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THE ROYAL SOCIETY

Documenting the diet in ancient human populations through stable isotope analysis of hair

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Fundamental to the understanding of human history is the ability to make interpretations based on artefacts and other remains which are used to gather information about an ancient population. Sequestered in the organic matrices of these remains can be information, for example, concerning incidence of disease, genetic defects and diet. Stable isotopic compositions, especially those made on isolates of collagen from bones, have been used to help suggest principal dietary components. A significant problem in the use of collagen is its long-term stability, and the possibility of isotopic alteration during early diagenesis, or through contaminating condensation reactions. In this study, we suggest that a commonly overlooked material, human hair, may represent an ideal material to be used in addressing human diets of ancient civilizations.

Through the analysis of the amino-acid composition of modern hair, as well as samples that were subjected to radiation (thus simulating ageing of the hair) and hair from humans that is up to 5200 years old, we have observed little in the way of chemical change. The principal amino acids observed in all of these samples are essentially identical in relative abundances and content. Dominating the compositions are serine, glutamic acid, threonine, glycine and leucine, respectively accounting for approximately 15%, 17%, 10%, 8% and 8% of the total hydrolysable amino acids. Even minor components (for example, alanine, valine, isoleucine) show similar constancy between the samples of different ages. This constancy clearly indicates minimal alteration of the amino-acid composition of the hair. Further, it would indicate that hair is well preserved and is amenable to isotopic analysis as a tool for distinguishing sources of nutrition.

Based on this observation, we have isotopically characterized modern individuals for whom the diet has been documented. Both stable nitrogen and carbon isotope compositions were assessed, and together provide an indication of trophic status, and principal type (C_3 or C_4) of vegetation consumed. True vegans have nitrogen isotope compositions of about 7‰ whereas humans consuming larger amounts of meat, eggs, or milk are more enriched in the heavy nitrogen isotope. We have also analysed large cross-sections of modern humans from North America and Europe to provide an indication of the variability seen in a population (the supermarket diet). There is a wide diversity in both carbon and nitrogen isotope values based at least partially on the levels of seafood, corn-fed beef and grains in the diets. Following analysis of the ancient hair, we have observed similar trends in certain ancient populations. For example, the Coptics of Egypt (1000 BP) and Chinchorro of Chile (5000–800 BP) have diets of similar diversity to those observed in the modern group but were isotopically influenced by local nutritional sources. In other ancient hair (Egyptian Late Middle Kingdom mummies, *ca.* 4000 BP), we have observed a much more uniform isotopic signature, indicating a more constant diet. We have also recognized a primary vegetarian component in the diet of the Neolithic Ice Man of the Oetztaler Alps (5200 BP). In certain cases, it appears that sulphur isotopes may help to further constrain dietary interpretations, owing to the good preservation and sulphur content of hair. It appears that analysis of the often-overlooked hair in archaeological sites may represent a significant new approach for understanding ancient human communities.

Keywords: stable isotopes; mummy; amino acids; Coptic; Chinchorro; Ice Man

1. INTRODUCTION

Establishing the diets of ancient human populations is an integral component of most archaeological studies. Stable isotope analysis of well preserved bone, bone extracts, or bone collagen has been one of the most direct approaches for a general assessment of palaeodiet. However, this method has been limited by the scarcity of well-preserved skeletal materials for this type of destructive analysis. Hair is preserved at many burial sites or in mummification, but has been overlooked as an alternative material for isotopic analysis. Here we report the stable carbon, nitrogen and sulphur isotope values for the hair from a variety of mummified humans. Whereas previous investigations have focused on bone collagen, the stable isotope composition of hair may prove to be a reliable proxy for palaeodiet reconstruction, in particular when skeletal remains are not well preserved and additional archaeological artefacts are unavailable.

Traditional methods for the determination of palaeodiets are often based on incomplete and sometimes misleading archaeological records of, e.g. faunal and floral remains, artefacts or other cultural evidence and dental records of ancient populations. Subsequent to early reports that the stable isotope compositions of animals tend to reflect the isotope compositions of their respective diets (DeNiro & Epstein 1978, 1981), there have been numerous attempts to use this approach for dietary reconstructions, including that of ancient human populations (e.g. Schoeninger *et al*. 1983, 1984; Spielman *et al*. 1990; White 1993) and even Mesozoic age reptiles (Ostrom *et al*. 1993). Work on ancient populations has generally focused on the stable isotopic analysis of bone collagen, the assumption being that the preservation of bone collagen is sufficient to retain the original isotopic signal of the organism. However, even under the best of circumstances, bone collagen tends to be altered by diagenetic reactions such as hydrolysis, decarboxylation and deamination (Macko & Engel 1991). Thus, how closely the stable isotope composition of residual collagen is likely to reflect that of the original biomolecule has been the topic of much debate (e.g. Sillen *et al*. 1989).

An alternative proteinaceous material that is commonly encountered in ancient human burials is hair (J. Hantman, personal communication). Consisting of α-keratins, which include hair and fingernails, as well as horn, skin and wool (Lehninger *et al*. 1994), hair is composed of hydrophobic proteins that are not easily degraded (Lubec *et al*. 1987). The microbial degradation of these insoluble macromolecules depends on the secretion of extracellular enzymes with the ability to act on the surface. Keratins have a mechanical stability dependent on the tight-packing of the α-helix and can be degraded by a few species of parasitic and saprotrophic fungi as well as thermophilic anaerobic bacteria (Bockle *et al*. 1995; Friedrich & Antranikian 1996).

Numerous studies have used stable isotope analyses of tissues from living organisms and have documented the capacity for estimating the diet of an organism as well as its trophic level in a food web (Ostrom & Fry 1993). There exists a strong similarity between the isotopic composition of an organism and that of its diet as a result of biosynthesis, and the incorporation of nitrogen, carbon or sulphur from the diet into the tissues of the consumer. In the case of carbon, the isotopic composition of the diet will reflect the biosynthetic pathways used (C_3 or C_4) in the conversion of CO_2 into plant carbon. It is well established that C_4 plants have $\delta^{13}C$ compositions averaging -12.5‰, whereas C_3 plants have average values of -26.5‰ (Tieszen 1991; Koch *et al*. 1994). Further, the sources of carbon to the primary producers (bicarbonate by marine plants or atmospheric CO_2 used by terrestrial plants) are reflected in the organisms from those environments, with marine values typically being approximately -20‰ (Koch *et al*. 1994). Small fractionations have been reported for carbon (+1‰) relative to the diet (Ostrom & Fry 1993). The nitrogen isotopic composition of an organism also reflects the source nitrogen of the primary production, with average values for marine and terrestrial plants being +6 and 0‰, respectively. An approximate 3‰ enrichment is typically observed in ^{15}N with each trophic level increase in natural food chains (Macko & Engel 1991). Sulphur isotopic compositions have not been used as often as either carbon or nitrogen. The sulphur isotope content of an organism directly reflects the ^{34}S composition of its diet, without significant fractionation (MacAvoy *et al*. 1998), and can be useful for distinguishing marine (*ca*. +15 to +20‰) from terrestrial (*ca*. +5‰) end members. Dietary interpretations using isotopic data obtained on human archaeological remains are based on comparisons with the isotopic compositions of modern plants and animals (Vogel & van der Merwe 1977; Vogel 1978; Tauber 1981; Chisholm *et al*. 1982, 1983). The observation that isotopic compositions can be used to interpret modern natural food webs necessitated laboratory studies to establish alterations or fractionations of isotopic compositions at both the molecular biosynthetic level and at the bulk organism trophic level (DeNiro & Epstein 1978, 1981; Tieszen *et al*. 1983; Nakagawa *et al*. 1985; Tieszen & Boutton 1988; Katzenberg & Krouse 1989; Ambrose & Norr 1993; Tieszen & Fagre 1993). These research efforts have resulted in the necessary information about the ultimate fates of carbon, nitrogen and sulphur during feeding and metabolism and have provided the basic framework to use this powerful tool in palaeodietary reconstruction.

Relative to other modern animals, the effects of dietary variation on the isotopic values of human tissues has been little studied, owing to a need for destruction of the human tissue for analysis. Archaeological isotopic analyses of human remains are usually made on well-preserved bone collagen because bones are often the only part of the body recovered after significant burial periods. As a result, numerous animal isotopic studies have concentrated on bone collagen and the relationship between bone collagen isotopic values and diet (see Ambrose 1993; Ambrose & Norr 1993). An advantage of using bone collagen for palaeodietary analysis is that it has a long turnover period, and is therefore thought to reflect the diet consumed over a period of about ten years (Stenhouse & Baxter 1979). However, bone is not a readily available sample material from most modern living humans. In most investigations of modern living humans, hair has been used as a representative sample material for all body tissues (Webb *et al*. 1980; Nakamura *et al*. 1982; Minagawa 1992; Yoshinga *et al*. 1991, 1996), although urine and blood have also been used (Schoeller *et al*. 1986; Katzenberg & Krouse 1989). Hair keratin has been used because the limited animal data suggest that keratin isotopic values closely reflect diet. For adult male beard hair, an isotopic shift has been observed to result from a change in diet in as little as 6–12 days (Nakamura *et al*. 1982). This observation follows the fact that it takes a minimum of six days for the growing hair to emerge from the skin (Saitoh *et al*. 1969).

The carbon isotopic values of well-preserved collagen and the hair protein keratin correlate well with diet (DeNiro & Epstein 1978; Jones *et al*. 1981; Katzenberg & Krouse 1989; Hare *et al*. 1991; Ambrose & Norr 1993;

Tieszen & Fagre 1993). The nitrogen isotopic values are also very similar and correlate well with diet, being typically enriched by about 3% (DeNiro & Epstein 1981; Nakagawa *et al*. 1985; Sealy *et al*. 1987; Ambrose 1993). In agreement with the available animal isotopic data, these studies of modern human hair suggest that hair isotopic values do reflect the isotopic composition of the diet and its variation (Webb *et al*. 1980; O'Connell 1996). Isotopic analysis has been suggested as a method for establishing animal protein consumption in archaeological humans. This is because δ^{15}N values increase as the food chain is ascended, with carnivores having higher δ^{15}N compositions than the herbivores on which they feed, and herbivores having higher δ^{15}N values than their plants. It has been argued that a diet low in meat would produce lower δ^{15}N values in the body proteins of the consumer and one high in meat would produce higher δ^{15}N values in the consumer (Schoeninger & DeNiro 1984). Estimation of the amount of meat or animal protein intake in the diet of archaeological populations is important in establishing the type of subsistence, i.e. agricultural as opposed to a pastoral or hunting society.

Based on these and our own isotopic studies of modern hair (Macko *et al*. 1998), we suggest that it is possible to estimate the palaeodiets of ancient humans based on the stable isotope compositions of their hair and that the stable carbon, sulphur and nitrogen isotope compositions of modern human hair are both reliable and powerful indicators of the diets of these ancient individuals, especially when used together on the same sample.

Here we report a test of this hypothesis, focusing on reconstruction of the palaeodiets of two different large populations of mummified humans, the Coptics of Egypt (1000 BP) and Chinchorro of Chile (5000–800 BP), as well as other individual mummies. These isolated samples include the Ice Man, preserved in a glacier of the Oetztaler Alps during the Neolithic, *ca*. 5200 years ago and nine samples from Egyptian mummies of the Late Middle Kingdom Dynasty (*ca*. 4000 BP).

2. MATERIALS AND METHODS

(a) *Hair samples*

Coptic mummy hair and authentic dried foods from the Hofburg Emperors' collection were provided by Dr M. Teschler-Nicola of the Department of Anthropology, University of Vienna. Late Middle Kingdom samples were donated by Dr Eugen Strouhn, University of Prague, Czech Republic. Samples of Chinchorro mummy hair and authentic dried foods from the Chinchorro sites were provided by Dr Bernardo Arriaza, University of Nevada, Las Vegas. Hair samples of the Ice Man and associated grass and goat hair were provided by Dr G. Wortmann, Deutsches Wollforschungs Institut, Aachen, Germany. For comparison, modern hair samples were collected from vegans (individuals having no animal protein in their diet) and from individuals subsisting on vegetarian (having egg or milk protein as well as plants) or omnivorous (eating meat, secondary animal products and plants) diets. All hair samples were wiped with a Kimwipe tissue and cleaned with distilled ethanol to remove any possible superficial debris. One sample of modern hair was subdivided into 10 mg aliquots and irradiated at 23 °C using a ^{60}Co source (Gammacell, Nordion Int. Co., Canada) with an activity of 20 kCu and equipped with three lead attenuators for the reduction of the dose rate. Dosimetry was carried out by the Ficke principle. The shell organic matter from a Chinchorro site was isolated from the carbonate matrix through acidification, filtration and drying on an ashed glass fibre filter.

(b) *Amino-acid composition and stereochemistry*

With the expectation that even well-preserved ancient hair keratin will likely have undergone alteration to some minor extent (Lubec *et al*. 1994), samples of modern hair and that of a large number of the samples from the mummified remains were analysed for their respective amino-acid distributions as a first approximation of relative preservation. The individual hair samples were characterized with respect to their amino-acid distributions through hydrolysis in quartz-distilled 6N HCl in tubes which were sealed under N_2 and heated at 100 °C for 24 h. An aliquot of the acid liquor was diluted with quartz-distilled water for injection into an amino-acid analyser. Separation and quantification of the amino acids was done by high performance liquid chromatography (HPLC) using a cation-exchange column (5 μ resin, St John Associates), with stepwise isocratic elution and post-column derivatization with ortho-phthaldialdehyde (OPA) for fluorescence detection and integration (Hare *et al*. 1985). Although proline is a component of keratin, it could not be detected by the chromatographic method employed using OPA. Proline, however, was observed in the analyses done by gas chromatography (GC). Aliquots of the hydrolyzates of the hair samples were derivatized to trifluoroacetyl isopropyl esters and analysed by GC using an optically active stationary phase (Chirasil-Val) to determine the extent to which the amino acids may have been racemized. Details of the GC method are reported elsewhere (Serban *et al*. 1988; Macko *et al*. 1997).

(c) *Isotope analysis*

The cleaned hair samples and authentic foods were converted to CO_2, N_2 and SO_2 for isotope analysis using a Carlo Erba elemental analyser which was coupled to an OPTIMA stable isotope ratio mass spectrometer (Micromass, Manchester, UK). The stable isotopic ratio is reported as follows

$$\delta^{N}E = (R_{sample}/R_{standard} - 1) \times 10^3 \text{ (‰)},$$

where N is the heavy isotope of the element E, and R is the abundance ratio of the heavy to light isotopes ($^{13}C/^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{32}S$) of that element. The standard for carbon is the Peedee Belemnite limestone (PDB), for nitrogen atmospheric N_2 (air), and for sulphur Canyon Diablo Troilite (CDT), all of which are assigned δ^{N}E values of 0.0‰. The reproducibility of the measurement is typically better than ±0.2‰ for the elements using the continuous flow interface on the OPTIMA. In the laboratory, the samples are commonly measured against tanks of CO_2, N_2 and SO_2 gases which have been calibrated against NBS 22, atmospheric N_2 and NBS 127, respectively.

3. RESULTS

(a) *Amino acids*

Compared to modern hair, the mummified samples exhibited slight decreases in the absolute abundances (figure 1*a*) of the more unstable amino acids (e.g. serine, threonine), this loss increasing with time (modern > Coptic > Ice Man). The principal amino acids observed in all of these samples are, however, essentially identical in relative

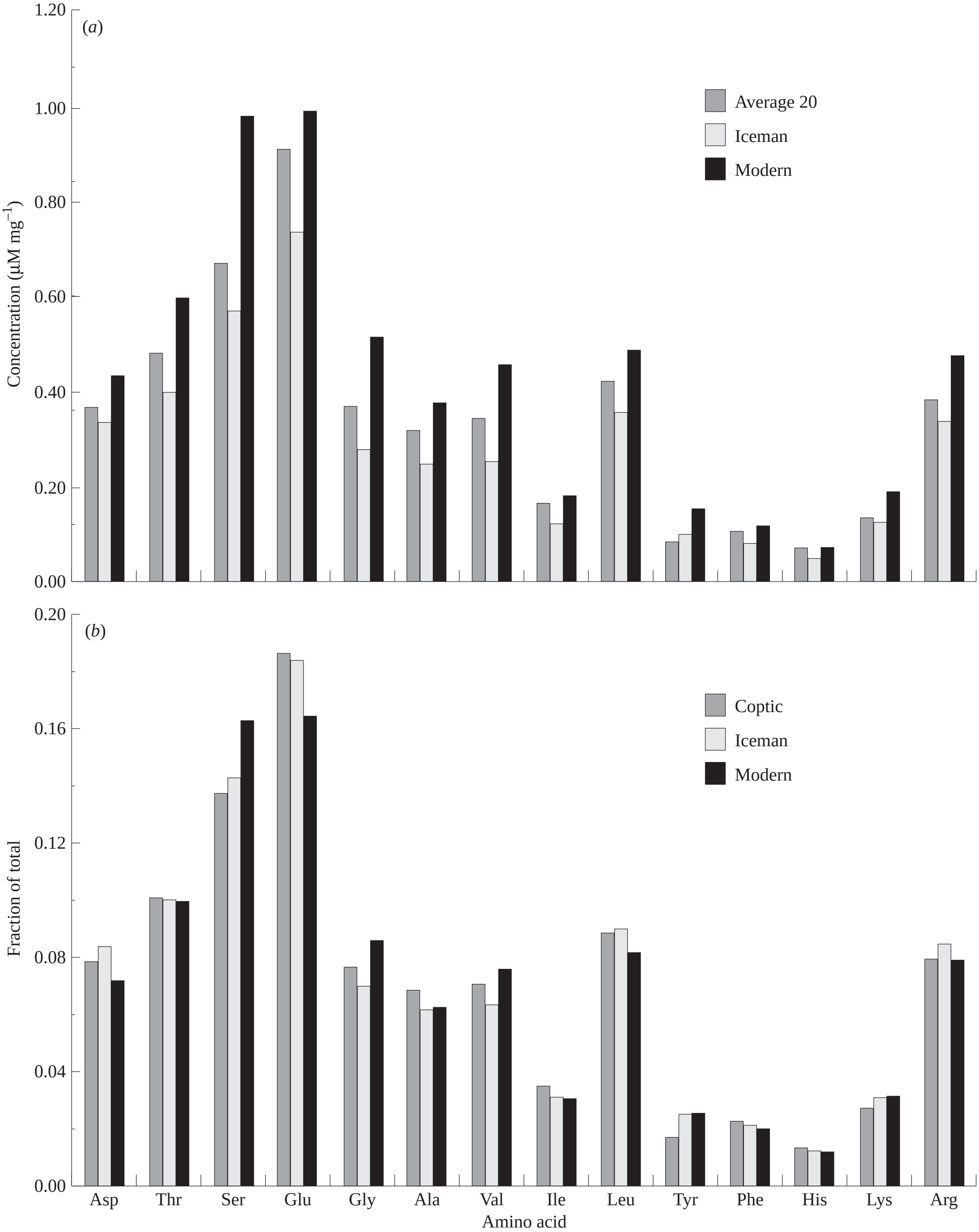

Figure 1. (*a*) Absolute abundances of individual amino acids in hair from Coptic (average of 20 samples) and Ice Man mummies, as well as modern hair. (*b*) Relative fractional abundances of individual amino acids in hair from Coptic (average of 20 samples) and Ice Man mummies, as well as modern hair.

abundances and content. Dominating the compositions are serine, glutamic acid, threonine, glycine and leucine, respectively accounting for approximately 15%, 17%, 10%, 8% and 8% of the hydrolysable amino acids. Even minor components (e.g. alanine, valine, isoleucine) show similar constancy between the samples of different ages. Additionally, the relative percentages of individual amino acids that comprise the modern hair and these ancient samples (the Ice Man and Coptics) were remarkably similar (figure 1*b*; table 1). The elevated percentages of hydrophobic amino

Table 1. *Relative levels of individual amino acids to total amino acids (molar basis) in modern and ancient hair*

amino acid	modern	Coptic	Ice Man
Asp	0.07	0.08	0.08
Thr	0.10	0.10	0.10
Ser	0.16	0.14	0.14
Glu	0.16	0.19	0.18
Gly	0.09	0.08	0.07
Ala	0.06	0.07	0.06
Val	0.08	0.07	0.06
Ile	0.03	0.03	0.03
Leu	0.08	0.09	0.09
Tyr	0.03	0.02	0.03
Phe	0.02	0.02	0.02
His	0.01	0.01	0.01
Lys	0.03	0.03	0.03
Arg	0.08	0.08	0.08
total	1.00	1.00	1.00

acids (e.g. leucine, alanine, glycine, valine, isoleucine) in these hair samples are consistent with the distributions observed for modern hair keratin, and may in part account for its resistance to hydrolysis subsequent to interment. The irradiation treatment had no observable effect either on the amino acid content or the stereochemistry of the hair. The fact that none of the amino-acid constituents of the mummified hair exhibited any indication of racemization above background levels also supports the suggested excellent preservation of these ancient hair keratins.

(b) *Stable isotopes*

(i) *Modern hair*

There was no significant difference in hair $\delta^{13}C$ values among the population of Austrian vegetarians in this study (figure 2*a*). A similar study of modern human hair from the people of Oxford, UK, also indicated no significant differences among the carbon signals of vegans, vegetarians and omnivores, but clear enrichments in ^{15}N were seen between the individuals reporting to be on vegan diets and those on vegetarian and omnivorous diets (table 2). The isotopic compositions of carbon among hair from modern vegetarians and omnivores have not been observed to be statistically different (Webb *et al.* 1980; O'Connell 1996). Clearly, the availability of C_4 foods to the population makes it difficult to assess any change in $\delta^{13}C$ related solely to levels of dietary intake. The Austrian population of vegetarians consisted of two true vegans and eight vegetarians, the latter reportedly deriving some portion of their nutrition from milk products or eggs. The true vegans had $\delta^{15}N$ values of *ca.* 7‰, whereas the other vegetarians were significantly more enriched in ^{15}N. Clearly, the supplementation of diet with eggs and milk products yields ^{15}N isotope signatures in the consumers that are similar to those of omnivores. In fact, in some of the ovo-lacto-vegetarians, the heightened ^{15}N signals were nearly the same as individuals who reported high levels of meat in their diets (O'Connell 1996). The variability in the ^{15}N signals would reflect the amounts of these supplements to the basic vegan diet. The observations for the Austrian group were nearly identical

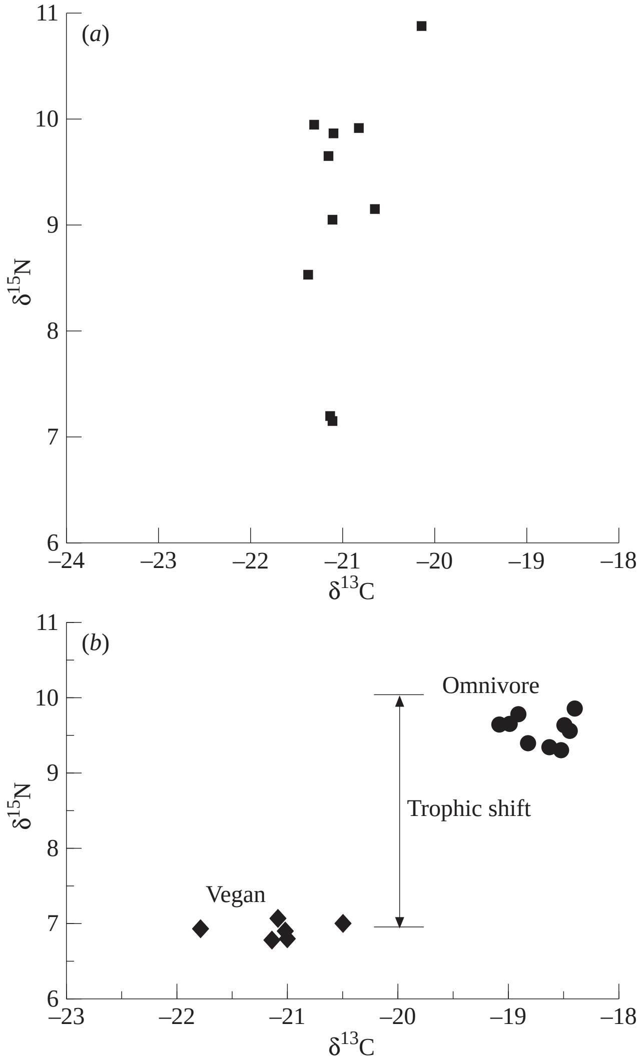

Figure 2. (*a*) Distribution of carbon and nitrogen isotopes in a modern population of Austrians identified as vegetarians or vegans. (*b*) Change in nitrogen isotope content of modern individual, with change in diet from omnivore to vegan.

to those seen in the individuals from the UK, with the groups overlapping in ^{13}C, and the true vegans having less ^{15}N than either the ovo-lacto-vegetarians or omnivores (table 2).

The long-term effect of a change from an omnivorous to a vegan diet on the isotopic values of hair from an individual gave similar results (figure 2*b*). A comparison of nitrogen isotopic values from hair samples cut approximately 20 years apart, shows a difference of nearly 3‰ between hair grown when the individual was an omnivore and that grown as a vegan. No dependence of isotopic values of body tissues on age of adult humans has been found (Minagawa & Wada 1984; Lovell *et al.* 1986; White & Schwarcz 1989; Katzenberg *et al.* 1993). Therefore, this decrease in $\delta^{15}N$ can be ascribed to a decrease in animal protein in the diet. The striking change in carbon isotopic values, is less simple to explain, but is consistent with a change from a diet higher in C_4 influenced meats and marine foods to a vegan C_3 based diet.

Radiation had no significant effect on the stable nitrogen or carbon isotopic compositions of the hair, with the irradiated samples being essentially indistinguishable

Table 2. *Mean isotopic analyses of carbon and nitrogen in a modern population from Oxford, UK, showing dietary preference groups*

group	individuals	δ^{13}C	s.d.	δ^{15}N	s.d.
vegans	8	−20.9	0.8	6.9	0.5
vegetarians	6	−21.0	0.3	8.7	0.5
omnivores	14	−20.2	0.7	8.8	0.6

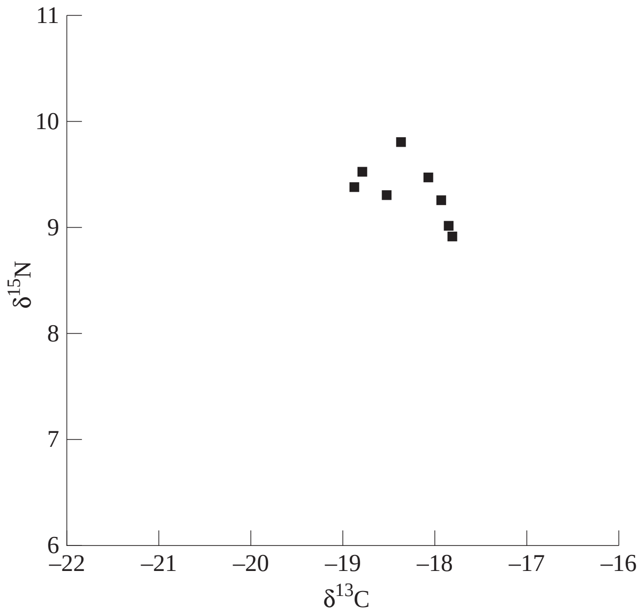

Figure 3. Effect of radiation on the isotope composition of hair, simulating ageing of the α-keratin. Included in the plot are both irradiated and control (non-irradiated) samples.

from non-irradiated controls. The variations observed were within the distributions observed for an individual (figure 3) reflecting the variability of a modern individual's diet over the time the hair grew.

(ii) *Coptic mummies*

The distribution of isotope signatures seen in the hair from the Coptic mummies (figure 4) is striking in both the ranges of both carbon and nitrogen, and the enrichments in ^{15}N. Authentic foods of these individuals obtained from this site show a normal C_3 signal in carbon (table 3). Rape seed, a material rich in oil is the most depleted in ^{13}C, which is likely to be the result of a contribution of ^{13}C depleted lipid to the total carbon signal of the seed. The ^{15}N of the foods is slightly enriched relative to the expected values observed in modern plants (which is likely a result of the present use of Haber process fertilizers). The date seed is somewhat depleted in nitrogen isotope content relative to the other ancient plants, perhaps reflecting the phreatophytic nature of this plant. The nitrogen used by the date tree would reflect a source completely different from that of the plants grown agriculturally. The sulphur signals are fairly typical of terrestrial materials, and are quite uniform for all of the plants.

(iii) *Chinchorro mummies*

The Chinchorro mummy hair samples are divided into groupings based on the location of the burial sites. The

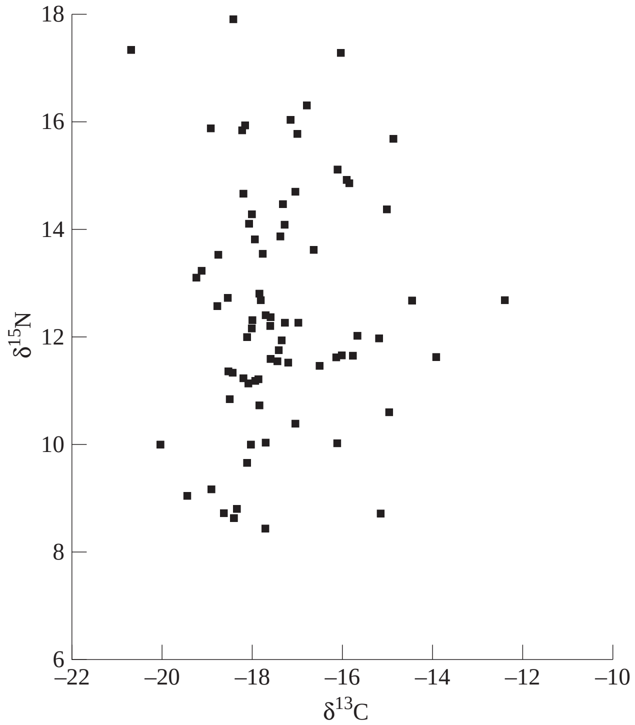

Figure 4. Stable nitrogen and carbon isotopic compositions of the Coptic mummy hair.

Table 3. *Authentic foods derived from the sites of the mummy burials:* (*a*) *Emperors' Collection, Hofburg* (ca. 1000 BP); (*b*) *Arica Region, Chile* (5000–800 BP)

(nd, not determined.)

	code	^{13}C	^{15}N	^{34}S
(*a*) Coptic food				
rape seed	708	−27.9	8.6	5.6
emmer wheat	407	−24.9	12.0	7.3
date seed	404	−25.5	1.6	7.6
mustard seed	658	−25.5	12.3	5.9
watermelon	474	−24.5	11.5	nd
castor bean	725	−23.0	10.2	nd
grape seed	700	−24.3	11.0	8.6
cow bean	725	−24.7	8.9	nd
(*b*) Chinchorro food				
jiquina (tuber)	AZ6	−23.9	14.3	6.8
maize (Azapa Valley)		−9.5	10.5	5.9
fish (Morro 1)[a]	M1T28C19	−16.1	24.3	15.6
shell (Estrato B)[b]	CAM PN	−22.5	21.6	16.8
maize	AZ141T31	−8.8	9.6	6.2

[a] Tissue.
[b] Shell organic component.

Morro site is on the coast, and the Azapa Valley site is inland. The Maderas Enco site is also coastal, but located between the Morro and Azapa Valley sites (Arriaza 1995). The authentic foods found in these sites (table 3) include C_3 plants, C_4 plants and fish/shellfish. The isotope signals of these materials in general are typical of such materials in the modern world, with the exception of the ^{15}N content. The samples of the plants and animal tissues were highly enriched in nitrogen isotope compositions as compared to

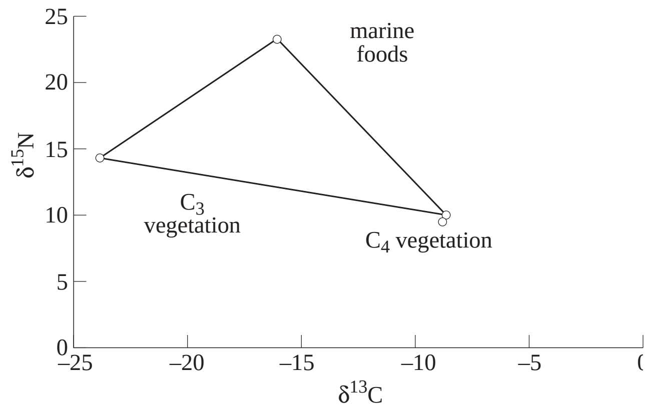

Figure 5. Isotopic analyses of carbon and nitrogen for authentic foods from the Arica, Chile region, representing possible food sources. Lines establish potential mixing relationships and expected field for diets.

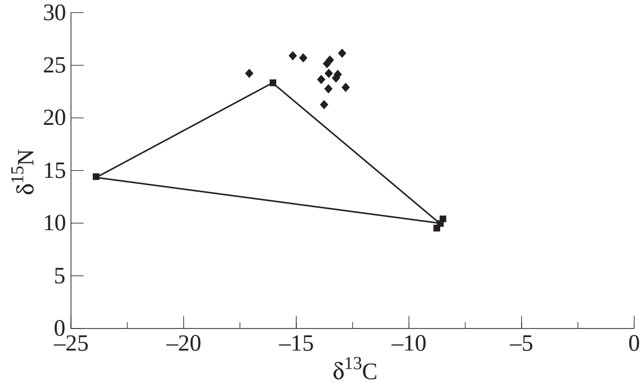

Figure 6. Isotopic analyses of carbon and nitrogen of mummy hair from Morro site from the Arica, Chile region, a coastal site. Lines establish potential mixing relationships and expected field for diets.

all other similar materials presently grown or found in aquatic settings (figure 5). One would expect the 'dietary field' established by connecting these end member foods, to include much of the potential range of diets for the Chinchorro mummies. As can be seen for the Morro site (figure 6), the isotopic compositions of the hairs of the mummies all lie outside the field. This is completely expected, owing to the fact that there are well-recognized fractionations with trophic level, probably the result of synthesis and metabolism (see §1). If the carbon and nitrogen isotope compositions of the potential diets are increased by 1‰ and 3‰, respectively, the field for potential consumers is established (figure 7*a*). Some elevation in the ^{15}N signals of the individuals using terrestrial foodstuffs (Azapa Valley or Maderas Enco sites) could be the result of consumption of animal proteins (llamas for example, after Arriaza (1995)). It was not possible to inlude these terrestrial animal end members in the authentic foods from this location. However, the use of terrestrial animal proteins would only slightly alter the dietary field (figure 5). Essentially all of the Morro samples lie on a line between C_4 vegetation and the marine foods, near the marine end member. The samples from the Maderas Enco site also show a similar location on the mixing line connecting these two end members (figure 7*b*). The samples from the Azapa Valley (figure 7*c*) samples lie more to the central portion of this dietary field, and suggest a greater contribution of terrestrial C_4 and C_3 plants to their diets.

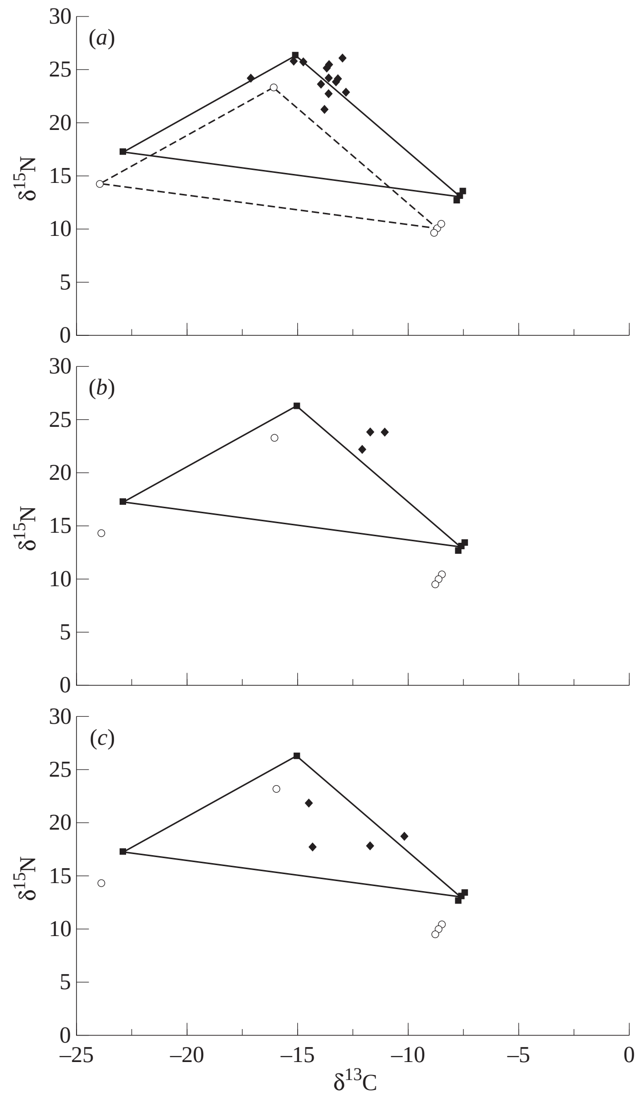

Figure 7. (*a*) Isotopic analyses of carbon and nitrogen of mummy hair from coastal Morro site from the Arica, Chile region. Lines establish potential mixing relationships and expected field for diets (open squares, dashed lines), but are shifted for suggested fractionation of carbon and nitrogen by consumer (closed squares, solid lines). (*b*) Isotopic analyses of carbon and nitrogen of mummy hair from Maderas Enco site from the Arica, Chile region, a coastal site. Lines establish potential mixing relationships for diets (open squares), but are shifted for suggested fractionation of carbon and nitrogen by consumer (closed squares, solid lines). (*c*) Isotopic analyses of carbon and nitrogen of mummy hair from Azapa Valley site from the Arica, Chile region, an inland site. Lines establish potential mixing relationships for diets (open squares), but are shifted for suggested fractionation of carbon and nitrogen by consumer (closed squares, solid lines).

(iv) *Late Middle Kingdom mummies*

By comparison to the previous three populations (modern, Coptic and Chinchorro hair) the hair of the mummies from the Late Middle Kingdom are extremely uniform (figure 8). The entire range is approximately 4‰ in carbon and approximately 2.5‰ in nitrogen for these

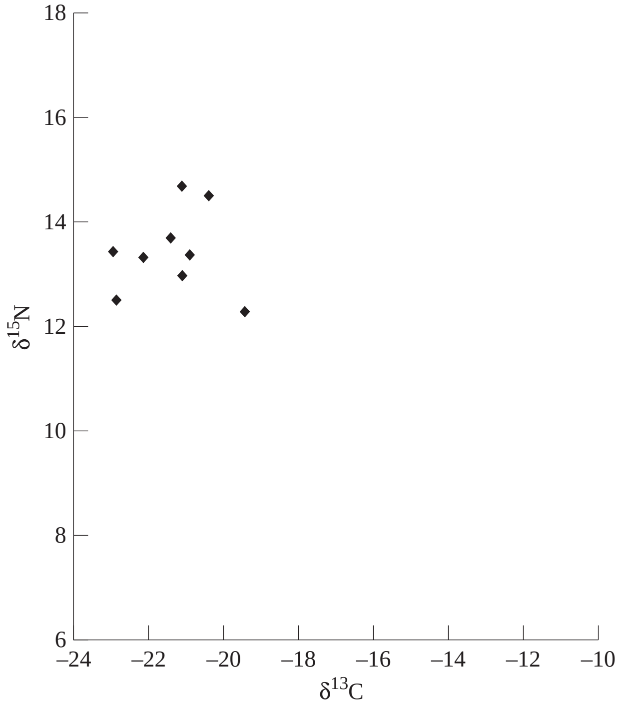

Figure 8. Isotopic analyses of carbon and nitrogen of mummy hair from Late Middle Kingdom, Egypt.

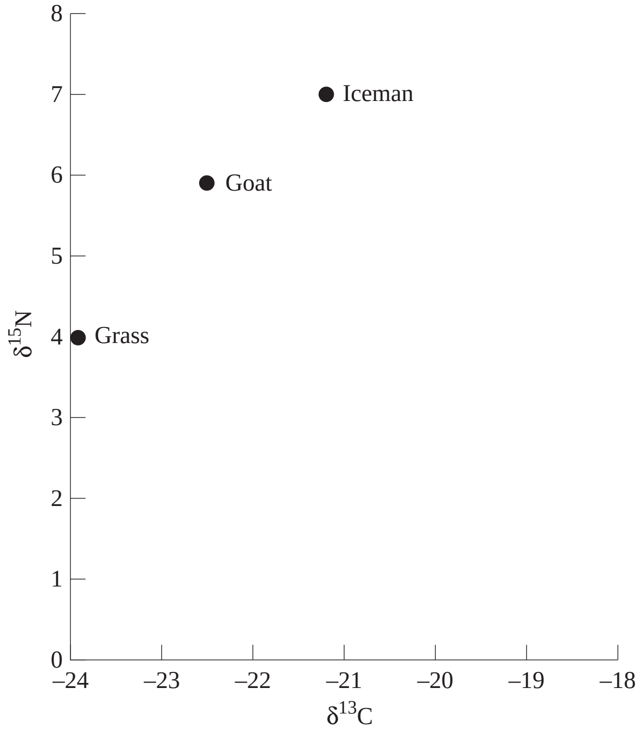

Figure 9. Isotopic analyses of carbon and nitrogen of hair from Ice Man mummy, associated grass, and goat hair, all from Oetztaler Alps.

nine samples. This narrow range reflects perhaps a select diet of foods that may have been available to these individuals. This is not to say that the foods were not present in the society, but that the foods consumed by these individuals, being of a higher socio-economic status, were perhaps more restricted. The carbon isotopes indicate that the foods consumed included a general influence of C_3 plants (a comparison can be made with the authentic foods in table 3 from the region, although younger in age).

(v) *Ice Man*

The stable carbon and nitrogen isotope values for the Ice Man's hair and the associated remains of goat fur and grass-like plants (figure 9) provide some significant clues with respect to his diet. Depending on the complexity of a food web, δ^{13}C values of an organism may show slight to moderate enrichment in ^{13}C relative to diet (Macko & Engel 1991). However, once again, the key for distinguishing the trophic level of a heterotroph is the stable nitrogen isotope composition. With increasing progression up a food chain, the δ^{15}N values of the organism become *ca.* 3‰ enriched relative to the diet (Macko & Engel 1991). As will be discussed below, δ^{15}N value of 7.0‰ for the Ice Man clearly points to a diet which is essentially vegan, and is consistent with the tooth abrasion observed in this specimen (Hoepfel & Spindler 1992). Plant and pollen found associated with the Ice Man confirms the availability of cereals (Hoepfel & Spindler 1992) with farming widespread throughout Europe and crops being readily available (Whittle 1985).

4. DISCUSSION

(a) *Modern hair*

Modern hair is highly reflective of the diet of an individual and shows expected trophic level shifts from the associated diet. It had been surmised that there is little effect of shampoo on the stable isotope ratios of hair, nor has there been observed any difference in values of grey and non-grey hair from members of the same family with the same diet (Minagawa 1992; O'Connell 1996). The amino acids in hair can be affected by dyes and bleaching (Baba *et al.* 1973) which in turn can alter the isotopic composition (O'Connell 1996). There have been no statistically significant variations between the isotopic values or C:N ratios of different types of body hair (scalp, axillary and pubic) from the same individual (O'Connell 1996). This present study suggests that the long-term diagenetic effects may also be minimal in both the amino-acid content and isotopic composition of the hair, as evidenced by the irradiation results.

(b) *Coptic mummies*

The Coptic mummy hair indicates the influence of both C_3 and C_4 vegetation on the diet of these individuals. It is likely that in addition to the foods listed, other vegetation based on C_4 synthesis (sorghum, millet) was a significant portion of their diets (White & Schwarcz 1994). The enrichments seen among these individuals in ^{15}N likely reflect the large variation in foods and meats they accessed. A wide variety of meats were available, including camel, horse, sheep, goat, pig, chicken, fish and shellfish such as mussels and snails (Hofmann 1967; Haaltzka 1985; Rowley-Conway 1989). There is some evidence of variation in diet among the Coptics owing to differences based on socio-economic status, with peasants not eating pork, others not consuming fish, etc. Additional confounding evidence, however, may be that there were long fasting periods, and the influence of this fasting on the isotope composition of an individual is not well

Table 4. *Sulphur isotope compositions of mummy hair*

	$\delta^{34}S$
Coptic	
K14	8.9
K23	8.3
K45	9.6
K52	7.9
K68	8.6
K76	8.6
Chinchorro	
MO1R2	16.5
MO1T28C14	15.6
MO16T22	15.9
MO16T53	14.8
Maderas Enco C1	15.8
Maderas Enco C3	13.6
CAM15DT23	14.3
AZ140T38	14.2
AZ140T055	8.2
AZ140TXPB2A	9.6
AZ140T118	9.1

established. Elevation in the nitrogen isotope signal in the plants could also have resulted from the use of animal wastes to fertilize the plants, although this is not documented. The observation of the degree of variability in these signals indicates that a wide range of foods was available, and the diets of this population were perhaps similar in this context to that seen in modern populations with the 'supermarket' range in potential foods (Macko *et al*. 1998). A number of these individuals had isotopic compositions of both carbon and nitrogen typical of more modern diets, without the high enrichments in ^{15}N. Sulphur isotopic compositions on the hair samples were similar to values obtained from authentic foods of the region (tables 3 and 4).

(c) *Chinchorro mummies*

The samples obtained from the Maderas Enco site and from the Morro site are believed to be coastal dwellers, and were users of the coastal foods, including fish and shellfish (Arriaza 1995). The Azapa Valley site, located inland from the Morro and the Maderas Enco sites, is thought to have been more agricultural (Arriaza 1995). The isotope compositions are consistent with mixtures of the foods for these locations and for the general food types analysed in the authentic foods. The results also suggest that perhaps 90% of the protein in the diets of the individuals from the Morro site was derived from marine sources. The Morro site appears to have a slightly greater influence of the coastal materials in their diet than the Maderas Enco site. The Azapa Valley individuals, with the exception of one sample (AZ140T38), are consistent with more terrestrial foods. As further support for these observations, sulphur isotope compositions show that the coastal sites reflect closely the marine signal, and the Azapa Valley site individuals are similar to the authentic foods from that location (tables 3 and 4). However, the hair of the individual showing the strong marine dietary influence in carbon and nitrogen isotopes at the Azapa Valley site also showed a strong marine sulphur signal. This verification of the marine signal would suggest that this individual may have been a recent coastal immigrant to the community, or a visitor, whose diet in the Azapa Valley had not been influenced long enough to alter the composition of the portion of the hair we measured. This also indicates that the two regions did have some level of interaction, and exchange. Previously, higher nitrogen isotope compositions had been reported for plants from this area. (DeNiro & Epstein 1981; DeNiro & Hastorf 1985.) The highest plants are from Peruvian coastal desert archaeological sites and dry caves in the arid Tehuacan Valley of Mexico (DeNiro & Epstein 1981; DeNiro & Hastorf 1985). These high values have been considered to be the result of unknown diagenetic processes. However, environmental factors and cultural practices may have contributed to high plant $\delta^{15}N$ values in Peru. It is also possible that local use of marine algae to fertilize the cultivated plants, or fertilization with guano, possibly in seawater irrigation of the plants, has contributed to this enrichment. It is clear, however, that the enrichment is on a regional scale, influencing the C_3 plants, C_4 plants, and the nearshore fish and shellfish.

(d) *Late Middle Kingdom mummies*

The slightly elevated nitrogen isotope signals (about 3–6‰ above modern humans) are probably indicative of the similar ^{15}N enrichments seen in the Coptics from this same region. Because the base primary production nitrogen isotope compositions are elevated (table 3), this in turn would influence the higher trophic levels, and thus exhibit itself in the omnivorous consumers these individuals likely were. It is not presently known if this enrichment is the result of natural nitrogen isotope enrichments for the soils of the region, or reflect agricultural practices of fertilization with animal wastes. The carbon isotope signals indicate little C_4 of marine food influence on their diet; however, if a significant component of the diet were derived from freshwater fish, this would account for the enrichments seen here in nitrogen isotopes.

(e) *Ice Man*

With the remains of goat fur found on this site, one might intuitively suggest that goat meat was a significant component of the Ice Man's diet. However, if this were the case, the stable nitrogen isotope composition of his hair should have been 3‰ enriched relative to the goat $\delta^{15}N$ value, and clearly this is not the case. Rather, the $\delta^{15}N$ value for the Ice Man's hair is approximately 3‰ enriched relative to the $\delta^{15}N$ values of plants (figure 9), indicating that plants were likely a primary component of his diet at the time of his death. The stable nitrogen isotope composition of the Ice Man is consistent with that observed for modern vegans (figure 2*a*, table 2), which are depleted in ^{15}N by several per mil relative to modern humans on omnivorous diets (figure 2*b*, table 2). The goat should reflect the vegan diet as well, being enriched by 3‰ over primary production. In all likelihood it does, but represents an integration of browsing on a variety of terrestrial plants, some of which may be more depleted in ^{15}N owing to nitrogen fixation.

The fact that the $\delta^{13}C$ value of the Ice Man was depleted in ^{13}C reflects a high percentage of grains in the diet that can be attributed to plants which used the C_3 pathway for photosynthesis. A similar preference for C_3 plants (or for organisms that subsisted on C_3 plants) was reflected in the diets of the other omnivores, all of which are expected to be a few per mil enriched in ^{13}C relative to their respective C_3 sources.

5. SUMMARY

Stable isotopes provide a powerful probe for the reconstruction of palaeodiets of organisms. Our initial results indicate that under burial conditions such as natural or artificial mummification, where hair is well preserved, its stable isotope compositions can be used to help reconstruct the palaeodiet of the individual. The combination of three different isotopes, sulphur, carbon and nitrogen, further enhances the capabilities of this probe to gain insights in the diet of ancient human populations.

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REFERENCES

Ambrose, S. H. 1991 Effects of diet, climate and physiology on nitrogen isotope abundances in terrestrial foodwebs. *J. Arachaeol. Sci*. **18**, 293–317.

Ambrose, S. H. 1993 Isotopic analysis of palaeodiets: methodological and interpretive considerations. In *Investigations of ancient human tissue* (ed. M. K. Sandford), pp. 59–130. Langhorne, PA: Gordon & Breach Science Publishers.

Ambrose, S. H. & Norr, L. 1993 Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. In *Prehistoric human bone—archaeology at the molecular level* (ed. J. B. Lambert & G. Grupe), pp. 1–37. Berlin: Springer.

Arriaza, B. T. 1995 *Beyond death.* Washington, DC: Smithsonian Institution Press.

Baba, N., Nakayama, Y., Nozaki, F. & Tamura, T. 1973 Denaturation and release of amino acids in hairs treated with cold-waving lotions, hair-dyes, hair-bleaching solutions and depilatories. *J. Hyg. Chem*. **19**, 47.

Bockle, B., Galunsky, B. & Muller, R. 1995 Characterization of a keratinolytic serine proteinase from *Streptomyces pactum* DSM 40530. *Appl. Environ. Microbiol*. **61**, 3705–3710.

Chisholm, B. S., Nelson, D. E. & Schwarcz, H. P. 1982 Stable-carbon isotope ratios as a measure of marine versus terrestrial protein in ancient diets. *Science* **216**, 1131–1132.

Chisholm, B. S., Nelson, D. E. & Schwarcz, H. P. 1983 Dietary information from $\delta^{13}C$ AND $\delta^{15}N$ measurements on bone collagen. *J. Europ. Study Group Phys. Chem. Math. Tech. Appl. Archaeol*. **8**, 391–397.

DeNiro, M. J. & Epstein, S. 1978 Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* **42**, 341–351.

DeNiro, M. J. & Epstein, S. 1981 Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim. Cosmochim. Acta* **45**, 341–351.

DeNiro, M. J. & Hastorf, F. C. A. 1985 Alteration of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios of plant matter during the initial stages of diagenesis: studies using archeological specimens from Peru. *Geochim. Cosmochim. Acta* **49**, 97–115.

Friedrich, A. & Antranikian, G. 1996 Keratin degradation by *Fervidobacterium pennavorans*, a novel thermophilic anaerobic species of the order Thermotogales. *Appl. Environ. Microbiol*. **62**, 2875–2882.

Haaltzka, M. R. M. 1985 *Quelien zur Wirtschaft Aegyptens aus fruebarabischer Zeit in koptischer Sprache*. PhD thesis, University of Vienna, Austria.

Hare, P. E., St John, P. A. & Engel, M. H. 1985 Ion-exchange separation of amino acids. In *Chemistry and biochemistry of the amino acids* (ed. G. C. Barrett), pp. 415–425. New York: Chapman & Hall.

Hare, P. E., Fogel, M. L., Stafford, T. W. Jr, Mitchell, A. D. & Hoering, T. C. 1991 The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. *J. Arachaeol. Sci*. **18**, 277–292.

Hoepfel, F. W. & Spindler, K. 1992 *Der Mann im Eis*. Innsbruck: Eigenverlag der Universitaet.

Hofmann, I. 1967 *Die Kulturen des Nitals von Aswan bis Senner, Vom Messilithikum bis zum Ende der christlichen Epoche*. Kommissionsveriag Cram. Hamburg: De Gruyter & Co.

Jones, R. J., Ludlow, M. M., Troughton, J. H. & Blunt, C. G. 1981 Changes in natural carbon isotope ratio of the hair from steeds fed diets of C4, C3 and C4 species in sequence. *Search* **12**, 85–87.

Katzenberg, M. A. & Krouse, H. R. 1989 Application of stable isotope variation in human tissue to problems in identification. *Can. Soc. Forens. Sci. J*. **22**, 7–19.

Katzenberg, M. A., Saunders, S. R. & Fitzgerald, W. R. 1993 Age differences in stable carbon and nitrogen isotope ratios in a population of prehistoric maize horticulturalists. *Am. J. Phys. Anthropol*. **90**, 267–281.

Koch, P. L., Fogel, M. L. & Tuross, N. 1994 Tracing the diets of fossil animals using stable isotopes. In *Stable isotopes in ecology and environmental science* (ed. K. Lajtha & R. H. Michener), pp. 63–92. Oxford: Blackwell Scientific Publications.

Lehninger, A. L., Nelson, D. L. & Cox, M. M. 1994 *Principles of biochemistry.* New York: Worth Publishers.

Lovell, N. C., Nelson, D. E. & Schwarcz, H. P. 1986 Carbon isotope ratios in palaeodiet: lack of age or sex effect. *Archaeometry* **28**, 51–55.

Lubec, G., Nauer, G., Seifert, K., Strouhal, E., Portecler, H., Szilvassy, I. & Teschler, M. 1987 Structural stability of hair 4000 years old. *J. Arch. Sci*. **14**, 113–120.

Lubec, G., Weninger, M. & Anderson, S. R. 1994 Racemization and oxidation studies of hair protein in the *Homo tirolensis*. *FASEB J*. **8**, 1166–1169.

MacAvoy, S. E., Macko, S. A. & Garman, G. C. 1998 Tracing marine biomass into tidal freshwater ecosystems using stable sulfur isotopes. *Naturwissenschaften*. (In the press.)

Macko, S. A. & Engel, M. H. 1991 Assessment of indigeneity in fossil organic matter, amino acids and stable isotopes. *Phil. Trans. R. Soc. Lond.* B **333**, 367–374.

Macko, S. A., Uhle, M. E., Engel, M. H. & Andrusevich, V. 1997 Stable nitrogen isotope analysis of amino acid enantiomers by gas chromatography/combustion/isotope ratio mass spectrometry. *Analyt. Chem*. **69**, 926–929.

Macko, S. A., Engel, M. H. & Freeman, K. 1998 Variability of isotope compositions in modern and fossil organic matter. *Chem. Geol.* **152**, 1–2.

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.

Minagawa, M. & Wada, E. 1984 Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between metabolism. *Biomed. Mass Spectrom.* **12**, 502–506.

Nakagawa, A., Kitagawa, A., Asami, M., Nakamura, K., Schoeller, D. A., Slater, R., Minagawa, M. & Kaplan, I. R. 1985 Evaluation of isotope ratio (IR) mass spectrometry for the study of drug metabolism. *Biomed. Mass Spectrom.* **12**, 502–506.

Nakamura, K., Schoeller, D. A., Winkler, F. J. & Schmidt, H.-L. 1982 Geographical variations in the carbon isotope content of the diet and hair of contemporary man. *Biomed. Mass Spectrom.* **9**, 390–394.

O'Connell, T. C. 1996 *The isotopic relationship between diet and body proteins: implications for the study of diet in archaeology.* DPhil thesis, University of Oxford, UK.

Ostrom, P. H. & Fry, B. 1993 Sources and cycling of organic matter within modern and prehistoric food webs. In *Organic geochemistry* (ed. M. H. Engel & S. A. Macko), pp. 785–794. New York: Plenum Press.

Ostrom, P. H., Macko, S. A., Engel, M. H. & Russell, D. A. 1993 Assessment of trophic structure of Cretaceous communities based on stable nitrogen isotope analyses. *Geology* **21**, 491–494.

Rowley-Conway, P. 1989 Nubia AD 0–550 and the 'Islamic' agricultural revolution: preliminary botanical evidence from Qasr Ibrim, Egyptian Nubia. *Archeologie du Nil Moyen* **3**, 131–138.

Saitoh, M., Uzuka, M., Sakamoto, M. & Kobori, T. 1969 Rate of hair growth. In *Hair growth* (ed. W. Montagna & R. L. Dobson), pp. 183–201. New York: Pergamon Press.

Schoeller, D. A., Minagawa, M., Slater, R. & Kaplan, I. R. 1986 Stable isotopes of carbon, nitrogen and hydrogen in the contemporary North American human food web. *Ecol. Food Nutr.* **18**, 159–170.

Schoeninger, M. J. & DeNiro, M. J. 1984 Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochim. Cosmochim. Acta* **48**, 625–639.

Schoeninger, M. J., DeNiro, M. J. & Tauber, H. 1983 Stable nitrogen isotope ratios of bone collagen reflect marine and terrestrial components of prehistoric human diet. *Science* **220**, 1381–1383.

Sealy, J. C., Van der Merwe, N. J., Lee-Thorp, J. A. & Lanham, J. L. 1987 Nitrogen isotopic ecology in southern Africa: implications for environmental and dietary tracing. *Geochim. Cosmochim. Acta* **51**, 2707–2717.

Serban, A., Engel, M. H. & Macko, S. A. 1988 The distribution, stereochemistry and stable isotopic composition of amino acid constituents of fossil and modern mollusk shells. *Org. Geochem.* **13**, 1123–1129.

Sillen, A., Sealy, J. C. & Van der Merwe, N. J. 1989 Chemistry and paleodietary research: no more easy answers. *Am. Antiquities* **54**, 504–512.

Spielmann, K. A., Schoeninger, M. J. & Moore, K. 1990 Plains-Pueblo interdependence and human diet at Pecos Pueblo, New Mexico. *Am. Antiquities* **55**, 745–765.

Stenhouse, M. J. & Baxter, M. S. 1979 The uptake of bomb ^{14}C in humans. In *Radiocarbon dating* (ed. R. Berger & H. E. Suess), pp. 324–341. University of California Press.

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

Tieszen, L. L. 1991 Natural variations in the carbon isotope values of plants: implications for archaeology, ecology, and ealeoecology. *J. Arachaeol. Sci.* **18**, 227–248.

Tieszen, L. L. & Boutton, T. W. 1988 Stable carbon isotopes in terrestrial ecosystem research. In *Stable isotopes in ecological research* (ed. P. W. Rundel, J. R. Ehleringer & K. Nagy), pp. 167–195. Berlin: Springer.

Tieszen, L. L. & Fagre, T. 1993 Effect of diet quality and composition on the isotopic composition of respiratory CO_2, bone collagen, bioapatite, and soft tissues. In *Prehistoric human bone—archaeology at the molecular level* (ed. J. B. Lambert & G. Grupe), pp. 121–155. Berlin: Springer.

Tieszen, L. L., Boutton, T. W., Tesdahl, K. G. & Slade, N. A. 1983 Fractionation and turnover of stable isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. *Oecologia* **57**, 32–37.

Vogel, J. C. 1978 Isotopic assessment of the dietary habits of ungulates. *S. Afr. J. Sci.* **74**, 298–301.

Vogel, J. C. & Van der Merwe, N. J. 1977 Isotopic evidence for early maize cultivation in New York State. *Am. Antiquities* **42**, 238–242.

Webb, Y., Minson, D. J. & Dye, E. A. 1980 A dietary factor influencing ^{13}C content of human hair. *Search* **11**, 200–201.

White, C. D. 1993 Isotopic determination of seasonality in diet and death from Nubian mummy hair. *J. Archaeol. Sci.* **20**, 657–666.

White, C. D. & Schwarcz, H. P. 1989 Ancient Maya diet: as inferred from isotopic and elemental analysis of human bone. *J. Archaeol. Sci.* **16**, 451–474.

White, C. D. & Schwarcz, H. P. 1994 Temporal trends in stable isotopes for Nubian mummy tissues. *Am. J. Phys. Anthropol.* **93**, 165–187.

Whittle, A. 1985 *Neolithic Europe, a survey.* New York: Cambridge University Press.

Yoshinaga, J., Minagwa, M., Suzuki, T., Ohtsuka, R., Kawabe, T., Hongo, T., Inaoka, T. & Akimichi, T. 1991 Carbon and nitrogen isotopic characterization for Papua New Guinea foods. *Ecol. Food Nutr.* **26**, 17–25.

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 Gidran-speaking Papuans. *Am. J. Phys. Anthropol.* **100**, 23–34.

Discussion

R. P. Ambler (*University of Edinburgh, UK*). What sort of damage to hair would affect results? For instance chemical treatment during mummification or cosmetic treatments.

S. A. Macko. You are quite right in the assumption that the isotopic composition of the hair could be altered by the mummification processes used. We have observed no differences in hair that is washed with a variety of solvents or soaps, nor between grey hair and coloured hair. T. C. O'Connell has noted a small difference in some hairs that are bleached. Since the hair appears to consist entirely of well-preserved amino acids, we suspect that the natural drying we saw in the Chinchorro, Ice Man and Coptic mummies has maintained the original signatures. The Middle Kingdom were likely exposed to natron, which as salt, should not have affected the organic composition. The hairs had no evidence of bitumen on them.

B. Sykes (*University of Oxford, UK*). If the Ice Man was a vegan, why was he carrying a bow and arrow with him?

S. A. Macko. I believe he also had an axe with him. The presence of these tools did not require that he used them. Nor that he was proficient at their use. (Some have suggested he was a 'priest' of some sort.) If he was a successful hunter, the isotope signals of the hair simply state that at the period of his death he had a diet overwhelmingly dominated by vegetable material. This was likely longer term than a few months, given the wear of his teeth. The bow could also have been for protection, although I understand he did not have a bowstring on the bow, and it was not a functional bow—it was unfinished.

J. B. Griffiths (*London, UK*). Would a child drinking its mother's milk show up as a carnivore?

S. A. Macko. Yes. A suckling child is a veritable predator.

J. Bada (*University of California—San Diego, USA*). Some recent work on the stable isotopic composition of primate hair has indicated that the nutritional and disease status have an effect on the isotopic composition. These usually shift the isotopic numbers towards heavier values. How can you sort out these effects from the normal dietary isotopic shift?

S. A. Macko. There is some evidence that starvation can affect the ^{15}N signal. The individuals for this study had all died before hair could be sampled and there is no documentation of individual disease or nutritional status. However, it would be unlikely that they all died under nutritional stress or from disease. Furthermore, the ^{15}N signal could change with the stress but the ^{13}C does not appear to. Thus the individual isotope composition would lie off the mixing lines, which was not seen.

G. Eglinton (*University of Bristol, UK*). The hair molecular composition—the α-keratin—certainly means that the α-keratin molecules are in very stable conformations, packed regularly and closely together. Damage can therefore be expected to be limited—for example, hydrolysis into shorter polymer lengths—but oxidation and radiation damage will still occur. It would be really important to know what damage exactly does occur in this very special array of mainly α-keratin molecules—evidence of damage: racemization, reaction of unstable alpha amino acids (e.g. serine), chain fission and generation of new end groups, etc.

S. A. Macko. At present we do not know if there exist differences in the molecular weights of the α-keratins of different age hairs. The evidence that these hairs are fairly unaltered remains that there is essentially no racemization, and that the amino-acid spectrum and content are the same as modern hair. Radiation does not appear to change any of these parameters; assessing the molecular weights present would be an excellent new piece of information.