. Because components of the calculus dissolved when placed in water, the decision was made to not wash them prior to analysis. They were placed in a drying oven, after which they were powdered without further preparation. This same strategy was employed by Scott and Poulson (2012) for ancient calculus.

The samples for hair and nails weighed approximately 1 mg. With the exceptions

of Samples 19 and 28, all samples had enough nail material to be analyzed. All samples had enough hair for analysis. The quantities of dental calculus were the most varied as some individuals had pronounced calculus while others had slight or modest amounts. The goal for the calculus samples was a target weight of approximately 5 mg although some did not reach this level.

The hair samples were typically larger than what was needed for analysis. Some studies use hair from particular distances from the head, since a single hair can form over a long period of time, allowing for dietary variation along a strand. This study was looking at broad trends, so position of hair relative to the scalp was not a focus. Hair was taken by the dental hygienist according to the convenience of the subjects without noting distance from the scalp. The hair samples were cut and mixed together. Similarly, the nail samples with multiple nails were cut and mixed for each sample.

The stable isotope analysis for $\delta^{13}C$ and $\delta^{15}N$ was run at the Stable Isotope Laboratory of the Mineral Science Department, University of Nevada, Reno. The elemental concentrations and stable isotope compositions were acquired from a Eurovector elemental analyzer interfaced with an Isoprime stable isotope ratio mass spectrometer (Werner et al., 1999). For the stable isotope analysis, an acetanilide elemental analysis standard was used (Costech Analytical Technologies Inc., Valencia, CA).

5.0 RESULTS

Table 4 lists the results of the stable carbon and nitrogen isotope analysis for the modern calculus samples. Tables 5 and 6 list the stable isotope analysis results for modern hair and modern fingernails. These tables include the complete summaries of the stable isotope results for the three biomaterials, including sample weights, elemental compositions and atomic C:N (carbon to nitrogen) ratios. The overall mean and standard deviation are given for all columns except sample weights. Samples with replicates are listed with their replicate number in parentheses. The fingernail samples for Subject 7 had anomalously low weight percent values for carbon and nitrogen. This is likely the result of a weighing error, so the $\delta^{13}C$ and $\delta^{15}N$ values are given, but it is not included in the summary statistics. Similarly, the fingernail sample for Subject 13 was excluded from the analysis because of an abnormal C:N ratio.

Table 4. Elemental concentrations and stable isotope compositions (δ13C and δ15N) of modern dental calculus for Subjects 1-33. Samples too small to give results are marked with ***.

Sample #	Sample Weight (mg)	wt. % C	wt. % N	$\delta^{13}C$ (‰ vs. VPDB)	$\delta^{15}N$ (‰ vs. air)	Atomic C/N
Sample 1	2.034	12.79	2.61	-22.3	8.0	5.72
Sample 2	2.893	33.18	7.15	-19.4	7.1	5.41
Sample 3 (1)	5.294	11.70	2.64	-21.0	8.2	5.17
Sample 3 (2)	2.466	11.75	2.64	-21.0	8.2	5.19
Sample 3 (3)	3.549	14.05	3.13	-21.0	8.9	5.24
Sample 4	1.032	17.09	3.77	-17.9	6.8	5.29
Sample 5	5.822	20.81	4.02	-21.7	6.1	6.04
Sample 6	2.040	44.71	8.92	-21.2	4.3	5.85
Sample 7	1.019	17.59	3.70	-20.2	7.0	5.54
Sample 8	0.066	***	***	***	***	***
Sample 9	5.759	18.91	4.03	-20.3	7.6	5.47
Sample 10	2.574	16.28	3.80	-19.6	11.3	5.00
Sample 11	1.318	19.15	4.20	-20.0	8.0	5.32
Sample 12	0.087	***	***	***	***	***
Sample 13	2.515	26.32	5.95	-21.4	7.6	5.16
Sample 14 (1)	2.749	8.34	1.85	-18.0	7.5	5.26
Sample 14 (2)	3.856	8.43	1.82	-18.0	7.3	5.40
Sample 14 (3)	4.380	8.57	1.86	-18.1	7.8	5.37
Sample 15	1.694	33.71	7.89	-19.6	5.5	4.98
Sample 16	1.131	33.25	7.45	-19.6	5.9	5.21
Sample 17	3.740	44.46	9.58	-17.8	4.9	5.41
Sample 18	2.776	37.01	8.61	-18.7	7.1	5.01
Sample 19	1.837	40.10	9.65	-18.4	5.3	4.85
Sample 20	1.023	41.43	9.05	-19.3	6.2	5.34
Sample 21 (1)	6.134	5.98	1.14	-19.4	8.7	6.12
Sample 21 (2)	5.270	6.05	1.15	-19.4	8.4	6.14
Sample 22	1.741	41.57	9.65	-20.2	7.0	5.02
Sample 23 (1)	4.707	10.70	2.45	-18.5	9.5	5.09
Sample 23 (2)	3.595	14.01	3.38	-18.7	8.4	4.83
Sample 24	4.787	18.77	4.29	-18.1	7.5	5.10

Sample 25	1.469	20.78	4.88	-19.5	7.8	4.97
Sample 26	1.799	35.96	8.63	-18.9	6.9	4.86
Sample 27 (1)	5.124	10.38	2.05	-18.9	7.4	5.91
Sample 27 (2)	5.709	10.44	2.10	-19.0	7.5	5.80
Sample 28	2.497	34.32	7.85	-22.0	6.0	5.10
Sample 29	1.488	25.39	5.59	-20.7	5.6	5.30
Sample 30	1.547	45.48	8.17	-19.1	4.1	6.49
Sample 31	0.423	22.16	5.04	-16.9	3.7	5.13
Sample 32	1.685	45.57	9.38	-21.8	5.1	5.67
Sample 33	5.001	8.27	1.61	-18.6	5.2	5.99
Overall Mean		23.038	5.04	-19.58	6.970	5.387
Standard Deviation		13.093	2.87	1.35	1.577	0.408

Table 5. Elemental concentrations and stable isotope compositions (δ13C and δ15N) of modern hair for Subjects 1-33.

Sample #	Sample Weight (mg)	wt. % C	wt. % N	$\delta^{13}C$ (‰ vs. VPDB)	$\delta^{15}N$ (‰ vs. air)	Ratio C/N
Sample 1	1.027	46.40	15.80	-18.0	8.1	3.43
Sample 2	1.065	46.50	15.83	-18.0	8.1	3.43
Sample 3	1.139	48.22	14.91	-18.5	8.8	3.77
Sample 4	0.543	44.59	14.75	-16.8	8.6	3.53
Sample 5	1.113	48.27	15.79	-18.9	8.7	3.57
Sample 6	1.073	48.10	15.60	-18.4	8.0	3.60
Sample 7 (1)	0.991	47.94	15.68	-18.7	8.5	3.57
Sample 7 (2)	1.040	47.69	15.70	-18.7	8.5	3.54
Sample 8	1.075	48.22	15.86	-19.0	8.2	3.55
Sample 9 (1)	1.096	48.13	15.81	-17.1	9.2	3.55
Sample 9 (2)	1.016	47.94	15.72	-17.1	9.3	3.56
Sample 10	1.039	49.11	15.07	-18.8	8.8	3.80
Sample 11	1.008	46.49	15.52	-17.6	8.8	3.49
Sample 12	1.093	48.39	14.92	-18.2	8.8	3.78
Sample 13	1.026	47.22	16.10	-18.2	7.8	3.42
Sample 14	1.006	45.86	15.14	-16.9	9.1	3.53
Sample 15 (1)	1.023	48.85	15.69	-18.2	8.8	3.63
Sample 15 (2)	1.003	47.43	15.29	-18.2	8.7	3.62
Sample 16	1.084	49.26	15.21	-19.2	7.7	3.78
Sample 17 (1)	1.083	48.37	16.55	-17.3	8.9	3.41
Sample 17 (2)	1.054	47.50	16.22	-17.3	8.8	3.42
Sample 18 (1)	1.047	47.71	16.00	-16.2	9.1	3.48
Sample 18 (2)	1.014	47.80	16.11	-16.2	9.1	3.46
Sample 19	1.020	47.95	16.35	-16.5	8.6	3.42
Sample 20	1.030	48.04	16.42	-17.0	8.6	3.41
Sample 21	1.014	51.55	16.97	-17.7	9.1	3.54
Sample 22	1.131	47.78	15.03	-17.5	8.1	3.71
Sample 23	1.085	49.59	15.13	-18.2	9.1	3.82
Sample 24	1.039	49.10	15.92	-17.4	9.1	3.60
Sample 25	1.022	47.74	16.43	-17.6	8.5	3.39
Sample 26	1.008	49.10	15.74	-17.6	8.3	3.64
Sample 27	1.015	48.38	16.33	-17.0	9.2	3.46

Sample 28	1.090	49.02	15.72	-20.4	8.3	3.64
Sample 29	1.062	49.63	16.20	-18.5	8.4	3.57
Sample 30	0.993	48.93	15.91	-16.9	9.4	3.59
Sample 31	1.063	49.74	16.06	-15.9	9.3	3.61
Sample 32	1.072	49.62	16.73	-18.1	8.8	3.46
Sample 33	0.976	47.45	15.75	-17.9	8.6	3.51
Overall Mean		48.148	15.788	-17.78	8.67	3.559
Standard Deviation		1.233	0.527	0.94	0.43	0.120

Table 6. Elemental concentrations and stable isotope compositions (δ13C and δ15N) of modern fingernails for Subjects 1-33. C and N weight percentages suggesting a weighing error is marked by **. C/N ratio outside of the accepted range is marked by *.

Sample #	Sample Weight (mg)	wt. % C	wt. % N	$\delta^{13}C$ (‰ vs. VPDB)	$\delta^{15}N$ (‰ vs. air)	Ratio C/N
Sample 1 (1)	1.015	47.85	16.26	-18.7	9.0	3.43
Sample 1 (2)	1.018	47.83	16.28	-18.7	9.0	3.43
Sample 2 (1)	1.092	48.38	16.34	-18.4	9.0	3.45
Sample 2 (2)	1.073	48.54	16.37	-18.4	9.0	3.46
Sample 3 (1)	1.103	48.32	16.32	-18.3	9.3	3.45
Sample 3 (2)	1.001	48.44	16.32	-18.5	8.9	3.46
Sample 4	1.057	49.15	16.58	-16.9	9.0	3.46
Sample 5	1.051	48.24	16.33	-18.8	9.2	3.45
Sample 6	1.075	47.79	16.25	-18.5	8.7	3.43
Sample 7	**	10.62	3.52	-18.9	9.8	3.52
Sample 8	1.101	43.20	14.46	-19.1	9.0	3.48
Sample 9	1.007	48.42	16.12	-17.5	9.7	3.50
Sample 10	1.118	44.85	14.28	-20.5	8.8	3.66
Sample 11	1.080	41.58	13.97	-17.9	9.1	3.47
Sample 12	1.024	48.65	16.12	-18.2	9.0	3.52
Sample 13 *	1.112	49.58	14.06	-20.6	8.1	4.11*
Sample 14	1.009	48.67	16.50	-17.1	9.8	3.44
Sample 15	1.112	48.41	16.35	-18.5	9.3	3.45
Sample 16	1.059	49.05	15.91	-19.4	8.2	3.60
Sample 17	1.006	49.10	16.81	-17.5	9.5	3.41
Sample 18	1.064	49.19	16.74	-17.0	9.6	3.43
Sample 20	1.019	50.62	16.53	-18.0	9.1	3.57
Sample 21 (1)	1.146	49.60	16.85	-17.8	9.7	3.43
Sample 21 (2)	0.989	49.72	16.43	-18.0	9.6	3.53
Sample 22 (1)	0.984	49.93	16.91	-17.8	8.5	3.44
Sample 22 (2)	1.043	49.25	16.66	-17.8	8.5	3.45
Sample 23	0.997	50.69	16.24	-17.9	9.7	3.64
Sample 24	1.045	50.93	16.43	-18.3	9.4	3.62
Sample 25	1.001	49.66	16.76	-18.2	9.1	3.46
Sample 26	1.007	49.75	16.95	-17.0	9.5	3.42
Sample 27	1.025	49.31	16.66	-17.5	9.8	3.45

Sample 29	1.034	48.58	16.53	-18.4	9.0	3.43
Sample 30	1.020	47.35	16.25	-17.1	9.8	3.40
Sample 31	1.038	49.63	16.57	-16.2	10.0	3.49
Sample 32 (1)	1.025	49.35	16.57	-18.9	9.5	3.47
Sample 32 (2)	1.038	49.99	16.45	-19.1	9.5	3.54
Sample 33	0.994	49.80	16.78	-18.4	9.1	3.46
Overall Mean		48.594	16.221	-18.20	9.20	3.471
Standard Deviation		1.887	0.767	0.90	0.46	0.063

C:N ratios for hair were between 3.389 and 3.822. The C:N ratios for fingernails fell between 3.40 and 4.11. The C:N ratios for hair were similar to those from other studies which set the range for valid results between 3.0 and 3.8 (O'Connell, 1996; O'Connell and Hedges, 1999). The fingernail sample from Subject 13 has an unusually high C:N ratio at 4.12. Omitting this subject, the maximum C:N ratio from the fingernail samples was 3.66. This is in line with other research that provides ranges for C:N ratios (O'Connell et al., 2001).

Table 7 shows the $\Delta_{calculus\text{-}hair}$, $\Delta_{calculus\text{-}nails}$ and $\Delta_{hair\text{-}nails}$ for $\delta^{13}C$. Table 8 shows the $\Delta_{calculus\text{-}hair}$, $\Delta_{calculus\text{-}nails}$ and $\Delta_{hair\text{-}nails}$ for $\delta^{15}N$. For both tables, samples with replicates were averaged before values were compared between the three sample types. For the $\Delta_{calculus\text{-}hair}$, $\Delta_{calculus\text{-}nails}$ and $\Delta_{hair\text{-}nails}$ for $\delta^{13}C$ and $\delta^{15}N$, the statistical values given are the overall mean, standard deviation, standard error, and range. In these tables, it is noteworthy that the range for $\Delta_{calculus\text{-}hair}$ and $\Delta_{calculus\text{-}nails}$ is about twice as high for $\delta^{15}N$ than for $\delta^{13}C$. Not every calculus sample has a corresponding fingernail sample (Samples 19 and 28), but there is a corresponding hair sample for every calculus sample. Similarly, a few of the calculus samples were too small to analyze (Samples 8 and 12). Because the fingernail sample from Subject 7 had a good C:N ratio, it is included here despite a probable weighing error seen in the low weight percent values, but it is not used for the statistical values.

Table 7. Difference comparisons in $\delta^{13}C$ for modern calculus, hair and fingernails. * = Sample was unavailable; ** = Sample was too small to analyze; ***Sample excluded because of invalid C/N ratio

Sample #	Calculus $\delta^{13}C$	Hair $\delta^{13}C$	Nails $\delta^{13}C$	$\Delta_{calculus-hair}$	$\Delta_{calculus-nails}$	$\Delta_{hair-nails}$
Sample 1	-22.3	-18.0	-18.7	-4.3	-3.6	0.7
Sample 2	-19.4	-18.0	-18.4	-1.4	-1.0	0.4
Sample 3	-21.0	-18.5	-18.4	-2.5	-2.6	-0.1
Sample 4	-17.9	-16.8	-16.9	-1.1	-1.0	0.1
Sample 5	-21.7	-18.9	-18.8	-2.8	-2.9	-0.1
Sample 6	-21.2	-18.4	-18.5	-2.8	-2.7	0.1
Sample 7	-20.2	-18.7	-18.9	-1.5	-1.3	0.2
Sample 8	**	-19.0	-19.1			0.1
Sample 9	-20.3	-17.1	-17.5	-3.2	-2.8	0.4
Sample 10	-19.6	-18.8	-20.5	-0.8	0.9	1.7
Sample 11	-20.0	-17.6	-17.9	-2.4	-2.1	0.3
Sample 12	**	-18.2	-18.2			0.0
Sample 13	-21.4	-18.2	***	-3.2		
Sample 14	-18.0	-16.9	-17.1	-1.1	-0.9	0.2
Sample 15	-19.6	-18.2	-18.5	-1.4	-1.1	0.3
Sample 16	-19.6	-19.2	-19.4	-0.4	-0.2	0.2
Sample 17	-17.8	-17.3	-17.5	-0.5	-0.3	0.2
Sample 18	-18.7	-16.2	-17.0	-2.5	-1.7	0.8
Sample 19	-18.4	-16.5	*	-1.9		
Sample 20	-19.3	-17.0	-18.0	-2.3	-1.3	1.0
Sample 21	-19.4	-17.7	-17.9	-1.7	-1.5	0.2
Sample 22	-20.2	-17.5	-17.8	-2.7	-2.4	0.3
Sample 23	-18.6	-18.2	-17.9	-0.4	-0.7	-0.3
Sample 24	-18.1	-17.4	-18.3	-0.7	0.2	0.9
Sample 25	-19.5	-17.6	-18.2	-1.9	-1.3	0.6
Sample 26	-18.9	-17.6	-17.0	-1.3	-1.9	-0.6
Sample 27	-19.0	-17.0	-17.5	-2.0	-1.5	0.5
Sample 28	-22.0	-20.4	*	-1.6		
Sample 29	-20.7	-18.5	-18.4	-2.2	-2.3	-0.1
Sample 30	-19.1	-16.9	-17.1	-2.2	-2.0	0.2
Sample 31	-16.9	-15.8	-16.2	-1.1	-0.7	0.4
Sample 32	-21.8	-18.1	-19.1	-3.7	-2.7	1.0
Sample 33	-18.6	-17.9	-18.4	-0.7	-0.2	0.5
Overall Mean				-1.87	-1.49	0.32

Standard Deviation	0.99	1.07	0.45
Standard Error	0.18	0.20	0.08
Range	3.9	4.5	2.3

Table 8. Difference comparisons in $\delta^{15}N$ for modern calculus, hair and fingernails. * = Sample was unavailable; ** = Sample was too small to analyze; ***Sample excluded because of invalid C/N ratio

Sample #	Calculus $\delta^{15}N$	Hair $\delta^{15}N$	Nails $\delta^{15}N$	$\Delta_{calculus\text{-}hair}$	$\Delta_{calculus\text{-}nails}$	$\Delta_{hair\text{-}nails}$
Sample 1	8.0	8.1	9.0	-0.1	-1.0	-0.9
Sample 2	7.1	8.1	9.0	-1.0	-1.9	-0.9
Sample 3	8.4	8.8	9.1	-0.4	-0.7	-0.3
Sample 4	6.8	8.6	9.0	-1.8	-2.2	-0.4
Sample 5	6.1	8.7	9.2	-2.6	-3.1	-0.5
Sample 6	4.3	8.0	8.7	-3.7	-4.4	-0.7
Sample 7	7.0	8.5	9.8	-1.5	-2.8	-1.3
Sample 8	**	8.2	9.0			-0.8
Sample 9	7.6	9.2	9.7	-1.6	-2.1	-0.5
Sample 10	11.3	8.8	8.8	2.5	2.5	0.0
Sample 11	8.0	8.8	9.1	-0.8	-1.1	-0.3
Sample 12	**	8.8	9.0			-0.2
Sample 13	7.6	7.8	***	-0.2		
Sample 14	7.5	9.1	9.8	-1.6	-2.3	-0.7
Sample 15	5.5	8.7	9.3	-3.2	-3.8	-0.6
Sample 16	5.9	7.7	8.2	-1.8	-2.3	-0.5
Sample 17	4.9	8.9	9.5	-4.0	-4.6	-0.6
Sample 18	7.1	9.1	9.6	-2.0	-2.5	-0.5
Sample 19	5.3	8.6	*	-3.3		
Sample 20	6.2	8.6	9.1	-2.4	-2.9	-0.5
Sample 21	8.5	9.1	9.6	-0.6	-1.1	-0.5
Sample 22	7.0	8.1	8.5	-1.1	-1.5	-0.4
Sample 23	9.0	9.1	9.7	-0.1	-0.7	-0.6
Sample 24	7.5	9.1	9.4	-1.6	-1.9	-0.3
Sample 25	7.8	8.5	9.1	-0.7	-1.3	-0.6
Sample 26	6.9	8.3	9.5	-1.4	-2.6	-1.2
Sample 27	7.4	9.2	9.8	-1.8	-2.4	-0.6
Sample 28	6.0	8.3	*	-2.3		
Sample 29	5.6	8.4	9.0	-2.8	-3.4	-0.6
Sample 30	4.1	9.4	9.8	-5.3	-5.7	-0.4
Sample 31	3.7	9.3	10.0	-5.6	-6.3	-0.7
Sample 32	5.1	8.8	9.5	-3.7	-4.4	-0.7
Sample 33	5.2	8.6	9.1	-3.4	-3.9	-0.5
Overall Mean				-1.94	-2.52	-0.57

Standard Deviation	1.65	1.75	0.26
Standard Error	0.30	0.33	0.05
Range	8.1	8.8	1.3

Overall, hair was enriched over fingernails in $\delta^{13}C$ by an average of 0.39‰ while fingernails were enriched over hair in $\delta^{15}N$ by an average of 0.56‰. For $\delta^{13}C$, hair was enriched over calculus by an average of 1.87‰, and fingernail samples were more enriched than calculus samples by an average of 1.46‰. With $\delta^{15}N$, hair was enriched by an average of 1.88‰ over calculus, and nail was enriched by an average of 2.39‰ over calculus.

Fig. 3 is a scatterplot showing the $\delta^{13}C$ comparisons for calculus, hair and fingernail. The three samples are plotted against each other giving three different linear regressions. The samples with replicates were averaged to compare sample values between the three samples types. For $\delta^{13}C$ comparisons, the coefficient of determination (r^2) was 0.48 for calculus and hair, 0.34 for calculus and fingernail, and 0.74 for hair and fingernail.

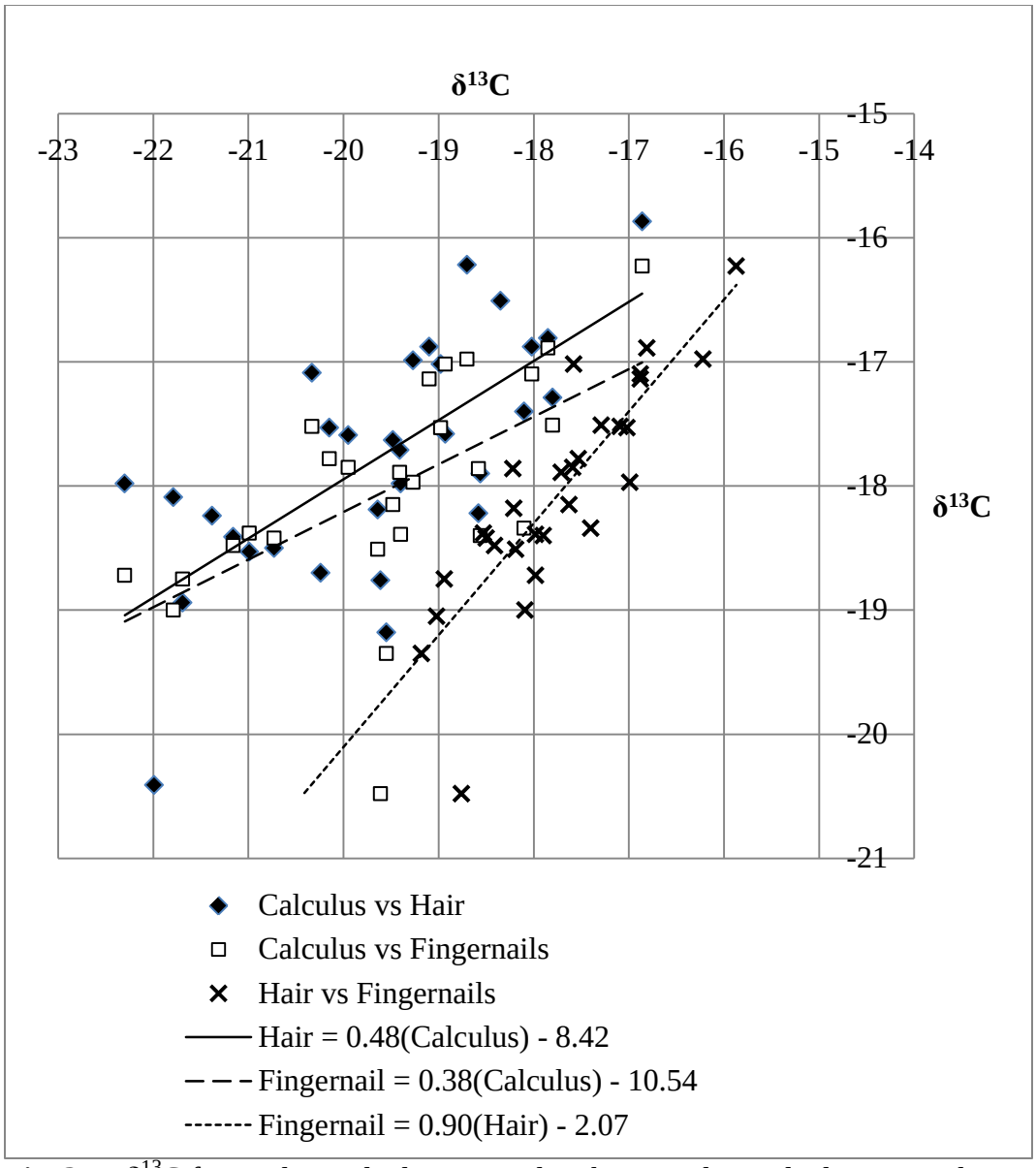

Fig. 3. $\delta^{13}C$ for modern calculus vs. modern hair, modern calculus vs. modern fingernail, and modern hair vs. modern fingernail.

Fig. 4 is a scatterplot showing the $\delta^{15}N$ comparisons for calculus, hair and fingernails. Again, the three samples are plotted against one another, yielding three regression lines. The samples with replicates were averaged to compare sample values between the three samples types. For $\delta^{15}N$ comparisons, the coefficient of determination was 0.00 for modern calculus and modern hair, 0.01 for modern calculus and modern fingernail, and 0.66 for modern hair and modern fingernail.

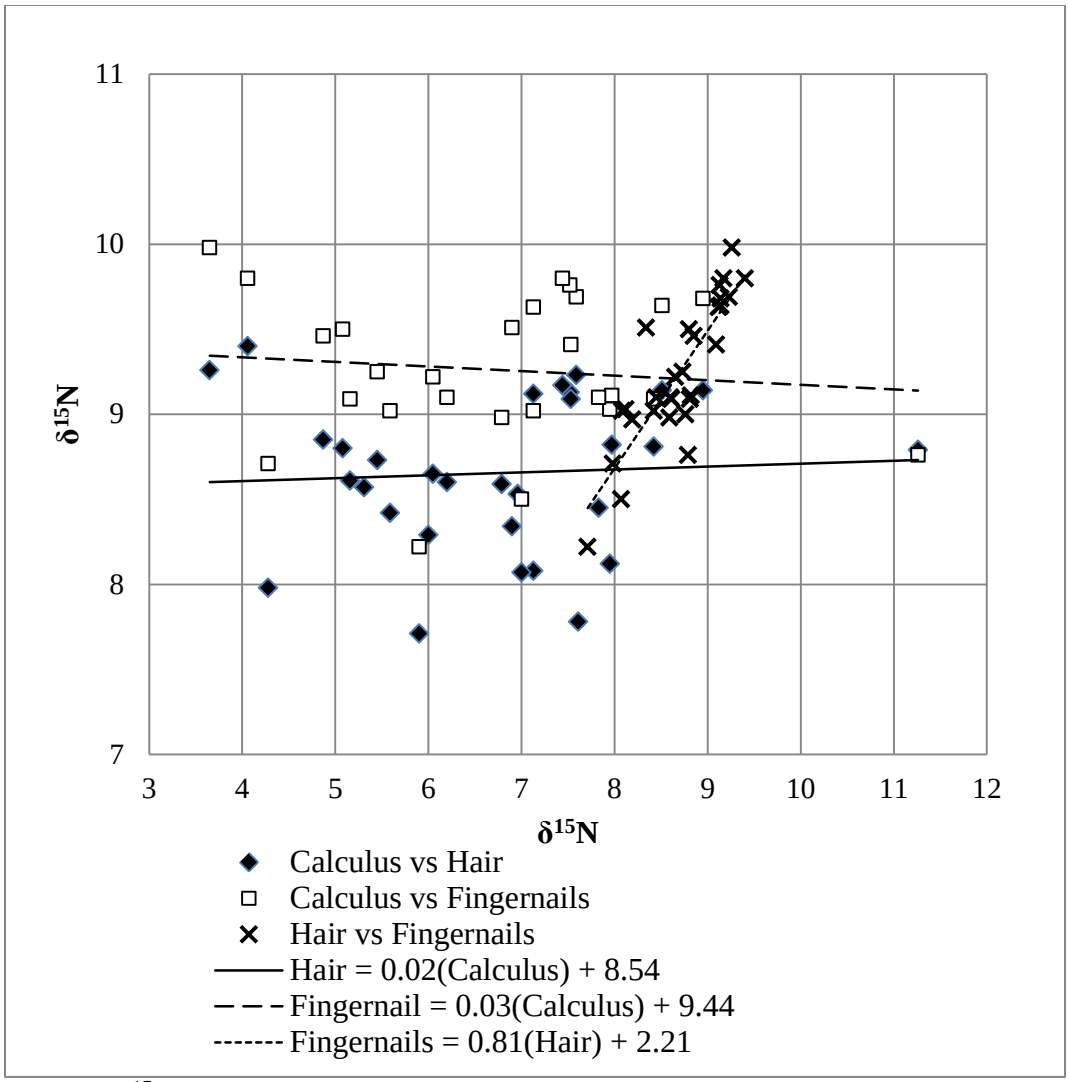

Fig. 4. $\delta^{15}N$ for modern calculus vs. modern hair, modern calculus vs. modern fingernail, and modern hair vs. modern fingernail.

One-on-one comparisons between the $\delta^{13}C$ values of calculus and those of hair and fingernails are shown in Figs. 5 and 6. Scatterplots show significant correlations for $\delta^{13}C$ compositions between calculus and hair ($r = 0.69$) and calculus and fingernail ($r= 0.58$). One-on-one comparisons between the $\delta^{15}N$ values of calculus and those of hair and fingernails are shown in Figs. 7 and 8. In contrast to carbon isotopes, there are low and nonsignificant correlations between calculus and hair ($r = 0.06$) and calculus and fingernail ($r = -0.11$). For $\delta^{15}N$ values, calculus has a much higher range (ca. 3.5 – 11.5) of values compared to the ranges for hair (7.5 – 9.5) and fingernail (8 and 10). Overall, the range of $\delta^{15}N$ values for calculus was 7.61‰, but only 1.69‰ for hair and 1.93‰ for fingernail. Contrasting the results for nitrogen, the ranges for $\delta^{13}C$ values are about the same for calculus (ca. 4) and hair/fingernail (ca. 5).

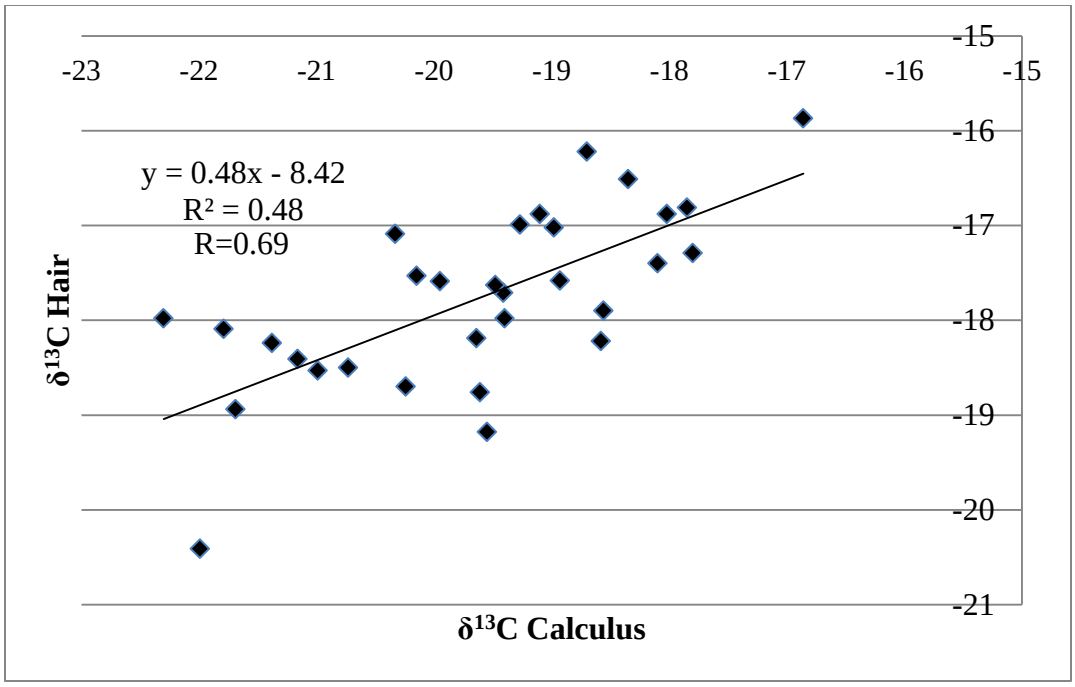

Fig. 5. $\delta^{13}C$ for modern calculus plotted against $\delta^{13}C$ for modern hair.

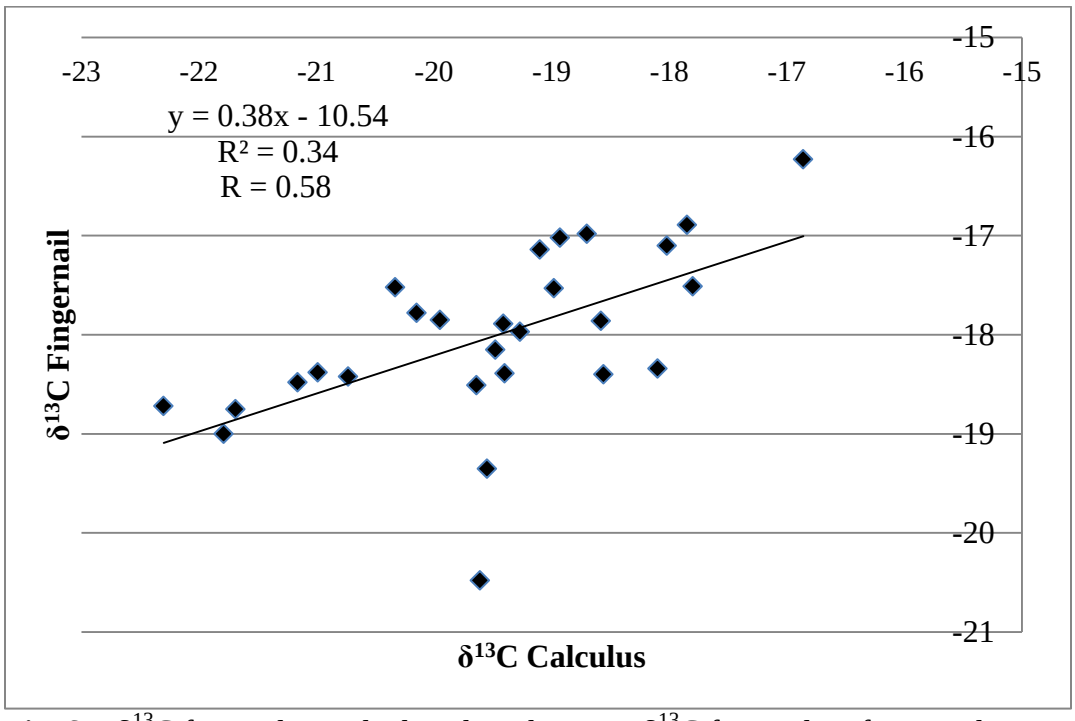

Fig. 6. $\delta^{13}C$ for modern calculus plotted against $\delta^{13}C$ for modern fingernail.

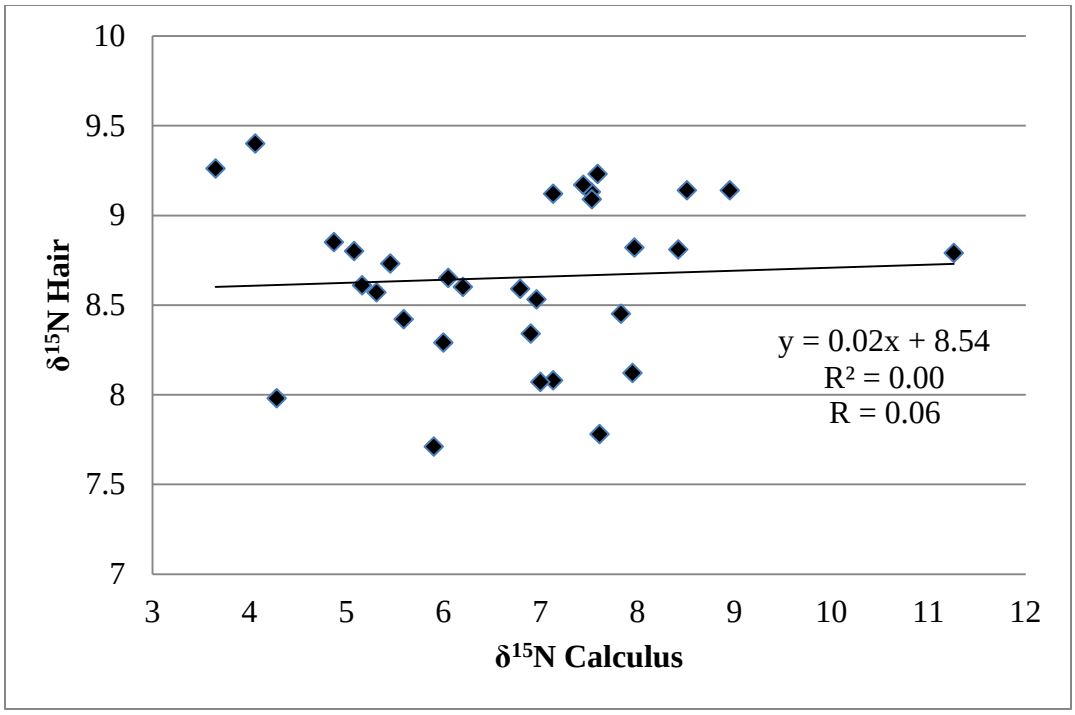

Fig. 7. $\delta^{15}N$ for modern calculus plotted against $\delta^{15}N$ for modern hair.

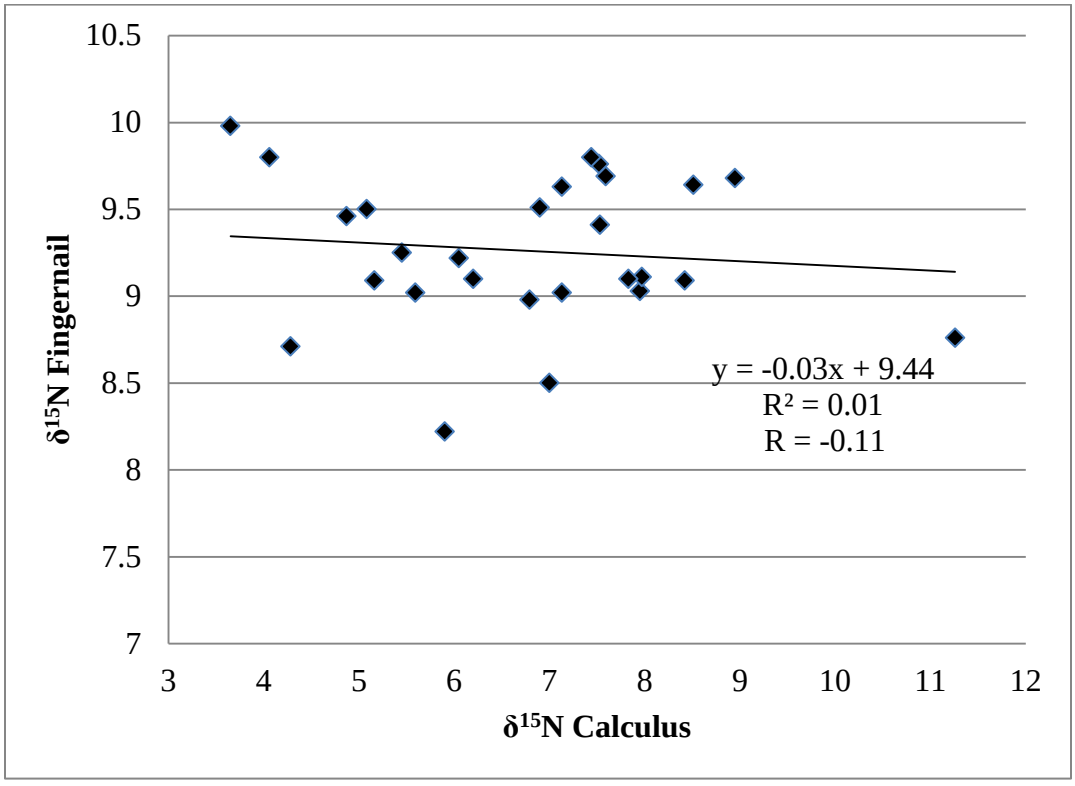

Fig. 8. $\delta^{15}N$ for modern calculus plotted against $\delta^{15}N$ for modern fingernail.

Fig. 9 shows $\delta^{13}C$ plotted against $\delta^{15}N$ for calculus, hair and fingernails, with replicates averaged. $\delta^{13}C$ and $\delta^{15}N$ comparisons for each biomaterial yielded small to moderate coefficients of determination for calculus (0.01), hair (0.29), fingernail (0.40).

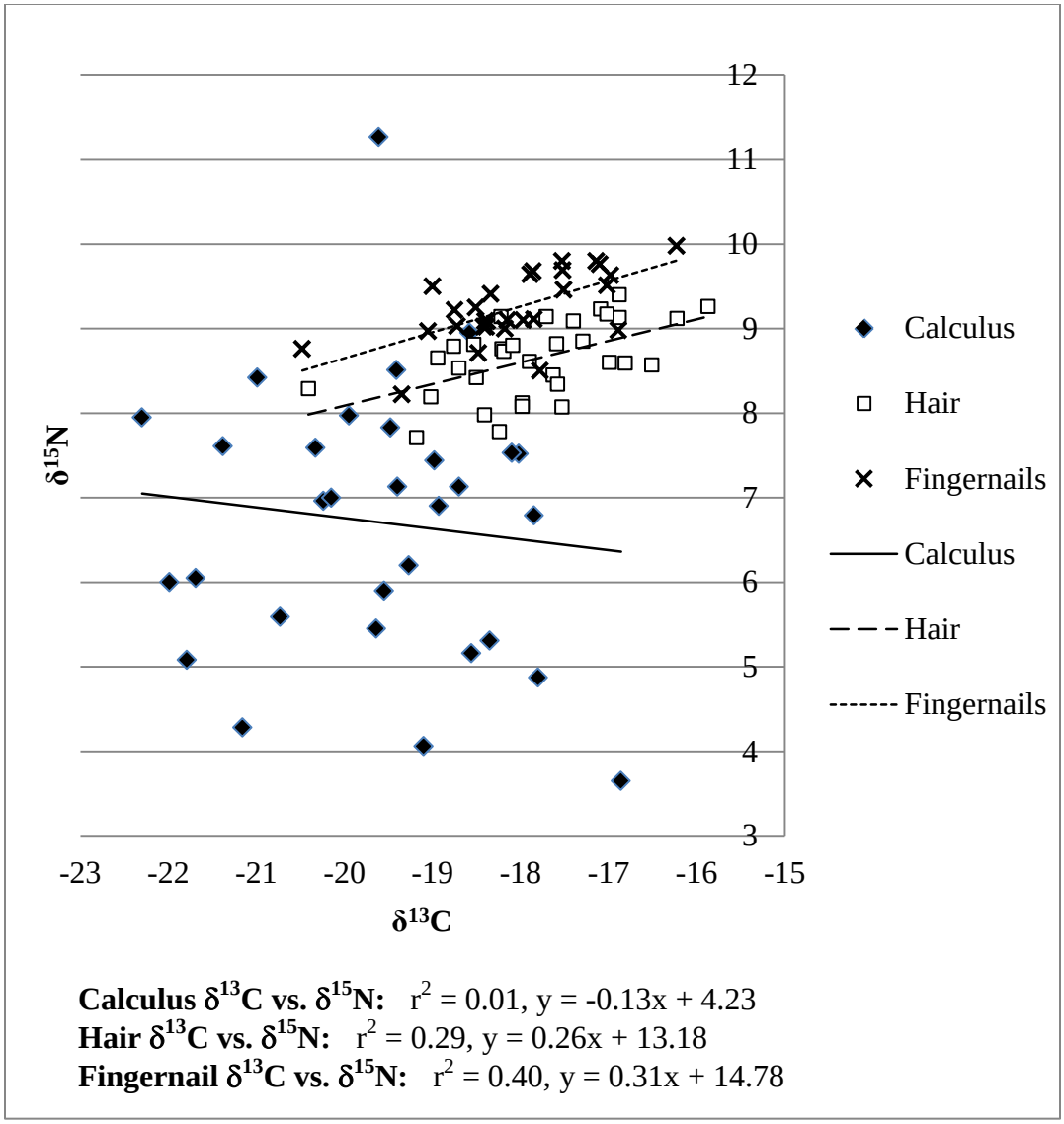

Fig. 9. $\delta^{13}C$ plotted against $\delta^{15}N$ for modern calculus, modern hair and modern fingernails.

Weight % carbon and nitrogen values vary more for calculus than for hair and nails. (Fig. 10). Even with a broad range of variation, the coefficient of determination was 0.98 for weight % carbon vs. weight % nitrogen values for calculus. Although stable carbon and nitrogen isotope compositions from calculus show no correlation, the actual amount of carbon and nitrogen in the calculus samples is strongly correlated. For comparative purposes, data from Scott and Poulson (2012) are included in Fig. 10 to show how weight % values differ between ancient and modern calculus. Although carbon and nitrogen are reduced significantly through diagenesis, even at lower levels they are still strongly correlated with one another in the calculus samples.

In contrast to the high correlation between weight % carbon and weight % nitrogen for modern calculus ($r^2 = 0.98$), the correlations are much weaker for both hair and fingernail. The coefficients of determination are only 0.16 for the hair samples and 0.55 for the fingernail samples (Fig. 11). This contrast is attributable to the much smaller ranges for weight % values of hair and fingernails compared to the broad range shown by modern calculus.

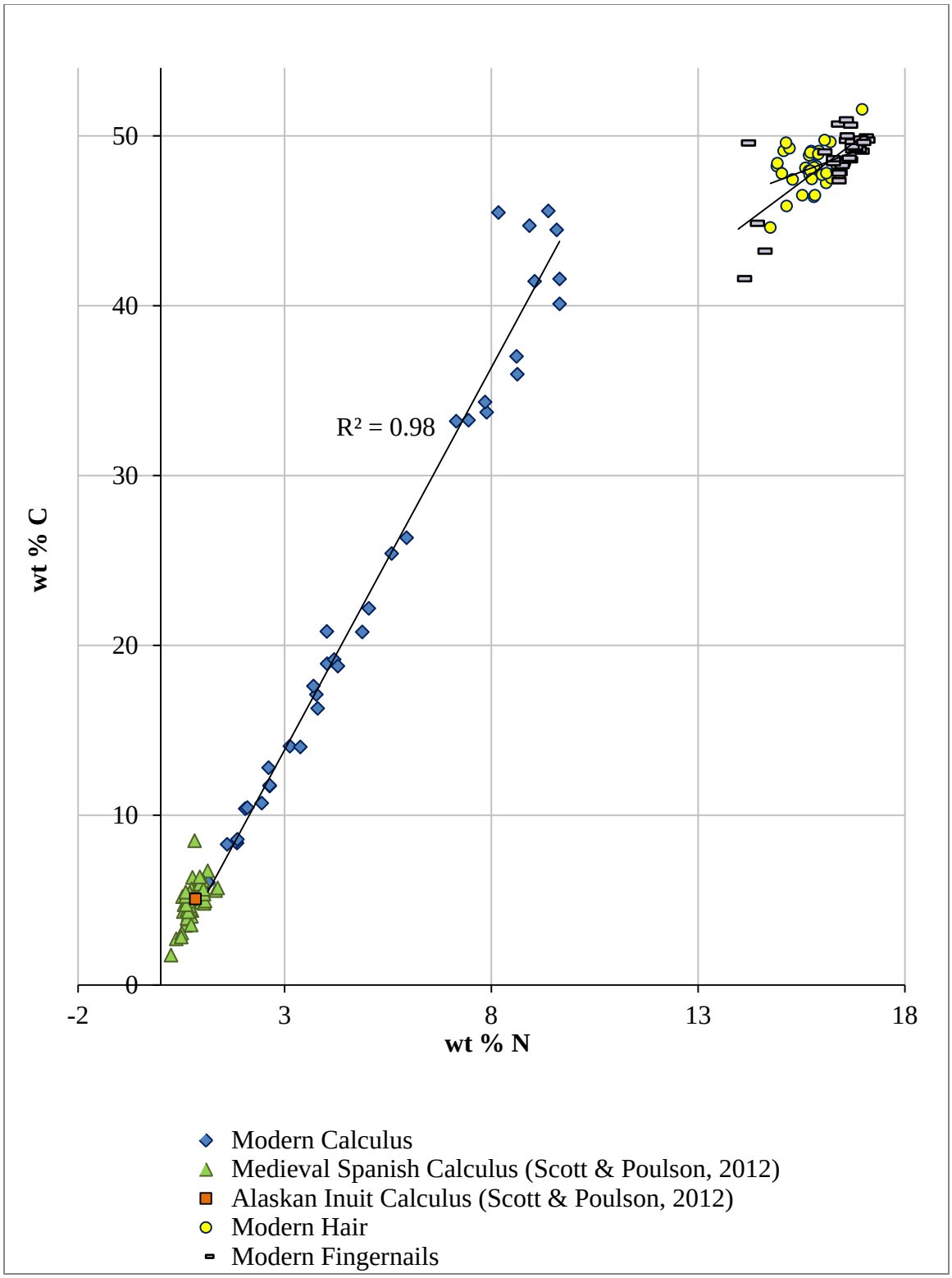

Fig. 10. Weight percent carbon plotted against weight percent nitrogen.

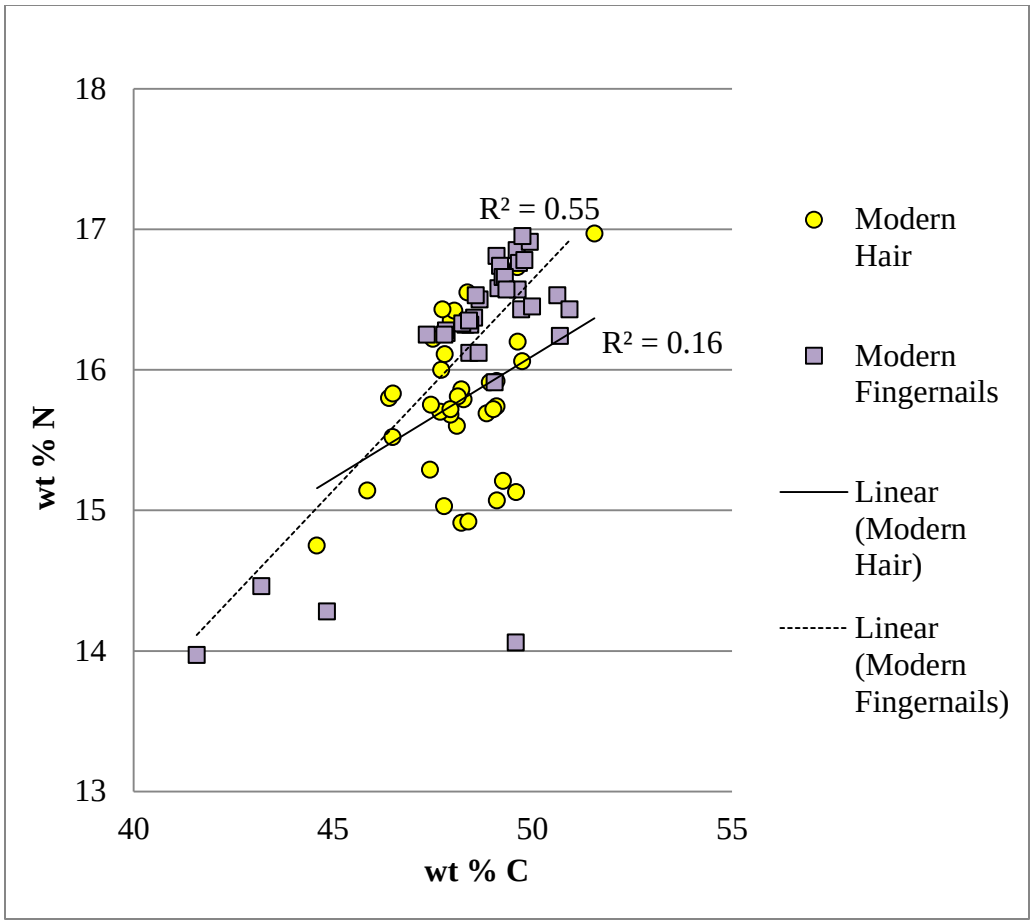

Fig. 11. Weight percent carbon plotted against weight percent nitrogen for modern calculus.

A few samples of calculus, hair and fingernails were large enough to allow for an evaluation of replicability. The results of the replicate analyses are shown in Table 9. The variation between calculus replicates is slight, and is larger for $\delta^{15}N$ than for $\delta^{13}C$. The greatest difference between replicates for both carbon and nitrogen isotopes was found in Sample 23 (1.1‰ difference for $\delta^{15}N$ and 0.3‰ difference for $\delta^{13}C$). The calculus replicates showed greater isotope disparities than for hair replicates where the average difference was 0.04‰ or less. The replicates for fingernails showed about the same level of difference as the calculus samples (between 0.07 - 0.11‰).

Table 9. Within sample variation of the samples with replicates for modern calculus, modern hair and modern fingernails.

Calculus $\delta^{13}C$	Difference $\delta^{13}C$	Difference $\delta^{15}N$
Sample 3 (Replicate 1-2)	0.0	0.0
Sample 3 (Replicate 2-3)	0.0	-0.7
Sample 3 (Replicate 1-3)	0.1	-0.7
Sample 14 (Replicate 1-2)	0.0	0.1
Sample 14 (Replicate 2-3)	0.1	-0.4
Sample 14 (Replicate 1-3)	0.1	-0.3
Sample 21 (Replicate 1-2)	0.1	0.3
Sample 23 (Replicate 1-2)	0.3	1.1
Sample 27 (Replicate 1-2)	0.1	-0.1
Overall Mean Difference	0.07	-0.08
Standard Deviation	0.08	0.54
Standard Error	0.03	0.18
Range	0.3	1.8

Hair $\delta^{13}C$	Difference $\delta^{13}C$	Difference $\delta^{15}N$
Sample 7 (Replicate 1-2)	0.0	0
Sample 9 (Replicate 1-2)	0.0	-0.1
Sample 15 (Replicate 1-2)	0.0	0.1
Sample 17 (Replicate 1-2)	0.0	0.1
Sample 18 (Replicate 1-2)	0.0	0.1
Overall Mean Difference	0.00	0.04
Standard Deviation	0.00	0.06
Standard Error	0.00	0.03
Range	0.0	0.2

Fingernail $\delta^{13}C$	Difference $\delta^{13}C$	Difference $\delta^{15}N$
Sample 1 (Replicate 1-2)	0.0	0.0
Sample 2 (Replicate 1-2)	0.1	0.0
Sample 3 (Replicate 1-2)	0.2	0.3
Sample 21 (Replicate 1-2)	0.2	0.1
Sample 22 (Replicate 1-2)	-0.1	0.0
Sample 32 (Replicate 1-2)	0.2	-0.1
Overall Mean Difference	0.11	0.07
Standard Deviation	0.12	0.15
Standard Error	0.05	0.06
Range	0.3	0.4

6.0 DISCUSSION

6.1 $\delta^{13}C$ and $\delta^{15}N$ of Hair and Fingernail

While the emphasis of this research is on $\delta^{13}C$ and $\delta^{15}N$ of calculus in relation to hair and fingernails, the correlation of stable isotope ratios between hair and fingernails is also significant. O'Connell et al. (2001) provides correlations between three biomaterials - hair, fingernails, and bone collagen. Since fingernail and hair correlations are provided in O'Connell et al. (2001), they are contrasted to findings in this research.

Fig. 12 shows $\delta^{13}C$ values of hair and fingernails plotted against each other for the two samples. For the present study, hair is enriched in ^{13}C by an average of 0.32‰, while O'Connell et al. (2001) found hair to be enriched in ^{13}C by an average of 0.21‰. Interestingly, there was no overlap in the distribution of ^{13}C in the two studies. In the present study, carbon isotope values varied from about -21 to -16 while the smaller O'Connell et al. (2001) sample had values between -23 and -21. One can only presume some contrast in the consumption of C_4 plants between the two samples. The regression for hair/nail was closer to 1.0 in the present study (0.90) compared to that of the O'Connell et al. (2001) sample (0.60).

$\delta^{15}N$ values for hair and fingernails are plotted in Fig. 13. In contrast to the $\delta^{13}C$ values, $\delta^{15}N$ values show almost complete overlap between the two samples where both vary between 8 and 10, with but one outlier. For nitrogen, there was also much closer correspondence in enrichment. That is, for the present study, nails were more ^{15}N enriched than hair by an average of 0.60‰, a value very similar to the 0.65‰ reported by O'Connell et al. (2001).

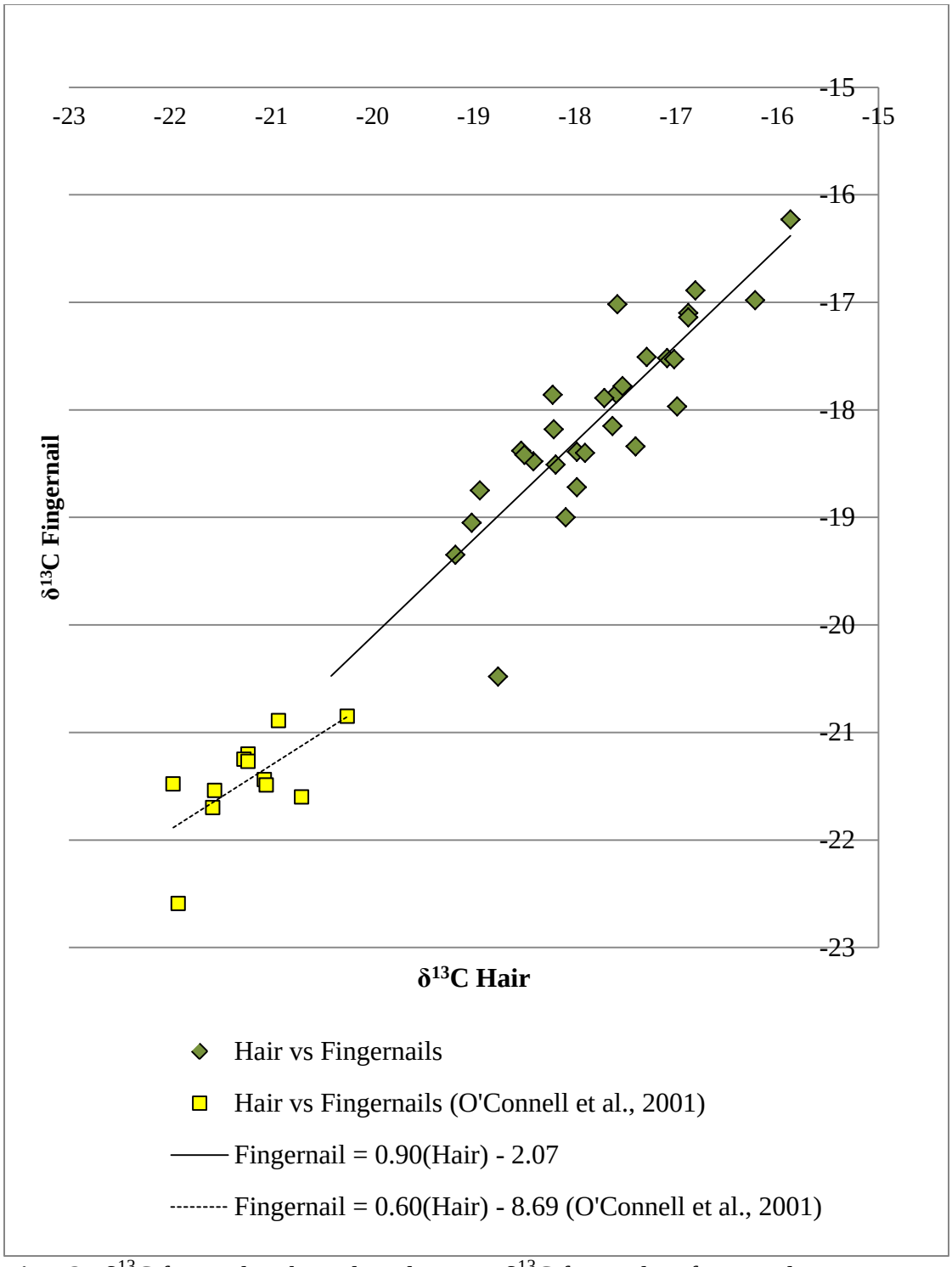

Fig. 12 $\delta^{13}C$ for modern hair plotted against $\delta^{13}C$ for modern fingernail.

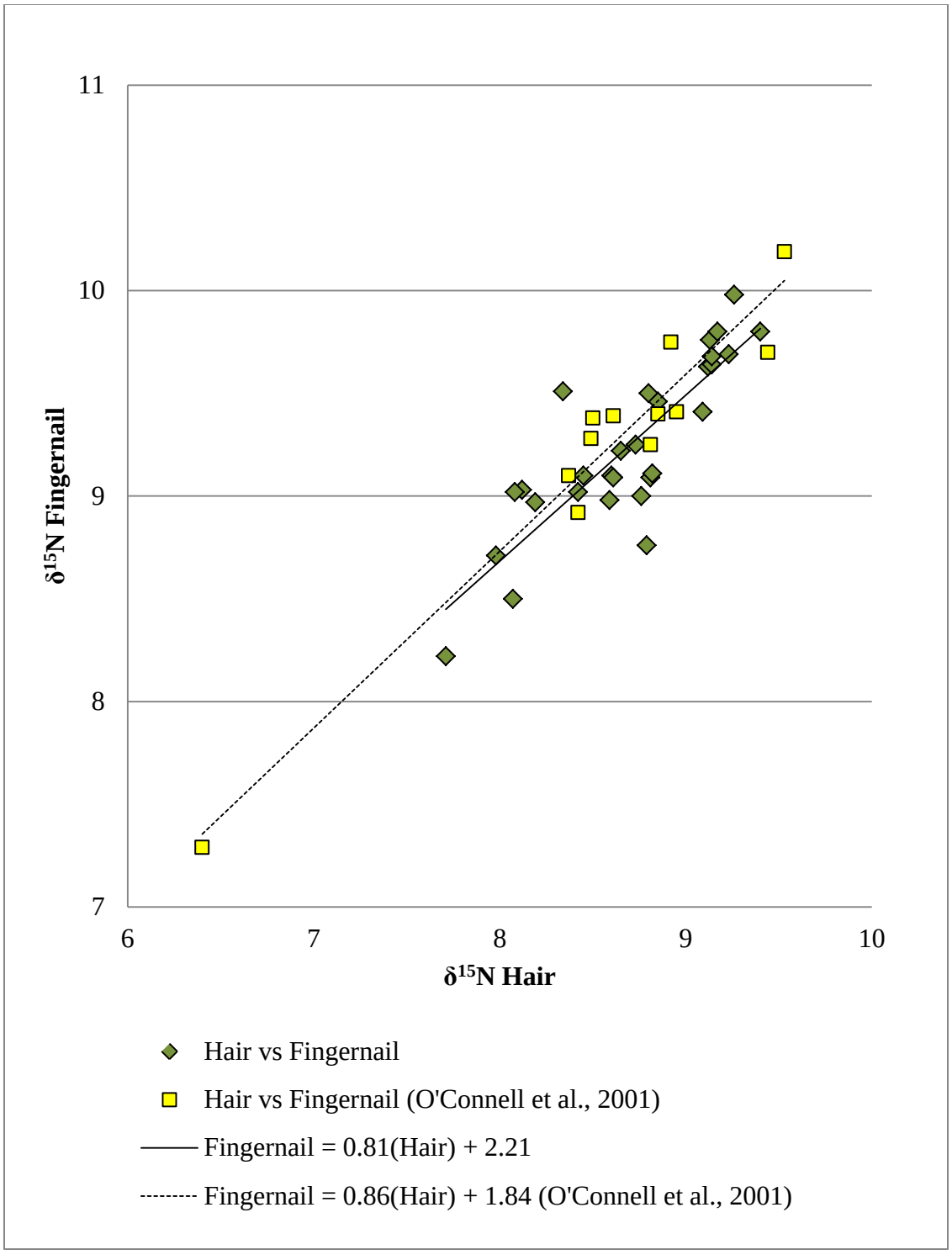

Fig. 13. $\delta^{15}N$ for modern hair plotted against $\delta^{15}N$ for modern fingernail.

While the average enrichments of this study are similar to those of O'Connell et al. (2001), the correlation for $\delta^{13}C$ between hair and fingernails was higher in the present study. In this study, the coefficient of determination (r^2) is 0.73, with a linear regression of y = 0.90x -2.07. In O'Connell et al. (2001), r^2 was 0.43 with a linear regression of y = 0.60x – 8.69. While both studies yield significant correlation values, the slopes of the regression equations are different. Because hair and nails are both keratin, the expectation is a 1 to 1 ratio and a regression coefficient of around 1. With a sample of 33 individuals, the present study yields a better 1 to 1 correlation between hair and nails. The deviation from the expected 1:1 ratio reported by O'Connell et al. (2001) is likely due to the narrow range of $\delta^{13}C$ for hair and nails (i.e., only 0.96‰). The plots for $\delta^{13}C$ from O'Connell et al. (2001) nonetheless cluster on the regression line found in this study.

Fingernails and hair were highly correlated for $\delta^{15}N$ compositions with an r^2 of 0.73. For $\delta^{15}N$ values, O'Connell et al. (2001) found even higher correlations between fingernails and hair ($r^2 = 0.94$). With $\delta^{15}N$ of hair on the x-axis, the regression equations for both studies are similar. In this study, the slope is 0.81 while O'Connell et al. (2001) derived a slope of 0.86. Both slopes are close to the ideal of 1. Overall, this study found generally similar results to O'Connell et al. (2001) for both $\delta^{13}C$ and $\delta^{15}N$ concerning hair and fingernails, except for the contrast in ranges for $\delta^{13}C$ in the two studies.

6.2 Plaque and Dental Calculus

Dental calculus has been analyzed for stable isotopes using ancient calculus and results are promising for both $\delta^{13}C$ and $\delta^{15}N$ (Scott and Poulson, 2012). Medieval Spanish samples yielded $\delta^{15}N$ values between 9.4‰ and 15.1‰, while a single Alaskan

Inuit had $\delta^{15}N$ at 17.5‰, in line with dietary expectations. Medieval Spanish samples gave $\delta^{13}C$ values between -24.1‰ and -17.4‰, while a single Inuit gave a $\delta^{13}C$ value of -19.5‰ (Scott and Poulson, 2012).

While the $\delta^{15}N$ range for medieval Spanish calculus was 5.7‰, it was 7.6‰ for the modern calculus samples. The modern calculus range seems high given the $\delta^{15}N$ ranges of 1.7‰ and 1.9‰, for hair and fingernails, respectively, suggesting individuals in the sample have generally similar diets. In contrast, the range of $\delta^{13}C$ values was 6.7‰ for the medieval Spanish sample and 5.44‰ for modern calculus. The range of $\delta^{13}C$ values for the modern hair samples was 4.21‰. The $\delta^{13}C$ range of calculus, while broader, is much more comparable to the ranges for hair/fingernail than found for $\delta^{15}N$.

The major difference between this study and Scott and Poulson (2012) is the presence of plaque in modern dental calculus. This raises the question as to why dental calculus can yield different stable isotope results when taken from living individuals as opposed to ancient remains. Despite the compositional differences between hair keratin and dental calculus, apparently the impact of plaque on the stable isotope ratios of modern calculus is not as pronounced for $\delta^{13}C$ as it is for $\delta^{15}N$.

Since plaque is a biofilm covering the tooth, it is likely that ancient calculus was exposed to the elements of a burial environment for some time and the non-mineralized biofilm was lost. In modern calculus, plaque and saliva are still retained. The initial strategy was to remove the biofilm by rinsing in water. Deionized water was added to two calculus samples, swirled, and put aside to decant. For both samples, a milky decant was noticeable. It was difficult to determine how much would be lost if decanting was

attempted. Initially, the samples were left out to see if particles would precipitate, but the milky appearance did not disappear. These samples were then put in a drying oven to avoid loss of sample material. Since calculus was not powdered at this point, the presence of bacterial film was the likely reason the rinsed samples had a milky appearance. After this, there were no additional attempts to rinse the calculus for fear of losing sample material in the process.

The two samples that were rinsed were of similar size. Sample 1 weighed 2.034 mg while Sample 2 weighed 2.893 mg. Despite comparable sample sizes, weight percent values were quite different between the two samples. Sample 1 had a weight % carbon of 12.79 and weight % nitrogen of 2.61. Sample 2 had a weight % carbon of 33.18 and weight % nitrogen of 7.15. If these values are compared to the weight percent values for ancient calculus, they are significantly higher in the percent of both carbon and nitrogen. For the medieval Spanish calculus, average weight % carbon was 4.90% and 0.77% for weight % nitrogen (Scott and Poulson, 2012). Since the composition of dental calculus is at most 15-20% organic, the elemental compositions from some of the modern calculus samples were impossibly high for pure dental calculus (Lieverse, 1999). This suggests the presence of organic elements derived from plaque and saliva.

The presence of plaque on the calculus samples was observed at an early stage of the experimental procedures. When the modern calculus samples were opened, they were put in a drying oven before there was any attempt to rinse them. There was a visual change in the volume of the samples before and after they were put in the drying oven. The largest samples changed little after 24 hours in the drying oven, but some samples dramatically decreased in volume. Since samples were not weighed prior to being placed

in the drying oven, it is impossible to say which samples changed the most. Two samples evaporated to the point where analysis was not possible (Samples 8 and 12). Even for larger samples, there was a discernible decrease in volume. This would not be the case if the samples were pure mineralized calculus.

The composition of plaque is fairly complex. It contains hundreds of bacterial species and is also protein rich. The nitrogen in plaque was estimated to be 12.6%, with all but 1.5% derived from proteins. Carbohydrates comprise up to 6% of plaque (Millen and Smith, 1961). These are the likely reasons for the abnormally high carbon and nitrogen levels in modern calculus samples.

While plaque made up the majority of the unmineralized portion of the samples, salivary residue was likely present as well. Saliva, like plaque, contains proteins and bacteria. Some bacteria present in saliva are the same bacteria that are most prevalent in plaque. Also similar to plaque, the quantity and types of proteins and bacteria as well as the lipids vary depending on the oral conditions. This is seen in the isolation and comparison of microflora (Cowan et al., 1992). In reality, most of the samples analyzed in this study are a mix of calculus and biofilm.

6.3 C:N Ratios of the Modern Calculus Samples

There is an established range for C:N values for uncontaminated hair and fingernail samples because these biomaterials are made up of keratin, a form of pure protein. The ratios of carbon and nitrogen are uniform in these samples because of their consistent elemental composition. Bone collagen, while not used in this study, is similar with regard to C:N ranges. The valid C:N range for bone collagen is between 2.9 and 3.6. Like hair and fingernails, collagen is a non-composite sample type that yields predictable amounts of

carbon and nitrogen when uncontaminated (O‘Connell and Hedges, 1999; Linderholm and Kjellström, 2011).

Modern dental calculus does not have an established range because no previous studies have analyzed this material for carbon and nitrogen isotopes. Results show that the C:N ratios are highly variable in modern calculus samples, ranging from 4.83 to 6.49 (Table 4). The 1.67 range of modern calculus C:N is almost four times greater than the range of hair C:N ratios (0.43) and over six times greater than the range of fingernail C:N ratios (0.27). The range and summary statistics of C:N values for modern calculus, hair, and fingernails along with one ancient calculus sample (Scott and Poulson, 2012) are shown in Table 10.

Table 10. Modern calculus C:N averages compared to available data from ancient calculus. Statistical values are also given for fingernail and hair samples obtained in this study. C:N statistics for Medieval Spanish calculus are representing ancient calculus. Medieval Spanish sample data taken from Scott and Poulson, 2012.

Modern Calculus C:N	
Overall Mean	5.39
Standard Deviation	0.41
Range	1.66
Medieval Spanish Calculus C:N	
Overall Mean	7.62
Standard Deviation	1.55
Range	7.26
Modern Fingernails C:N	
Overall Mean	3.50
Standard Deviation	0.12
Range	0.71
Modern Hair C:N	
Overall Mean	3.56
Standard Deviation	0.12
Range	0.43

Calculus may not provide consistent C:N ratios, but this is not surprising given its heterogeneous origin. While modern calculus samples are complicated by residual plaque and salivary components, the C:N range is still smaller for modern calculus than the range for ancient calculus (Scott and Poulson, 2012). This may reflect the situation where the carbon and nitrogen in dental calculus comes from a variety of compounds that get stuck in calculus by chance. These organic components come from food, various salivary proteins, lipids, carbohydrates and oral bacteria that are trapped during calculus formation. In a composite sample like calculus, variation in C:N values is expected.

6.4 Elemental Composition of Modern Calculus Samples

The weight % values for carbon and nitrogen in modern calculus show great variability. For modern calculus, weight % carbon values range from about 6% to 46%, while weight % nitrogen values range from about 1% to 9%. Fig. 6 showed that an increase in weight % carbon is strongly correlated with the elevation in weight % nitrogen.

Weight % carbon and nitrogen values from ancient calculus are both lower and more uniform than for modern calculus. In the 58 dental calculus samples from medieval Spain, weight % carbon ranged from 1.74% to 8.49%, while weight % nitrogen ranged from 0.25% to 1.38% (Scott and Poulson, 2012).

Based on the inclusion of dental plaque and saliva in modern calculus samples, it is likely that a biofilm is responsible for the higher carbon and nitrogen weight % values. For most of the samples, there was not enough sample material to run replicates. For most analyses, the entire sample was used to estimate elemental composition and isotope ratios. The dental hygienist collected as much calculus as was readily available from the subjects, but modern dental care does not allow the kinds of massive calculus accumulations that characterized past populations. There was no attempt to limit the amount of plaque collected with the calculus, but there was emphasis on collecting more than just a few milligrams if possible. As this is the first study of its kind, there was no way to know the $\delta^{13}C$ and $\delta^{15}N$ values would be different between plaque and calculus even though it was assumed that the weight percent values might vary.

For this study, the size of the sample is closely linked to the amounts of dental calculus in comparison to the plaque and saliva. While there is no way of deciphering exactly how much total calculus each subject had, the smallest samples reflect those of

individuals that did not have large accumulations of calculus. As would be expected, the larger samples had lower carbon and nitrogen weight percent values than the smaller samples (Fig. 14).

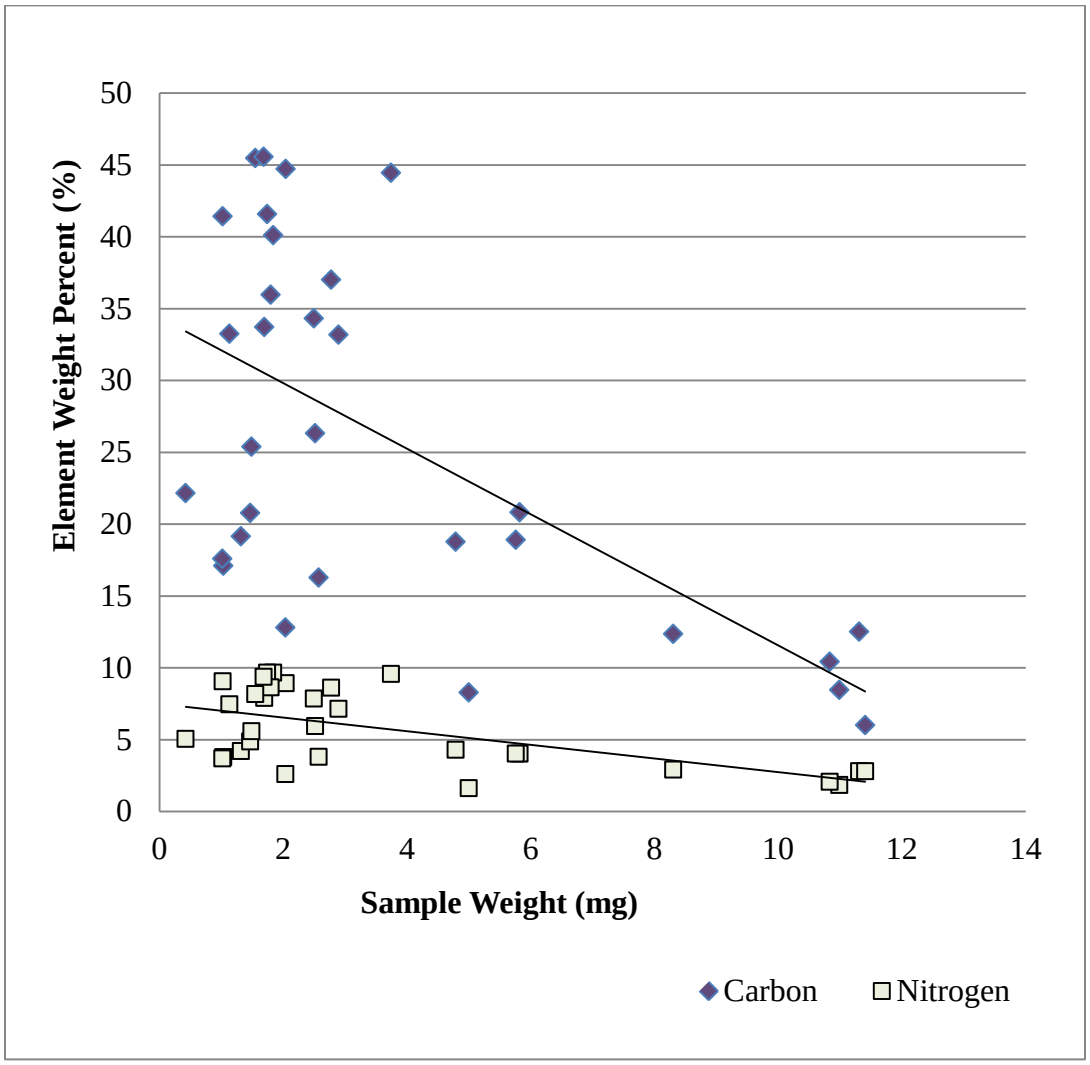

Fig. 14. The weight percent values of carbon and nitrogen for the modern calculus samples compared to the sample weights (mg).

The samples with less than 2 mg of calculus showed the highest weight % values and high variability. While calculus samples under 2 mg range from about 17% to 46% weight % carbon, calculus samples with more than 4 mg range from 6% to 21%. Similarly, calculus samples under 2 mg had weight % nitrogen that ranged from around 3.5% to 9.5%, while calculus samples over 4 mg ranged from around 1% to 4.5%. For both weight % carbon and nitrogen, the greatest ranges appear between 2 mg and 4 mg. This is likely because the amount of plaque compared to calculus is a matter of chance in samples from individuals with moderate calculus formation.

While there was no initial assumption that plaque and calculus would differ significantly in $\delta^{13}C$ and $\delta^{15}N$ values, the mixed results of these isotopic ratios suggests that the impact of plaque should be investigated. While the samples with the lowest weight % values show that plaque is less present, this does not address the affect plaque has on $\delta^{13}C$ and $\delta^{15}N$ values. Figs. 15 and 16 show the weight % carbon for the calculus samples plotted against the difference between the $\delta^{13}C$ values of hair and calculus (Fig. 15) and of fingernails and calculus (Fig. 16).

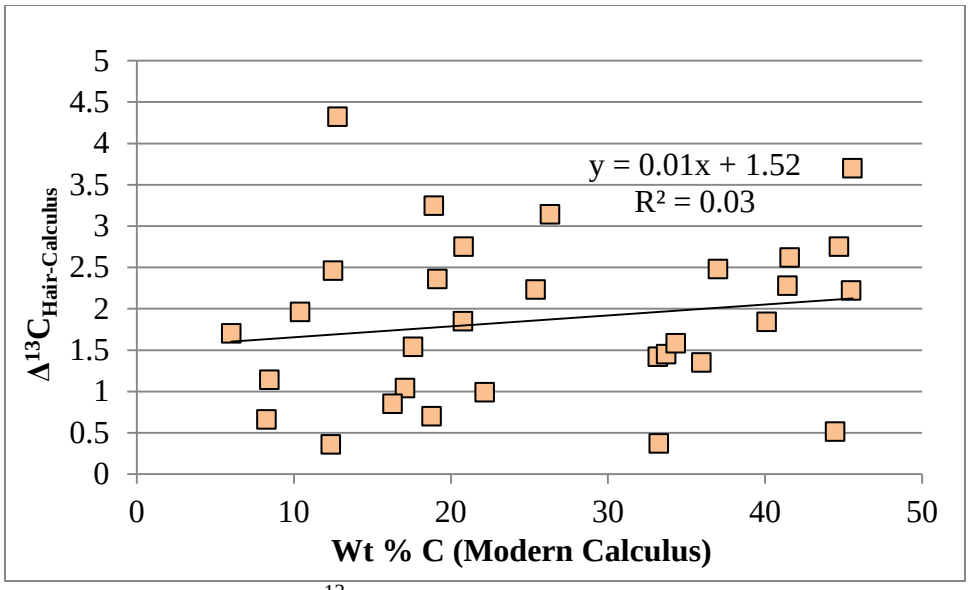

Fig. 15. Difference in $\delta^{13}C$ of hair and calculus plotted against the weight percent of the modern calculus samples.

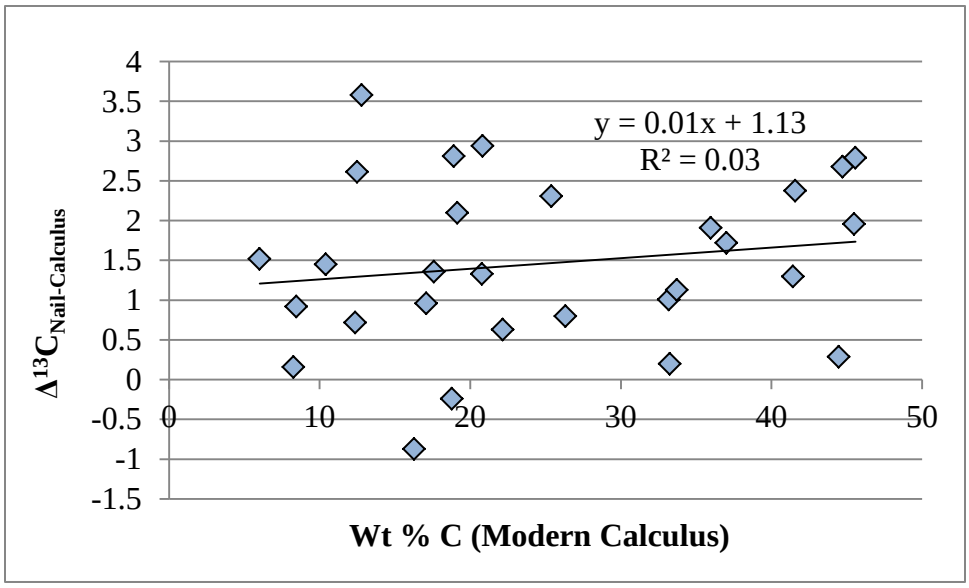

Fig. 16. Difference in $\delta^{13}C$ of fingernail and calculus plotted against the weight percent of the modern calculus samples.

Figs. 15 and 16 show that $\Delta^{13}C_{Hair\text{-}Calculus}$ and $\Delta^{13}C_{Nail\text{-}Calculus}$ differences get slightly closer to 0 as weight % carbon decreases. $\delta^{13}C$ values tend to be more positive for calculus samples with lower weight % carbon but by a very small margin. The coefficients of determination show a weak and non-significant relationship between $\Delta^{13}C$ and weight % carbon for calculus. The r^2 value is 0.03 between the weight % carbon and the $\Delta^{13}C_{Hair\text{-}Calculus}$ and 0.03 between the weight % carbon and the $\Delta^{13}C_{Nail\text{-}Calculus}$. While these regressions show a very slight decrease in $\Delta^{13}C$ as the weight % carbon of calculus lowers, these results are inconclusive.

Weight % nitrogen for calculus is compared with the difference between $\delta^{15}N$ values compared to hair and fingernails in Figs 17 and 18. On average, the $\Delta^{15}N_{Hair\text{-}Calculus}$ and $\Delta^{15}N_{Nail\text{-}Calculus}$ values decrease significantly as the weight % nitrogen of the sample decreases. Thus, Figs. 17 and 18 show that the $\delta^{15}N$ values move towards more positive values for calculus samples with lower weight % nitrogen. This shows a more significant trend than observed for the regressions of weight % carbon on $\Delta^{13}C$. The slope of the weight % nitrogen vs. $\Delta^{15}N$ regressions was 0.25 for $\Delta^{15}N_{Hair\text{-}Calculus}$ and 0.26 for $\Delta^{15}N_{Nail\text{-}Calculus}$. The r^2 value is 0.18 for the regression of weight % nitrogen and $\Delta^{15}N_{Hair\text{-}Calculus}$, and 0.16 for weight % nitrogen and $\Delta^{15}N_{Nail\text{-}Calculus}$. This suggests that the presence of plaque in calculus samples has a significant impact on $\delta^{15}N$ values.

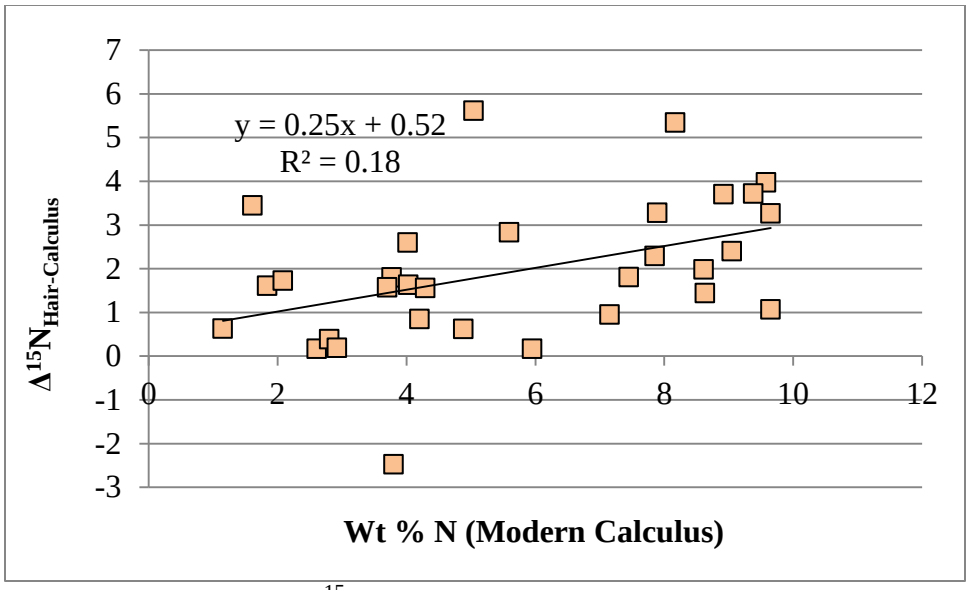

Fig. 17. Difference in $\delta^{15}N$ of hair and calculus plotted against the weight percent of the modern calculus samples.

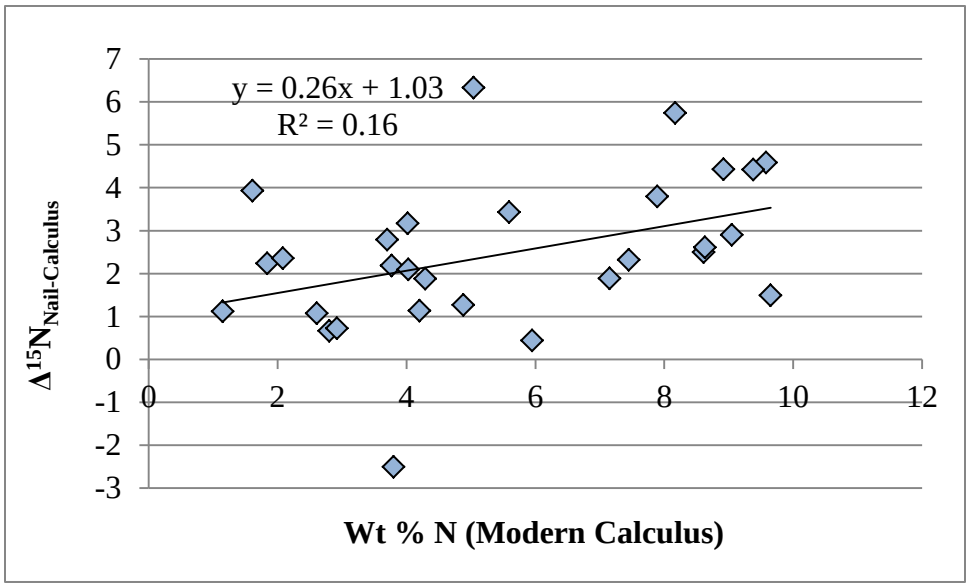

Fig. 18. Difference in $\delta^{15}N$ of fingernail and calculus plotted against the weight percent of the modern calculus samples.

To further address the issue of plaque, calculus samples were broken down into three groups according to whether their carbon and nitrogen weight percent values were high, moderate or low. Since weight % values are the only direct indicators of high organic content, this method is used to help tease out the contribution of plaque and calculus to $\delta^{13}C$ and $\delta^{15}N$ values.

The contrasts in carbon and nitrogen weight % values for modern calculus were previously shown in Fig. 10. At around 6% nitrogen and 30% carbon, there is a visible gap in the scatter plot contrasting the carbon and nitrogen weight percent values. There is a 6.8% gap for carbon and 1.2% gap for nitrogen. This gap was used to separate the calculus samples into those with weight percent values in the top 1/3 of the ranges for both carbon and nitrogen. At about 3.4% nitrogen and 15% carbon, there is a less dramatic gap in the weight percent values for carbon and nitrogen. This gap marks the lower 1/3 of the range. For this less dramatic separation, there is a 0.4% gap for nitrogen and a 3.0% gap for carbon. These natural separations are used in the following sections instead of assigned categories because natural separations ensure that all samples fall into the high, moderate or low group for both carbon and nitrogen weight % values. These separations are shown in Fig. 19, along with regression coefficients. This figure shows that the weakest correlations between weight % carbon and weight % nitrogen is for calculus with high carbon and nitrogen weight % values.

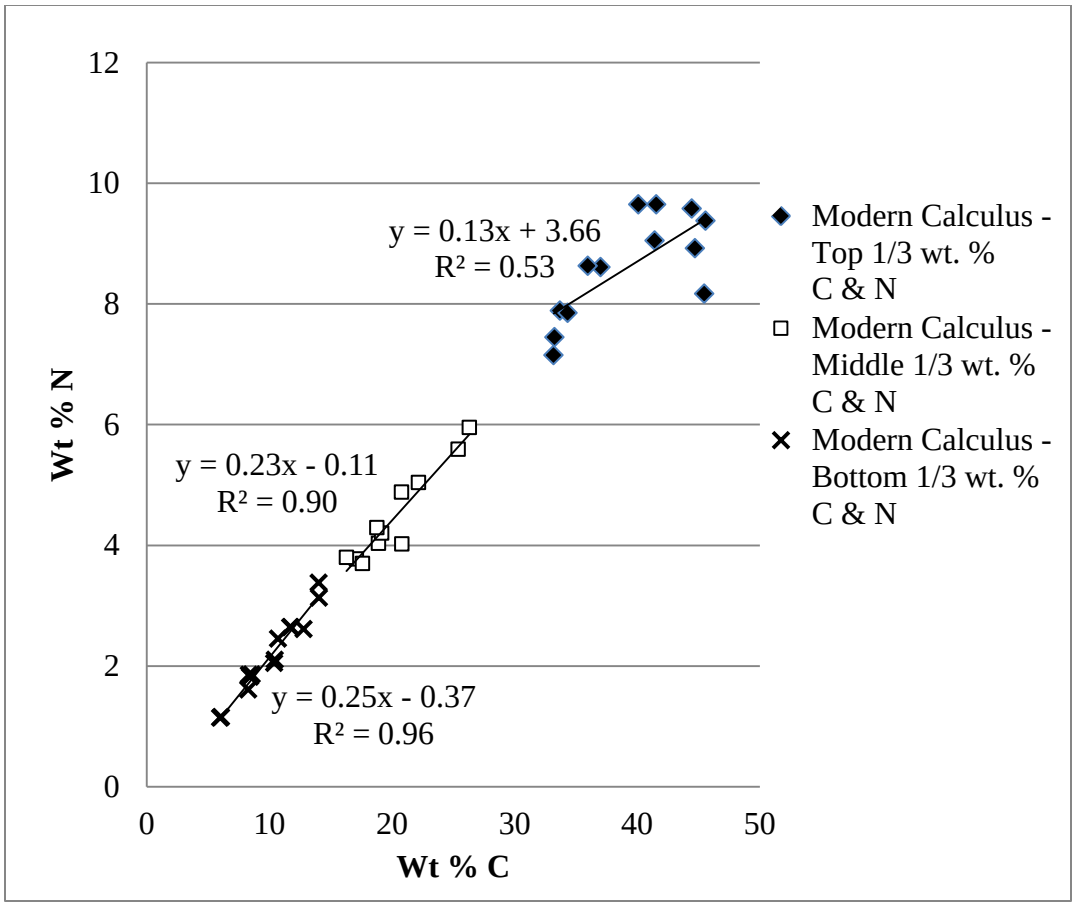

Fig. 19. Carbon and nitrogen weight percent comparisons for modern calculus broken into 3 groups.

6.5 $\delta^{13}C$ of Calculus Compared to $\delta^{13}C$ of Fingernails and Hair

While stable carbon isotope compositions for hair and fingernails are strongly correlated ($r^2 = 0.73$), the correlations between calculus and hair/fingernails for $\delta^{13}C$ are not as high but are nonetheless significant. The coefficients of determination (r^2) were 0.48 between calculus and hair and 0.34 between calculus and fingernails. Given the contrast in the two types of biomaterial, these correlations are higher than might be expected.

Since research involving comparisons of different biomaterials has been published,

the calculus correlations to hair and fingernails are compared to the correlation between bone collagen and hair provided by O'Connell et al. (2001). The coefficient of determination was 0.08 for $\delta^{13}C$ between hair and bone collagen, although sample size was small and the range of values was narrow. The range of $\delta^{13}C$ for hair was 0.96‰, a narrow range that would skew the correlation analysis (O'Connell et al., 2001). For the modern sample, the $\delta^{13}C$ range was 5.4‰ for calculus and 4.5‰ for hair. Hair and fingernails showed higher correlations with dental calculus than bone collagen, although this likely reflects the difference in $\delta^{13}C$ ranges between the two studies ($\delta^{13}C$ range of 5.4‰ for calculus and 1.43‰ for bone collagen).

When subjected to regression analysis, the slope of calculus (x) and hair (y) was 0.48, while the slope for calculus (x) and fingernail (y) was 0.38. For bone collagen, the slope of the bone (x) and hair (y) was only 0.22 (O'Connell et al, 2001). While a small range could explain the regression equation of O'Connell et al (2001), the calculus regressions are only minimally better with a significantly larger range. Since the correlations are relatively strong for calculus and hair/fingernails, the slope should be closer to 1.

To evaluate the impact of plaque, $\delta^{13}C$ and $\delta^{15}N$ values were analyzed in calculus samples broken into three groups based on weight percent values for carbon and nitrogen (Fig. 19). With modern calculus samples divided into three sections, these groups were compared with corresponding fingernail and hair samples for $\delta^{13}C$ and $\delta^{15}N$ values.

The enrichment of ^{13}C in hair and fingernail samples compared to modern calculus is shown in Table 11. When calculus samples are broken down into three groups relative

to carbon weight percent values, the difference of ^{13}C levels in calculus, compared to hair and fingernail, does not change significantly. Calculus with the highest weight percent values shows a difference of -1.89‰ relative to hair and -1.58‰ relative to fingernails. Calculus with moderate carbon weight percent values shows a difference of -1.88‰ compared to hair and -1.29‰ compared to fingernail. The group with the lowest weight percent values, and presumably the greatest amount of calculus in comparison to plaque, shows a 1.80‰ increase in ^{13}C of hair over calculus and a 1.57‰ increase of fingernails over calculus. All three calculus subgroups are similar to each other in relation to hair and fingernails despite relatively small samples sizes (n ranges from 7 to 13). In sum, the weight % carbon used to classify the three calculus subgroups does not appear to have a significant impact on $\delta^{13}C$ of the calculus samples.

Table 11. Modern calculus samples divided by weight percent carbon. The first group ranges from 33.18% to 45.57%. The second group ranges from 16.28% to 26.32%. The third group ranges from 5.98% to 14.05%. The calculus samples in these subgroups are compared here to their corresponding hair and fingernail samples.
Note: Grayed values are averages for samples with replicates.

Sample Number	Wt. % C	$\delta^{13}C$ Calculus	$\delta^{13}C$ Hair	$\delta^{13}C$ Fingernail	Δ13C Hair-Calculus	Δ13C Fingernail-Calculus
Sample 32	45.57	-21.8	-18.1	-19.0	3.7	2.8
Sample 30	45.48	-19.1	-16.9	-17.1	2.2	2.0
Sample 6	44.71	-21.2	-18.4	-18.5	2.8	2.7
Sample 17	44.46	-17.8	-17.3	-17.5	0.5	0.3
Sample 22	41.57	-20.2	-17.5	-17.8	2.7	2.4
Sample 20	41.43	-19.3	-17.0	-18.0	2.3	1.3
Sample 19	40.10	-18.4	-16.5		1.8	
Sample 18	37.01	-18.7	-16.2	-17.0	2.5	1.7
Sample 26	35.96	-18.9	-17.6	-17.0	1.3	1.9
Sample 28	34.32	-22.0	-20.4		1.6	
Sample 15	33.71	-19.6	-18.2	-18.5	1.4	1.1
Sample 16	33.25	-19.6	-19.2	-19.4	0.4	0.2
Sample 2	33.18	-19.4	-18.0	-18.4	1.4	1.0
Overall Mean					1.89	1.58
Standard Deviation					0.92	0.88
Standard Error					0.23	0.27

Sample Number	Wt. % C	$\delta^{13}C$ Calculus	$\delta^{13}C$ Hair	$\delta^{13}C$ Fingernail	Δ13C Hair-Calculus	Δ13C Fingernail-Calculus
Sample 13	26.32	-21.4	-18.2	-20.6	3.2	0.8
Sample 29	25.39	-20.7	-18.5	-18.4	2.2	2.3
Sample 31	22.16	-16.9	-15.9	-16.2	1.0	0.7
Sample 5	20.81	-21.7	-18.9	-18.8	2.8	2.9
Sample 25	20.78	-19.5	-17.6	-18.2	1.9	1.3
Sample 11	19.15	-20.0	-17.6	-17.9	2.4	2.1
Sample 9	18.91	-20.3	-17.1	-17.5	3.2	2.8
Sample 24	18.77	-18.1	-17.4	-18.3	0.7	-0.2
Sample 7	17.59	-20.2	-18.7	-18.9	1.5	1.3
Sample 4	17.09	-17.9	-16.8	-16.9	1.1	1.0
Sample 10	16.28	-19.6	-18.8	-20.5	0.8	-0.9
Overall Mean					1.88	1.29
Standard Deviation					0.93	1.21
Standard Error					0.28	0.36

Sample Number	Wt. % C	$\delta^{13}C$ Calculus	$\delta^{13}C$ Hair	$\delta^{13}C$ Fingernail	Δ13C Hair-Calculus	Δ13C Fingernail-Calculus
Sample 1	12.79	-22.3	-18.0	-18.7	4.3	3.6
Sample 3	12.50	-21.0	-18.5	-18.4	2.5	2.6
Sample 23	12.36	-18.6	-18.2	-17.9	0.4	0.7
Sample 27	10.41	-19.0	-17.0	-17.5	2.0	1.5
Sample 14	8.45	-18.0	-16.9	-17.1	1.1	0.9
Sample 33	8.27	-18.6	-17.9	-18.4	0.7	0.2
Sample 21	6.02	-19.4	-17.7	-17.9	1.7	1.5
Overall Mean					1.80	1.57
Standard Deviation					1.33	1.18
Standard Error					0.50	0.44

Comparisons between calculus samples and corresponding hair and fingernail samples for the three calculus weight percent groups are shown in Figs. 20 and 21. Calculus and hair appear to correlate best with the middle group of weight % carbon values where $r^2 = 0.65$ (Fig. 20). The weakest correlation between calculus and hair is for the group with weight % carbon values under 15% ($r^2 = 0.27$). The group with the highest weight % carbon had a coefficient of determination of 0.51. By contrast, calculus has a greater correlation with fingernail ^{13}C values as weight percent values for calculus get lower (Fig. 21). The correlation coefficients between calculus and fingernails for the groups with high, moderate, and low carbon weight percent values are, respectively, 0.39, 0.42, and 0.57. The slope of the regression line does not get significantly closer to 1 when examining calculus by weight percent groups (Figs. 20 and 21).

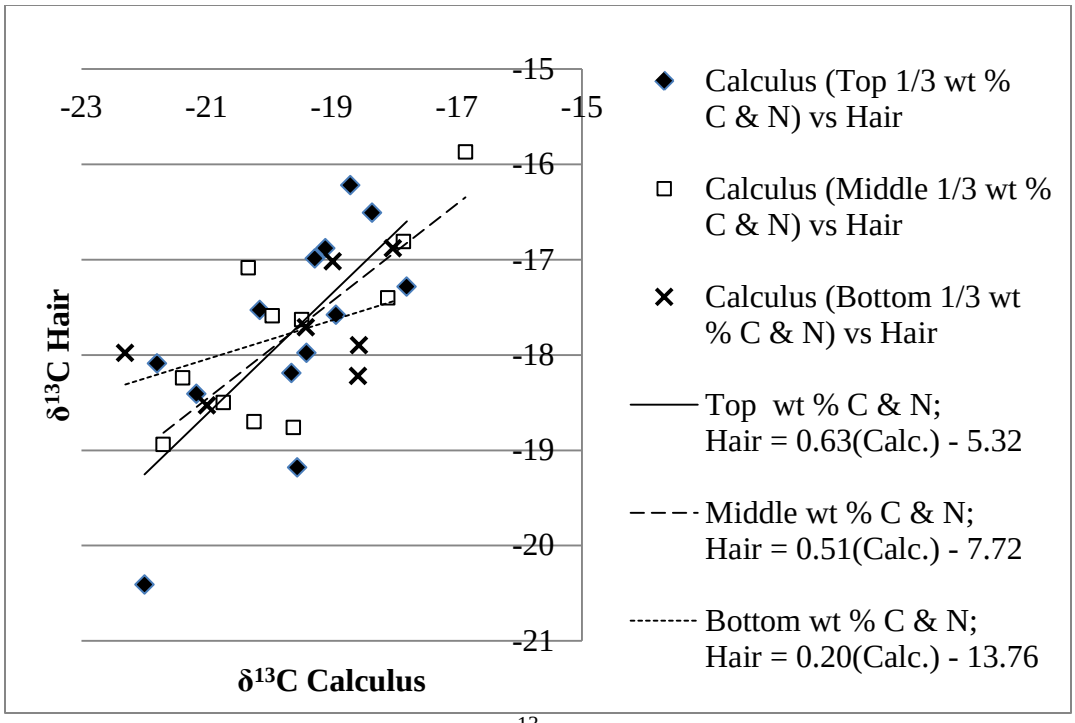

Fig. 20. Modern calculus vs. hair for $\delta^{13}C$ broken into the 3 groups based on their elemental compositions.

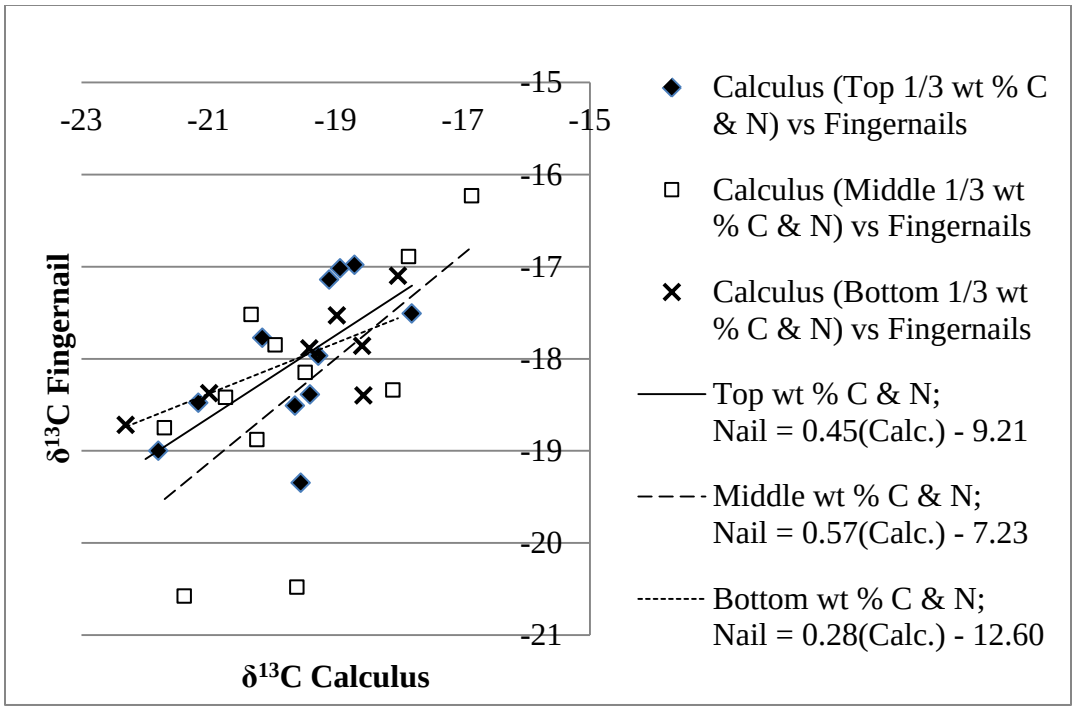

Fig. 21. Modern calculus vs. fingernails for $\delta^{13}C$ broken into the 3 groups based on their elemental compositions.

The differences in $\delta^{13}C$ between calculus and both hair and nails is not dictated by weight % carbon values. The mixed results in the correlation coefficients for the three calculus groups (Fig. 20 and 21) reflect the same thing as Figs. 15 and 16. If plaque is a greater component in calculus samples with the highest carbon weight percent values, this does not translate into significantly higher or lower ^{13}C levels.

6.6 $\delta^{15}N$ of Calculus Compared to $\delta^{15}N$ of Fingernails and Hair

While $\delta^{13}C$ isotope compositions of modern calculus are significantly correlated with those of fingernails and hair, $\delta^{15}N$ compositions show no such relationship. The coefficient of determination (r^2) for $\delta^{15}N$ was 0.00 between calculus and hair and 0.01 between calculus and nails. Given the correlations for $\delta^{13}C$ between calculus and both fingernails and hair, the low $\delta^{15}N$ correlations are surprising.

The $\delta^{15}N$ differences between calculus and hair/nail samples are more consistent than the coefficients of determinations suggest. On average, hair was ^{15}N enriched by 1.94‰ compared to calculus. Fingernail was 2.45‰ more ^{15}N enriched than calculus. From hair and fingernail analysis, fingernail is ^{15}N enriched by an average of 0.57‰ compared to hair. Only Sample 10 did not reflect depletion of ^{15}N in dental calculus compared to hair and fingernail. Samples 30 and 31 gave the most significant ^{15}N depletions of calculus compared to hair and fingernails (i.e., over 5‰). For the 30 remaining samples, all provided ^{15}N values for calculus that were depleted between 0.1‰ and 4.0‰ compared to hair and between 0.4‰ and 4.6‰ compared to fingernails. These enrichment results are consistent given the lack of statistical correlation between calculus and hair/nail for $\delta^{15}N$ compositions.

The ^{15}N enrichment of hair and fingernail compared to modern calculus was broken down into groups based on weight % nitrogen (Table 12). Weight % nitrogen has a noticeable effect on $\delta^{15}N$ values. The calculus group with the highest weight % nitrogen values has an average ^{15}N depletion of 2.71‰ compared to hair and 3.34‰ compared to fingernails. The calculus group with the middle weight % nitrogen values has an average ^{15}N depletion of 1.53‰ compared to hair and 2.02‰ compared to fingernails. The calculus group with the lowest weight % nitrogen values has an average ^{15}N depletion of 1.17‰ compared to hair and 1.73‰ compared to fingernails.

Table 12. Modern calculus samples divided by weight percent nitrogen. The first group ranges from 7.15% to 9.65%. The second group ranges from 3.70% to 5.95%. The third group ranges from 1.15% to 3.38%. The calculus samples in these subgroups are compared here to their corresponding hair and fingernail samples. Note: Grayed values are averages for samples with replicates.

Sample Number	Wt. % N	$\delta^{15}N$ Calculus	$\delta^{15}N$ Hair	$\delta^{15}N$ Fingernail	Δ15N Hair-Calculus	Δ15N Fingernail-Calculus
Sample 22	9.65	7.0	8.1	8.5	1.1	1.5
Sample 19	9.65	5.3	8.6		3.3	
Sample 17	9.58	4.9	8.9	9.5	4.0	4.6
Sample 32	9.38	5.1	8.8	9.5	3.7	4.4
Sample 20	9.05	6.2	8.6	9.1	2.4	2.9
Sample 6	8.92	4.3	8.0	8.7	3.7	4.4
Sample 26	8.63	6.9	8.3	9.5	1.4	2.6
Sample 18	8.61	7.1	9.1	9.6	2.0	2.5
Sample 30	8.17	4.1	9.4	9.8	5.3	5.7
Sample 15	7.89	5.5	8.7	9.3	3.2	3.8
Sample 28	7.85	6.0	8.3		2.3	
Sample 16	7.45	5.9	7.7	8.2	1.8	2.3
Sample 2	7.15	7.1	8.1	9.0	1.0	1.9
Overall Mean					2.71	3.34
Standard Deviation					1.30	1.34
Standard Error					0.36	0.40

Sample Number	Wt. % N	$\delta^{15}N$ Calculus	$\delta^{15}N$ Hair	$\delta^{15}N$ Fingernail	Δ15N Hair-Calculus	Δ15N Fingernail-Calculus
Sample 13	5.95	7.6	7.8	8.1	0.2	0.5
Sample 29	5.59	5.6	8.4	9.0	2.8	3.4
Sample 31	5.04	3.7	9.3	10.0	5.6	6.3
Sample 25	4.88	7.8	8.5	9.1	0.7	1.3
Sample 24	4.29	7.5	9.1	9.4	1.6	1.9
Sample 11	4.20	8.0	8.8	9.1	0.8	1.1
Sample 9	4.03	7.6	9.2	9.7	1.6	2.1
Sample 5	4.02	6.1	8.7	9.2	2.6	3.1
Sample 10	3.80	11.3	8.8	8.8	-2.5	-2.5
Sample 4	3.77	6.8	8.6	9.0	1.8	2.2
Sample 7	3.70	7.0	8.5	9.8	1.5	2.8
Overall Mean					1.53	2.02
Standard Deviation					1.97	2.16
Standard Error					0.59	0.65

Sample Number	Wt. % N	$\delta^{15}N$ Calculus	$\delta^{15}N$ Hair	$\delta^{15}N$ Fingernail	Δ15N Hair-Calculus	Δ15N Fingernail-Calculus
Sample 23	2.92	9.0	9.1	9.7	0.1	0.7
Sample 3	2.80	8.4	8.8	9.1	0.4	0.7
Sample 1	2.61	8.0	8.1	9.0	0.1	1.0
Sample 27	2.08	7.4	9.2	9.8	1.8	2.4
Sample 14	1.84	7.5	9.1	9.8	1.6	2.3
Sample 33	1.61	5.2	8.6	9.1	3.4	3.9
Sample 21	1.15	8.5	9.1	9.6	0.6	1.1
Overall Mean					1.17	1.73
Standard Deviation					1.20	1.18
Standard Error					0.45	0.45

Compared to both hair and fingernails, the calculus samples with the highest weight % nitrogen values have $\delta^{15}N$ that are significantly more negative than the samples with lower values. In sum, the higher the weight % nitrogen for a sample, the lower the corresponding $\delta^{15}N$ value.

In Figs. 22 and 23, the $\delta^{15}N$ and $\delta^{13}C$ enrichment of hair and fingernails compared to dental calculus is broken down into three groups based on elemental compositions. While weight % carbon does not significantly change $\delta^{13}C$ isotopic compositions, weight % nitrogen has a significant impact on the $\delta^{15}N$ isotopic compositions of calculus. This suggests that as the presence of plaque and oral fluids diminishes, the depletion of ^{15}N compared to hair/fingernails becomes more consistent.

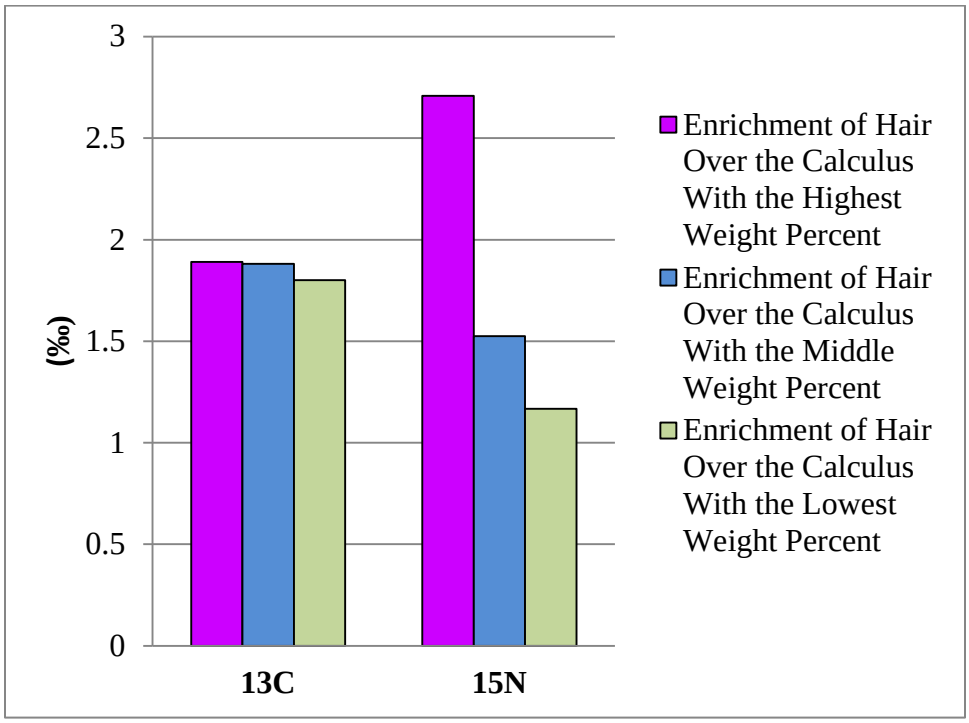

Fig. 22. The $\delta^{13}C$ enrichment of hair compared to modern calculus broken down into the 3 groups based on their elemental composition.

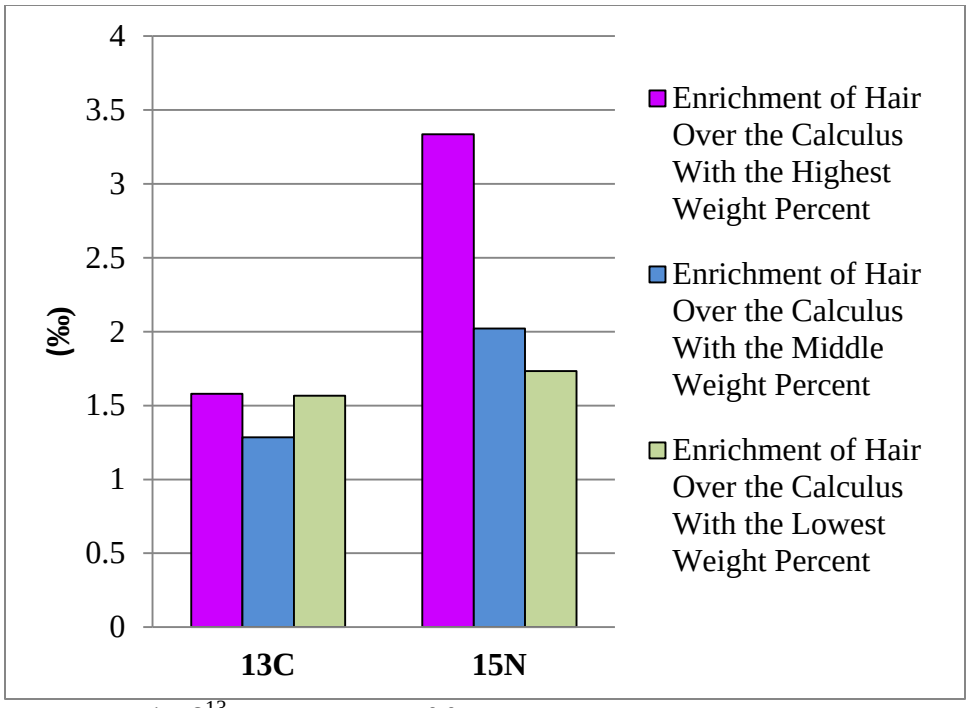

Fig. 23. The $\delta^{13}C$ enrichment of fingernail compared to modern calculus broken down into the 3 groups based on their elemental composition.

Comparisons between calculus samples and corresponding hair and fingernail samples for the three weight % nitrogen groups are shown in Figs. 24 and 25. All replicates for calculus, hair and fingernail samples were averaged. Only the calculus group with weight % nitrogen values under 3% has a positive correlation with hair and fingernails for ^{15}N, but this correlation is weak. For the lowest weight % nitrogen group, the slope was 0.09 when compared to both hair and fingernails, with coefficients of determination (r^2) of 0.09 with hair and 0.10 with fingernails.

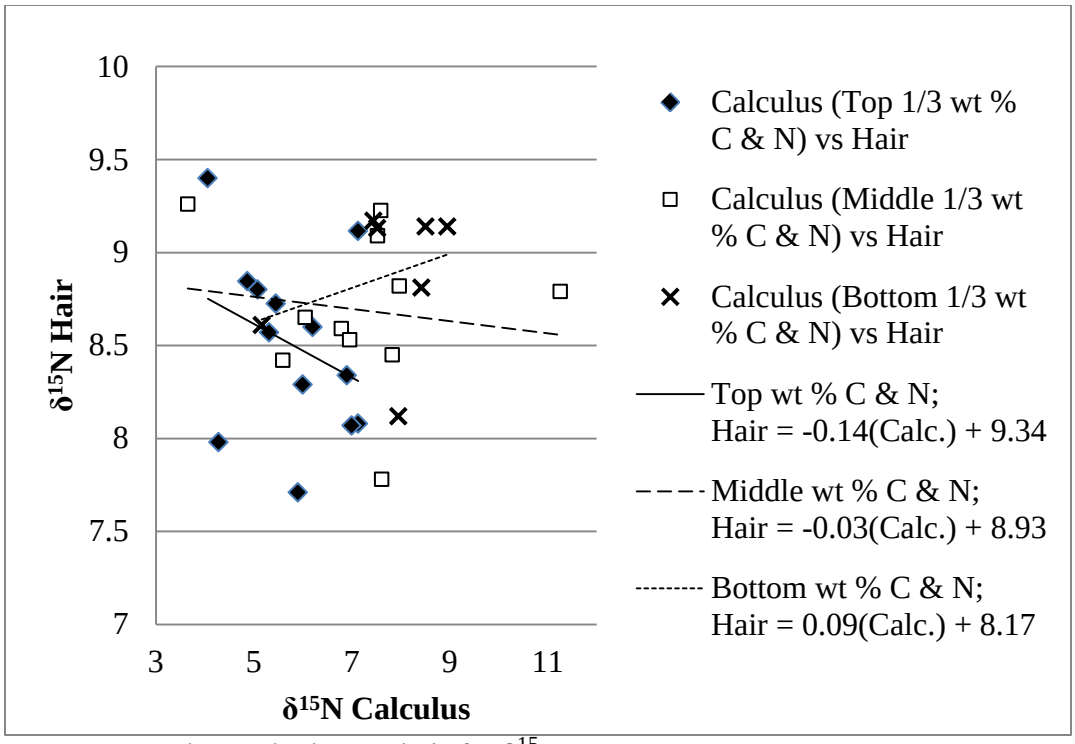

Fig. 24. Modern calculus vs. hair for $\delta^{15}N$ broken into the 3 groups based on their elemental compositions.

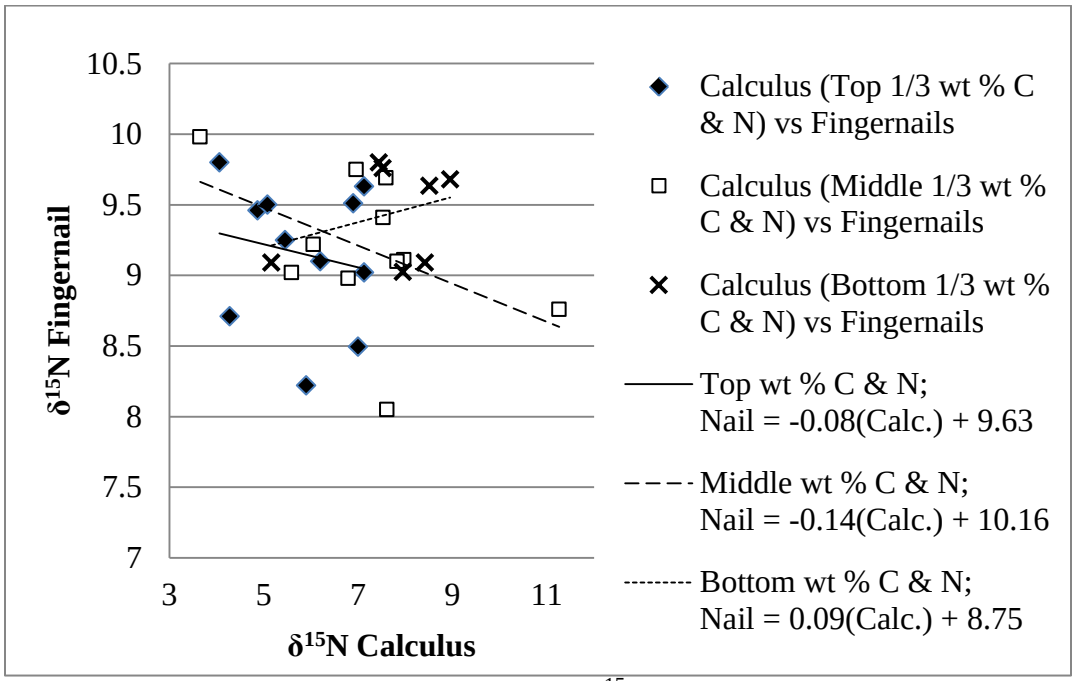

Fig. 25. Modern calculus vs. fingernails for $\delta^{15}N$ broken into the 3 groups based on their elemental compositions.

A histogram showing the dispersion of $\delta^{13}C$ and $\delta^{15}N$ compositions for calculus, hair and nails is shown in Fig. 26. Two points are evident in this figure. First, the $\delta^{13}C$ and $\delta^{15}N$ compositions for calculus show greater dispersion than shown for either hair or fingernails, both of which have comparable ranges of variation. Second, the means for both $\delta^{13}C$ and $\delta^{15}N$ compositions are about 2‰ lower than the corresponding means for hair and fingernails, which again, are very similar to one another. The variance of the calculus samples for $\delta^{13}C$ was 1.89, while variance was 0.89 for hair and 0.92 for fingernail. The $\delta^{15}N$ variance for calculus was 2.56, while variance was 0.20 for hair and 0.22 for fingernails. The greater variance for the calculus samples likely reflects its heterogeneous origins versus the more homogeneous nature of keratin, the protein that makes up hair and nails. More research is required to explain the lower isotopic compositions of calculus compared to hair/fingernail values.

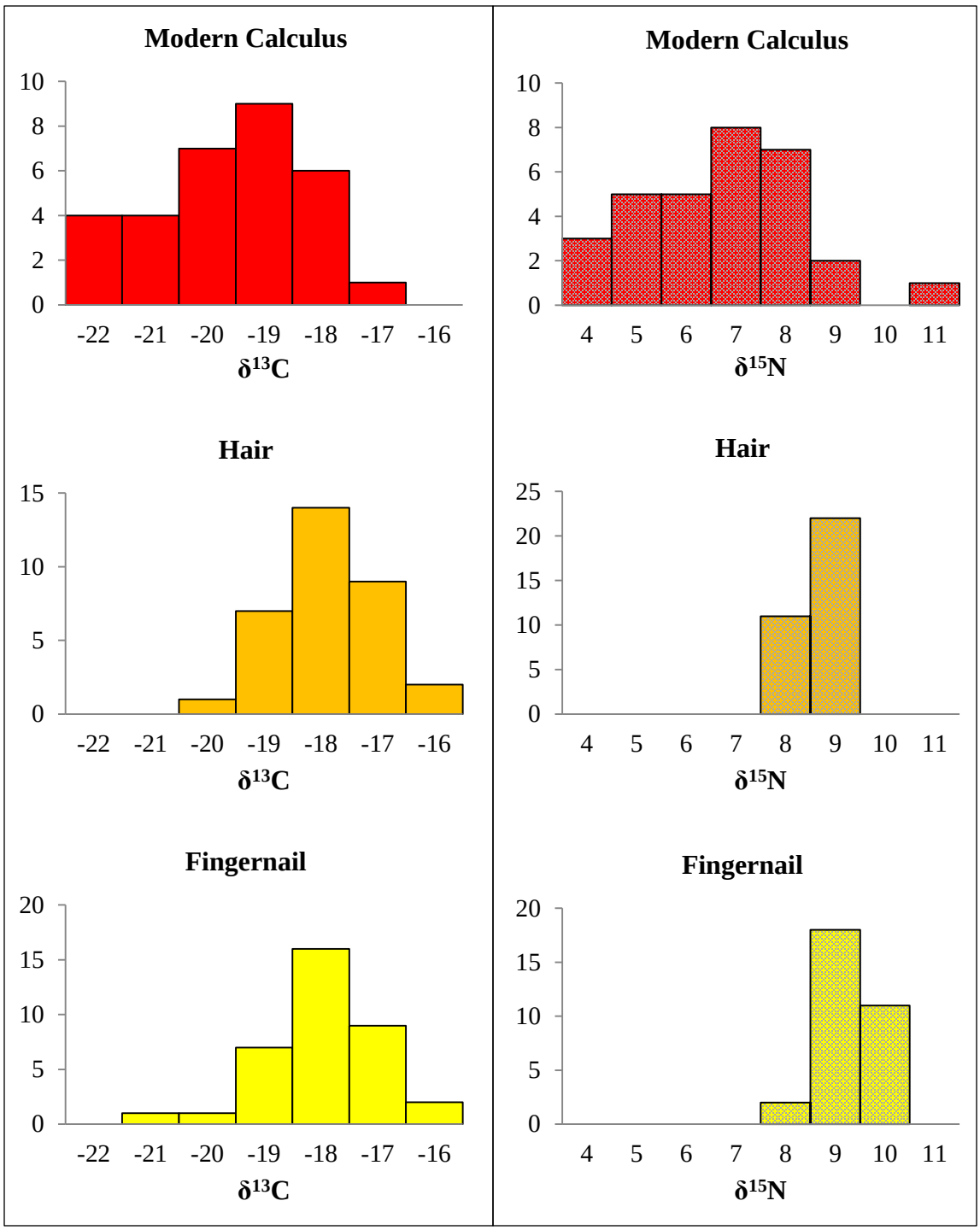

Fig. 26. Histograms of the $\delta^{13}C$ and $\delta^{15}N$ for calculus, hair and fingernail. (Calculus, n = 31; Hair, n = 33; Fingernail, n = 31)

7.0 CONCLUSIONS

This study examined stable carbon and nitrogen isotope values of modern dental calculus and compared them to values derived from fingernail and hair samples from the same individuals. A recent study demonstrated there was sufficient carbon and nitrogen in ancient dental calculus samples to get isotope compositions that could be replicated and were consistent with values derived from the traditional proxy, bone collagen (Scott and Poulson, 2012). To establish calculus as another proxy for stable isotope studies, several lines of research had to unfold. This research focused on the question of whether or not isotope ratios from modern calculus showed the same pattern as isotope values from two established proxies, hair and fingernails.

Calculus is not pure protein, like most isotope proxies, but is a biomaterial of mixed origins. Due to its mixed composition, dental calculus was not expected to produce the same resolution of stable isotope results as fingernails and hair. Despite that caveat, stable carbon and nitrogen isotope ratios for modern dental calculus show a reasonable correlation with hair and fingernails for $\delta^{13}C$ but not for $\delta^{15}N$. This complication may reflect the impact of biofilm on the $\delta^{15}N$ results seen in the varying elemental compositions for the modern calculus samples. As a biofilm, plaque has significantly greater amounts of carbon and nitrogen compared to highly mineralized calculus. While the addition of varying levels of plaque in the samples did not have a negative effect on the $\delta^{13}C$ values, it is a complicating factor for $\delta^{15}N$ values. Still, the combination of oral bacteria, salivary proteins, food particles and oral mucosa appear to produce signatures that are in line with dietary expectations. More research is needed to understand why dental calculus and plaque appear to yield different $\delta^{15}N$ values, while $\delta^{13}C$ values of modern dental calculus

gave good correlations to fingernail and hair. The one downside to the $\delta^{13}C$ correlations was that they did not show a one-to-one relationships to hair and fingernails, but this may represent the influence of biofilm.

The relationship between modern calculus $\delta^{13}C$ values and the $\delta^{13}C$ values from the hair and fingernail samples supports the position that dental calculus has potential for stable isotope analysis in prehistoric samples. While plaque residue may impact the $\delta^{15}N$ of the modern calculus samples, this would not be a factor in ancient calculus. The poor relationship between $\delta^{15}N$ of calculus and hair and nails cannot be used to support or oppose using calculus as an isotope proxy for ancient remains.

7.1 Future Research

When this work was initiated, it was novel and exploratory. There was no way to predict the stable isotope and elemental compositions of human dental calculus. Other workers have studied hair and fingernails so the expectations were more straightforward for these biomaterials. As it turned out, results for hair and fingernails were in line with expectations. The results for calculus, on the other hand, were unexpected. A number of findings require additional research to help explain the patterns of variation in the chemistry of calculus. Some questions that require follow up include:

1. Why is there a good correlation in $\delta^{13}C$ between calculus and hair/nails but no comparable correlation for $\delta^{15}N$.

2. Given the stark contrast in correlations, why do the mean values for $\delta^{13}C$ and $\delta^{15}N$ show fundamentally the same pattern when compared to hair and nail samples. For both carbon and nitrogen, calculus is about 1‰ to 3‰ lighter, with almost identical variance values.

3. Is dental calculus only suitable for stable nitrogen isotope analysis if plaque and gingival fluid are minimized.

4. What is the impact of plaque on both the elemental and stable isotope compositions of calculus. To address this question, larger samples with at least 5 mg of calculus will be required, along with ancillary biomaterials (i.e. fingernails or hair). Larger calculus samples could be cleaned using different rinsing methods to remove and thus reduce the impact of plaque. If the sample was broken down into groups that could be rinsed a varying number of times (once, twice, thrice, not at all), the $\delta^{13}C$ and $\delta^{15}N$ contrasts to other biomaterials (e.g., hair) would help determine what effect biofilm had on isotope ratios and elemental weight percentages. Following this approach, one would be in a better position to determine if there is a one-to-one correlation between the stable isotope ratios of calculus and those of hair and nails, a question the current study cannot fully resolve.

5. The primary goal of isotope research on human dental calculus is to establish its utility as a nondestructive isotope proxy for ancient human remains. More research is required on both ancient and modern samples to develop this new line of research.

BIBLIOGRAPHY

Abraham J, Grenón M, Sánchez HJ, Pérez C, Barrea R. 2005. A case study of elemental and structural composition of dental calculus during several stages of maturation using SRXRF. J Biomed Mater Res A 75:623-628.

Adams TS, Sterner RW. 2000. The effect of dietary nitrogen content on trophic level ^{15}N enrichment. Limnol Oceanogr 45:601-607.

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

Ambrose SH. 1986. Stable carbon and nitrogen isotopic analysis of human and animal diet in Africa. J Hum Evol 15:707-731.

Ambrose SH. 1990. Preparation and characterization of bone and tooth collagen for isotopic analysis. J Archaeol Sci 17:431-451.

Ambrose SH. 1991. Effects of diet, climate and physiology on nitrogen isotope abundances in terrestrial foodwebs. J Archaeol Sci 18:293-317.

Ambrose SH, Butler BM, Hanson DB, Hunter-Anderson RL, Krueger HW. 1997. Stable isotopic analysis of human diet in the Marianas Archipelago, Western Pacific. Am J Phys Anthropol 104:343-361.

Atahan P, Dodson J, Li X, Zhou X, Hu S, Chen L, Bertuch F, Grice K. 2011. Early Neolithic diets at Baijia, Wei River valley, China: stable carbon and nitrogen isotope analysis of human and faunal remains. J Archaeol Sci 38:2811-2817.

Aufderheide AC, Kelley MA, Rivera M, Gray L, Tieszen LL, Iversen E, Krouse HR, Carevic A. 1994. Contribution of chemical dietary reconstruction to the assessment of adaptation by ancient highlands immigrants (Alto Ramirez) to coastal conditions at Pisagua, North Chile. J Archaeol Sci 21:515-524.

Bell LS, Cox G, Sealy J. 2001. Determining isotope life history trajectories using bone density fractionation and stable isotope measurements: a new approach. Am J Phys Anthropol 116:66-79.

Benson AA, Calvin M. 1950. The path of carbon in photosynthesis, VII: respiration and photosynthesis. J Exp Bot I:63-68.

Blatt SH, Redmond BG, Cassman V, Sciulli PW. 2011. Dirty teeth and ancient trade: evidence of cotton fibres in human dental calculus from Late Woodland, Ohio. Int J Osteoarchaeol 21:669-678.

Buchardt B, Bunch V, Helin P. 2007. Fingernails and diet: stable isotope signatures of a marine hunting community from modern Uummannaq, North Greenland. Chem Geol 244:316-329.

Cerling TE, Harris JM, Ambrose SH, Leaky MG, Solounias N. 1997. Dietary and environmental reconstruction with stable isotope analyses of herbivore tooth enamel from the Miocene locality of Fort Ternan, Kenya. J Hum Evol 33:635-650.

Choy K, Ok-Ryun J, Fuller BT, Richards MP. 2010. Isotopic evidence of dietary variations and weaning practices in the Gaya Cemetery at Yeanri, Gimhae, South Korea. Am J Phys Anthropol 142:74-84.

Christersson LA, Grossi SG, Dunford RG, Machtei EE, Genco RJ. 1992. Dental plaque and calculus: risk indicators for their formation. J Dent Res 71:1425-1430.

Clayton F, Sealy J, Pfeiffer S. 2006. Weaning age among foragers at Matjes River Rock Shelter, South Africa, from stable nitrogen and carbon isotope analyses. Am J Phys Anthropol 129:311-317.

Coltrain JB, Hayes MG, O'Rourke DH. 2004. Sealing, whaling and caribou: the skeletal isotope chemistry of Eastern Arctic foragers. J Archaeol Sci 31:39-57.

Coltrain JB. Janetski JC, Carlyle SW. 2007. The stable- and radio-isotope chemistry of western basket maker burials: implications for early Puebloan diets and origins. Am Antiquity 72:301-321.

Cowan MM, Van der Mei HC, Rouxhet PG, Busscher HJ. 1992. Physico-chemical and structural properties of the surfaces of *Peptostreptococcus micros* and *Streptococcus mitis* as compared to those of *Mutans streptococci*, *Streptococcus sanguis* and *Streptococcus salivarius*. J Gen Microbio 138:2707-2714.

Craig H. 1953. The geochemistry of the stable carbon isotopes. Geochim Cosmochim Ac 3:53-92.

Craig H. 1957. Isotope standards for carbon and oxygen and correction factors for mass-spectrometric analyses of carbon dioxide. Geochim Cosmochim Ac 12:133-149.

DeNiro MJ. 1987. Stable isotopy and archaeology. Am Sci 75:182-191.

DeNiro MJ, Epstein S. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Ac 42:495-506.

DeNiro MJ, Epstein S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Ac 45:341-351.

DeNiro MJ, Schoeniger MJ. 1983. Stable carbon and nitrogen isotope ratios of bone collagen: variations within individuals, between sexes, and within populations raised on monotonous diets. J Archaeol Sci 10:199-203.

Drucker D, Bocherens H. 2004. Carbon and nitrogen stable isotopes as tracers of change in diet breadth during middle and upper Palaeolithic in Europe. Int J Osteoarchaeol 14:162-177.

Drucker DG, Henry-Gambier D. 2005. Determination of the dietary habits of a Magdalenian woman from Saint-Germain-la-Rivière in southwestern France using stable isotopes. J Hum Evol 49:19-35.

Dupras TL, Tocheri MW. 2007. Reconstructing infant weaning histories at Roman Period Kellis, Egypt using stable isotope analysis of dentition. Am J Phys Anthropol 134:63-74.

Ehleringer J, Björkman O. 1997. Quantum yields for CO_2 uptake in C_3 and C_4 plants dependence on temperature, CO_2, and O_2 concentration. Plant Physiol 59:86-90.

Ehleringer JR, Cerling TE, Helliker BR. 1997. C_4 photosynthesis, atmospheric CO_2, and climate. Oecologia 112:285-299.

Ehleringer JR, Sage RF, Flanagan LB, Pearcy RW. 1991. Climate change and the evolution of C_4 photosynthesis. Trends Ecol Evol 6:95-99.

Evans DT. 1973. A Preliminary evaluation of tooth tartar among the preconquest Maya of the Tayasal Area, El Peten, Guatemala. Am Antiquity 38:489-493.

Farnsworth P, Brady JE, DeNiro MJ, MacNeish RS. 1985. A re-evaluation of the isotopic and archaeological reconstructions of diet in the Tehuacan Valley. Am Antiquity 50:102-116.

Fox CL, Juan J, Albert RM. 1996. Phytolith analysis on dental calculus, enamel surface, and burial soil: information about diet and paleoenvironment. Am J Phys Anthropol 101:101-113.

Fuller BT, Richards MP, Mays SA. 2003. Stable carbon and nitrogen isotope variations in tooth dentine serial sections from Wharram Percy. J Archaeol Sci 30:1673-1684.

Fuller BT, Molleson TI, Harris DA, Gilmour LT, Hedges REM. 2006. Isotopic evidence for breastfeeding and possible adult dietary differences from Late/Sub-Roman Britain. Am J Phys Anthropol 129:45-54.

Gil AF, Neme GA, Tykot RH. 2011. Stable isotopes and human diet in central western

Argentina. J Archaeol Sci 38:1395-1404.

Gröcke DR. 1997. Stable-isotope studies on the collagen and hydroxyl apatite components of fossils: palaeoecological implications. Lethaia 30:65-78.

Hardy K, Blakeney T, Copeland L, Kirkham J, Wrangam R, Collins M. 2009. Starch granules, dental calculus and new perspectives on ancient diet. J Archaeol Sci 36:248-255.

Hatch MD, Slack CR. 1966. Photosynthesis by sugarcane leaves: A new carboxylation reaction and the pathway of sugar formation. Biochem J 101:103-111.

Hatch MD, Slack CR. 1967. Further studies on a new pathway of photosynthetic carbon dioxide fixation in sugarcane, and its occurrence in other species. The Biochemical Journal 102:417-422.

Hayashizaki J, Ban S, Nakagaki H, Okumura A, Yoshii S, Robinson C. 2008. Site specific mineral composition and microstructure of human supra-gingival dental calculus. Arch Oral Biol 53:168-174.

Hedges REM, Reynard LM. 2007. Nitrogen isotopes and the trophic level of humans in archaeology. J Archaeol Sci 34:1240-1251.

Henry AG, Piperno DR. 2008. Using plant microfossils from dental calculus to recover human diet: a case study from Tell al-Raqa'i, Syria. J Archaeol Sci 35:1943-1950.

Henry MG, Brooks AS, Piperno DR. 2011. Microfossils in calculus demonstrate consumption of plants and cooked foods in Neanderthal diets (Shanidar III, Iraq; Spy I and II, Belgium). PNAS 108:486-491.

Herrscher E, Le Bras-Goude G. 2010. Southern French Neolithic populations: isotopic evidence for regional specificities in environment and diet. Am J Phys Anthropol 141:259-272.

Hinrichs JE. 2006. The role of dental calculus and other predisposing factors. In: Newman MG, Takei H, Klokkevold P, Caranza FA, editors. Carranza's clinical periodontology. St. Louis, MO: WB Saunders. p 170-192.

Jay M, Fuller BT, Richards MP, Knüsel CJ, King SS. 2008. Iron Age breastfeeding practices in Britain: isotopic evidence from Wetwang Slack, East Yorkshire. Am J Phys Anthropol 136:327-337.

Katzenberg MA, Saunders SR, Fitzgerald WR. 1993. Age differences in stable carbon and nitrogen isotope ratios in a population of prehistoric maize horticulturists. Am J Phys Anthropol 90:267-281.

Kellner CM, Schoeninger MJ. 2007. A simple carbon isotope model for reconstruction prehistoric human diet. Am J Phys Anthropol 133:1112-1127.

Kusaka S, Hyodo F, Yumoto T, Nakatsukasa M. 2010. Carbon and nitrogen stable isotope analysis on the diet of Jomon populations from two coastal regions of Japan. J Archaeol Sci 37:1968-1977.

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

Lieverse AR. 1999. Diet and the aetiology of dental calculus. Int J Osteoarchaeol 9:219-232.

Lillie M, Richards MP, Jacobs K. 2003. Stable isotope analysis of 21 individuals from the Epipalaeolithic cemetery of Vasilyevka III, Dnieper Rapids region, Ukraine. J Archaeol Sci 30:743-752.

Lillie M, Budd C, Potekhina I. 2011. Stable isotope analyses of prehistoric population from the cemeteries of the Middle and Lower Dnieper Basin, Ukraine. J Archaeol Sci 38:57-68.

Linderholm A, Kjellström A. 2011. Stable isotope analysis of a medieval skeletal sample indicative of systemic disease from Siguna Sweden. J Archaeol Sci 38:925-933.

Loesche WJ. 1996. Microbiology of dental decay and periodontal disease. In: Baron S, editor. Medical microbiology (4^{th} edition). University of Texas Medical Branch at Galveston: p 1169-1184.

Macko SA, Engel MH, Andrusevich V, Lubec G, O'Connell TC, Hedges REM. 1999. Documenting the diet in ancient human populations through stable isotope analysis of hair. Philos Trans R Soc Lond 354:65-76.

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

Millin DJ, Smith MH. 1961. Nature and composition of dental plaque. Nature 189:664-665.

Minagawa M. 1992. Reconstruction of human diet from $\delta^{13}C$ and $\delta^{15}N$ in contemporary Japanese hair: a stochastic method for estimating multi-source contribution by double isotopic tracers. Appl Geochem 7:145-158.

Moher, FL. 1955. Reference samples of isotopic abundance. Science 122:334-335.

Naito YI, Honch NV, Chikaraishi Y, Ohkouchi N, Yoneda M. 2010. Quantitative evaluation of marine protein contribution in ancient diets based on nitrogen isotope ratios of individual amino acids in bone collagen: an investigation at the Kitakogane Jomon site. Am J Phys Anthropol 143:31-40.

Nardoto GB, Silva S, Kendall C, Ehleringer JR, Chesson LA, Ferraz ESB, Moreira MZ, Ometto JPHB, Martinelli LA. 2006. Geographical patterns of human diet derived from stable-isotope analysis of fingernails. Am J Phys Anthropol 131:137-146.

Nier AO, Gulbransen EA. 1939. Variations in the relative abundance of the carbon isotopes. J Am Chem Soc 61:697-698.

O'Connell TC, Hedges REM. 1999. Isotopic comparison of hair and bone: archaeological analyses. J Archaeol Sci 26:661-665.

O'Connell TC, Hedges REM. 2001. Isotopic comparison of hair, nail and bone: modern analyses. J Archaeol Sci 28:1247-1255.

Paritte JM, Kelly JF. 2009. Effect of cleaning regime on stable-isotope ratios of feathers in Japanese Quail (*Coturnix japonica*). Auk 126:165-174.

Pate, F.D. Brodie, R. & Owen, T.D. 2002. Determination of geographic origin of unprofaned aboriginal skeletal remains in South Australia employing stable carbon and nitrogen isotope analysis. Austral Archaeol 55:1-7.

Prowse TL, Schwarcz HP, Saunders SR, Macchiarelli R, Bondioli L. 2005. Isotopic evidence for age-related variation in diet from Isola Sacra, Italy. Am J Phys Anthropol 128:2-13.

Prowse TL, Schwarcz HP, Garnsey P, Knyf M, Macchiarelli R, Bondioli L. 2007. Isotopic evidence for age-related immigration to Imperial Rome. Am J Phys Anthropol 132:510-519.

Rajendran R, Sivapathasundharam B. 2009. Shafer's textbook of oral pathology (6^{th} edition). Elsevier India Put Ltd. p 417-420.

Reitsema LJ, Crews DE, Polcyn M. 2010. Preliminary evidence for medieval Polish diet from carbon and nitrogen stable isotopes. J Archaeol Sci 37:1413-1423.

Richards MP, Mays S, Fuller BT. 2002. Stable carbon and nitrogen isotope values of bone and teeth reflect weaning age at the medieval Wharram Percy Site, Yorkshire, UK. Am J Phys Anthropol 119:205-210.

Rightmire GP, Van der Merwe GP. 1976. Two burials from Phalaborwa and the

association of race and culture in the Iron Age of southern Africa. South Afr Archaeol Bull 31:147-52.

Roy DA, Hall R, Mix AC, Bonnichsen R. 2005. Using stable isotope analysis to obtain dietary profiles from old hair: A case study form Plains Indians. Am J Phys Anthropol 128:444-452.

Schoeninger MJ. 1995. Stable Isotope studies in human evolution. Evol Anthropol 4:83-98.

Schoeninger MJ. 2006. Population inferences from bone chemistry. In: Ubelaker DH editor. Handbook of North American Indians, vol 3: environment, origins, and population. Smithsonian Institution, Washington. p 640-644.

Schoeninger MJ. 2009. Stable isotope evidence for the adoption of maize agriculture. Curr Anthropol 50:633-640.

Schoeninger MJ. 2010. Diet reconstruction and ecology using stable isotope ratios. In: Larsen CS editor. A companion to biological anthropology. Blackwell Publishing Ltd. West Sussex, UK. p 445-464.

Schulting RJ, Blockley SM, Bocherens H, Drucker D, Richards M. 2008. Stable carbon and nitrogen isotope analysis on human remains from the Early Mesolithic site of La Vergne (Charente-Maritime, France). J Archaeol Sci 35:763-772.

Schurr MR, Powell ML. 2005. The role of changing childhood diets in the prehistoric evolution of food production: An isotopic assessment. Am J Phys Anthropol 126:278-294.

Schwarcz HP, Schoeninger MJ. 1991. Stable isotope analyses in human nutritional ecology. Yearb Physical Anthropol 34:283-321.

Scott GR, Poulson SR. 2012. Stable carbon and nitrogen isotopes of human dental calculus: a potentially new non-destructive proxy for paleodietary analysis. J Archaeol Sci 39:1388-1393.

Slomiany BL, Murty VLN, Aono M, Sarosiek J, Slomiany A, Mandel ID. 1983. Basic biological sciences: lipids of supragingival calculus. J Dent Res 62:862-865.

Slovak NM, Paytan A. 2009. Fisherfolk and farmers: carbon and nitrogen isotope evidence from Middle Horizon Ancón, Peru. Int J Osteoarchaeol 21:253-267.

Tafuri MA, Craig OE, Canci A. 2009. Stable isotope evidence for the consumption of millet and other plants in Bronze Age Italy. Am J Phys Anthropol 139:146-153.

Thompson AH, Chesson LA, Podlesak DW, Bowen GJ, Cerling TE, Ehleringer JR. 2010. Stable isotope analysis of modern human hair collected from Asia (China, India, Mongolia, and Pakistan). Am J Phys Anthropol 141:440-451.

Tsuda H, Arends J. 1993. Raman spectra of human dental calculus. J Dent Res 72:1609-1613.

Tykot, RH. 2006. Isotope analyses and the histories of maize. In: Staller JE, Benz RH, editors. Histories of maize: multidisciplinary approaches to the prehistory, linguistics, biogeography, domestication, and evolution of maize. Academic Press (Elsevier). p 131-142.

Tykot RH, Staller JE. 2002. The importance of early maize agriculture in coastal Ecuador: new data from La Emerenciana. Curr Anthropol 43:666-677.

Vogel JC, Van Der Merwe NJ. 1977. Isotopic evidence for early maize cultivation in New York State. Am Antiquity 42:238-242.

Van Der Merwe NJ. 1982. Carbon isotopes, photosynthesis, and archaeology: different pathways of photosynthesis cause characteristic changes in carbon isotope ratios that make possible the study of prehistoric human diets. Am Sci 70:596-606.

Van Der Merwe NJ, Vogel JC. 1978. ^{13}C content of human collagen as a measure of prehistoric diet in woodland North America. Nature 276:815-816.

Van Der Merwe NJ. 1992. Light stable isotopes and the reconstruction of prehistoric diets. P Brit Acad 77:247-264.

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

Werner RA, Bruch BS, Brand WA. 1999. ConFlo III – an interface for high precision $\delta^{13}C$ and $\delta^{15}N$ analysis with an extended dynamic range. Rappid Commun Mass Sp 13: 1237-1241.

Wesolowski V, de Souza SMFM, Reinhard KJ, Ceccantini G. 2010. Evaluating microfossil content of dental calculus from Brazillian sambaquis. J Archaeol Sci 37:1326-1338.

White CD, Schwarcz HP. 1994. Temporal trends in stable isotopes for Nubian mummy tissues. Am J Phys Anthropol 93:165-187.

White CD, Armelagos GJ. 1997. Osteopenia and stable isotope ratios in bone collagen of Nubian female mummies. Am J Phys Anthropol 103:185-199.

Williams LJ, White CD, Longstaffe FJ. 2005. Trophic level and macronutrient with the weaning process in the Postclassic Maya. Am J Phys Anthropol 128:781-790.

Williams LJ, White CD, Longstaffe FJ. 2011. Improving stable isotopic interpretations made from human hair through reduction of growth cycle error. Am J Phys Anthropol 145:125-136.

Wright LE, Schwarcz HP. 1998. Stable carbon and oxygen isotopes in human tooth enamel: identifying breastfeeding and weaning in prehistory. Am J Phys Anthropol 106:1-18.

Yesner DR, Torres MJF, Guichon RA, Borrero LA. 2003. Stable isotope analysis of human bone and ethno historic subsistence patterns in Tierra del Fuego. J Anthropol Archaeol 22:279-291.

Yoshinaga J, Minagawa M, Suzuki T, Ohtsuka R, Kawabe T, Inaoka T, Akimichi T. 1996. Stable carbon and nitrogen isotopic composition of diet and hair of Gidra-speaking Papuans. Am J Phys Anthropol 100:23-34.