14.23 Hair as a Geochemical Recorder: Ancient to Modern

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14.23.1 Introduction

Hair is frequently recovered during archaeological excavations and forensic investigations and its ability to record the geochemical environment of an individual presents a unique opportunity to reconstruct the environment, diet, and residential location of individuals. The noninvasive nature of sampling hair from both people and animals presents tremendous collection opportunities for modern ecological, medical, and anthropological studies. The relatively small sample size required for geochemical analyses also makes both historically collected and archaeological hair from museum collections potentially suitable for analysis. Over the past 20 years, there have been significant developments in the utility of hair within archaeological research and forensic science (Wilson, 2005; Wilson and Gilbert, 2007; Wilson and Tobin, 2010; Wilson et al., 2001). In archaeology, both nuclear (nDNA) and mitochondrial (mtDNA) DNA have been successfully recovered from the hair shaft and used in provenancing, to examine familial links, migration, and founder linkages, while DNA from human and nonhuman hairs is of wider significance to forensic casework (Bengtsson et al., 2012; Rasmussen et al., 2010). The use of hair in drug analysis and wider toxicology/pollutant studies has also been exploited in both archaeology and forensic science (Ogalde et al., 2009; Pragst and Balikova, 2006). The focus of this chapter, however, considers the value of geochemical information from hair for traditional archaeological aspects (e.g., seasonal or other influences on mummy hair), as well as modern anthropology (e.g., globalization of diets and drinks), forensic aspects (e.g., history of travel of individuals), physiology (e.g., human health, pollution), and a discussion on geographic and temporal variation in hair oxygen isotope ratios.

14.23.1.1 Structure and Composition of Hair

Much of our understanding of hair is derived from research allied to the textile and cosmetic industries (hair care/grooming) as well as with dermatology (hair graying, hair loss, and other dermatological conditions). Although much of the early work concerning hair form and function was focused on the properties of wool fibers for textiles rather than human hair, there are parallels between the various types of keratin fiber (Swift, 1997). They all possess an outer protective layer known as the cuticle, which is responsible for the optical properties of the fiber. This comprises overlapping cuticle cells arranged in a regularized pattern, one or more cells in thickness. The overall shape, size, and arrangement of these cuticle cells have traditionally been used in both forensic science and archaeology toward characterizing loose, dissociated fibers. Although these properties may vary along the fiber length, cuticle pattern is used together with other measurements such as mean fiber diameter to identify the species from which hairs are derived (Knecht, 2012). The structure
and form of the central medulla are also valuable in this process. The medulla is an air or fluid-filled structure at the core of the fiber that may be continuous, interrupted, or entirely absent along its length. Some animals have a particularly distinct form of medulla; for instance, Leporidae (hares and rabbits) have a characteristic ladder-like medulla and irregular petal-shaped cuticle. There are, however, promising signs that proteomics may offer a means of characterizing fibers where morphological characteristics are inconclusive, even in archaeological contexts (Hollemeyer et al., 2008). Extensive literature on the structure and chemical composition of human hair exists (e.g., Robbins, 2010; Tobin, 2005) but a brief summary is included here. The main bulk of the fiber, responsible for its mechanical and strength properties, is the fiber cortex. The cortex is made up of individual spindle-shaped cortical cells that interdigitate with one another along the fiber length. The cortex is in turn comprised of individual macrofibrils and pigment granules cemented together by nuclear remnants and an intermacrofibrillar matrix. A large proportion of hair consists of the fibrous, structural protein α-keratin, which provides mechanical properties such as elasticity to hair. Like all proteins, keratin is composed of amino acids, which are either derived from the diet (essential) or produced in the body (nonessential) and are condensed to form peptide chains that are ultimately assembled into tightly cross-linked filaments (Powell and Rogers, 1997). The relatively high proportion of cysteine residues in hair contributes to its highly stable structure by forming disulfide bridges between these filaments (Robbins and Kelly, 1970).

### 14.23.1.2 Hair Growth

The hair fiber is formed within the follicle – one of the most metabolically active tissues of the body, served by its own rich vasculature and innervations. As a complex mini-organ, the hair follicle undergoes many of the key physiological processes within the body, including cell differentiation/migration, programmed cell growth/death, and production of/response to a wide array of hormones. As the hair fiber continues to grow, the living cells undergo terminal differentiation to form the hard keratinized structure that eventually emerges from the scalp (Wilson and Tobin, 2010). With keratinization, which occurs above the bulge region of the follicle (see Figure 1), the cells harden and are modified and rearranged to form the different regions of the cortex, cuticle, and medulla within the hair shaft. Hair growth in mammals follows either a synchronous pattern of growth – in other words hair is naturally shed at the same time, as with molting in animals – or an asynchronous, or mosaic pattern, of growth as in humans, where different follicles are usually at different stages of the hair cycle, which may be observed microscopically. On the human scalp, the majority of the hair follicles will be in the active phase of hair growth known as anagen. During anagen, the hair follicle is programmed to grow for a continuous period of up to 10 years dependent on the individual. Anagen is then followed by a short period of resting known as catagen lasting roughly 3 weeks, before the follicle is gradually resorbed during telogen over roughly 3 months, leading eventually to natural shedding of the hair fiber during exogen. During anagen,

![Figure 1](image-url)  
**Figure 1** Schematic showing the hair fiber within the scalp. Detail (left) shows toluidine blue-stained transverse section through the emergent hair shaft to illustrate the relationship of the outer cuticle and cortex; Detail (right) transmission electron micrograph showing discernible pigment granules and cortical cell membrane complex.
fibers grow at a fairly uniform rate and in humans this ranges from about 0.26 mm day\(^{-1}\) in Africoid-type scalp hair to about 0.33 mm day\(^{-1}\) in Caucasoid-type scalp hair (Wilson and Gilbert, 2007). As such the forming hair captures a snapshot of information relating to the individual, which is based on the rate of hair growth and stage within the hair cycle (Appenzeller et al., 2007; Williams et al., 2011). Where possible, consideration should be given to the hair cycle when sampling hair, although it may not always be practical to retrieve the follicle from well-preserved human remains or from living patients, which does imply a resultant time-lag.

The literature describes three main racial categories – Africoid, Caucasoid (which also includes the majority of South Asian-type hair), and Asiatic hair. These categories are very broad in nature and relate largely to hair form – in other words shape in cross-section (Asiatic hair is largely round) and twist, pigment distribution, etc. (Swift, 1997). The human scalp carries approximately the same number of follicles during childhood as it does in adulthood; as such it is the relatively small size of these follicles on the smaller childhood scalp that is responsible for the fineness of hair in children. This therefore has an impact on the relative weight of fiber segments and quantities required with diachronic analysis. Although the growth rate varies according to location on the body and to the type of animal/type of fiber, it is only minimally affected by influences such as disease, pregnancy, and other stressors. Once hair is keratinized during growth, it does not undergo any further biogenic changes and so presents a chronological record of body chemistry that can subsequently be reconstructed. As hair grows, all of its components are derived from the available pool of amino acids, trace metals, and lipids that are present in the body. The analysis of hair as a geochemical recorder is based upon the assumption that this pool is in turn derived from an individual’s environment, taken in through diet, drinking water, and other environmental exposures. Knowledge of the relationship of a given isotopic or trace element component in hair to diet or environment varies greatly. Carbon and nitrogen isotope ratios in hair have been compared with better understood systems such as bone collagen (O’Connell and Hedges, 1999a), demonstrating that relationships between body tissues and diet are tissue-dependent, reflecting the tissue’s biochemical makeup and the metabolic routing of its components. Based upon observations of human hair hydrogen and oxygen isotope ratios, an understanding of the processes involved in the biosynthesis of hair and an ability to link large-scale, well-understood variations in environmental water isotope ratios, Ehleringer et al. (2008) produced a model predicting hair keratin isotopic values.

14.23.2 Survival of Hair in Archaeological and Forensic Contexts

We need to turn to the discipline of taphonomy to understand where and why hair can survive over archaeological timescales and within forensic contexts (Wilson, 2008; Wilson and Tobin, 2010). Taphonomy has its origins in palaeontology, although today its remit has broadened to encompass the study of decay processes and our understanding of the survival of soft and hard tissues (and associated artifacts) within both archaeology and forensic science (Janaway et al., 2009). Taphonomy is used to define the nature of the depositional environment in terms of soil conditions, moisture, pH, redox, temperature, and other biological/chemical constraints.

So robust is keratin, that few biological organisms can exploit it as a nutrient source, and those that do have evolved enzymatic means of digesting the keratin. There are for instance a number of zoophilic and geophilic keratinolytic fungi with wide geographic distributions that can exploit hair. The majority of these fungi are capable of tunneling through the protective cuticle to produce characteristic ovoid lesions on the fiber surface described as fungal tunnels (DeGaetano et al., 1992). Once these fungi have penetrated the cuticle they are then able to exploit the weaker underlying structures of the cortex and move laterally within the fiber, tracking along the medulla. The inherent weakness created by the formation of these fungal tunnels leads to embrittlement and often fragmentation along the fiber length (see Figure 2), which is a potential limitation for diachronic analyses.

Given that fungi are considered to be the primary decomposer organisms, the survival of hair over archaeological timescales usually occurs where fungal activity is inhibited. Such conditions occur where either oxygen or moisture are restricted under extreme environmental conditions such as waterlogging, freezing, or desiccation. Important examples where hair has been found include naturally desiccated remains from arid regions such as Egypt and the coastal desert of South America. Frozen examples include samples from Greenland and from high elevation shrines in the South Central Andes. Waterlogged remains include examples from deeply stratified urban archaeological sites, and midden or peat deposits. The earliest examples of artificial mummification come from the Chinchorro culture centered on the Azapa Valley in northern Chile, although many more cultures have understood the significance of evisceration and embalming practices in arresting putrefactive change (Guillen, 2004). Forensic samples may be recovered from buried remains, but are more usually associated with surface recoveries. Significant for both archaeology and forensic samples is the airflow, as the presence of an air void may influence desiccation and thus survival. Samples from living individuals/other animals may also be of value to geochemical analyses, as discussed later.

In order to understand its long-term survival, it is important to consider the heterogeneity and varied chemistry of the hair fiber. The outer surface of the cuticle cells (the epicuticle) for instance comprises a lipid–protein complex that has hydrophobic properties. Similarly, the condensation reactions that occur during keratinization produce hard, robust proteinaceous structures. However, several key weaknesses do exist. With shed and/or cut fibers the proximal and/or distal ends of the fibers may allow microbial/chemical ingress via the less-resistant central medulla, which is composed of trichohyalin. Furthermore, if the cuticle itself is damaged and the protective layers of the epicuticle/exocuticle are breached, then differential degradation may be observed (Wilson et al., 2007a). It is also important to recognize therefore that progressive changes toward the distal tip are a recognized feature of weathering arising from repeated grooming and the use of heat and chemical treatments. Within individual cuticle cells, the less
resistant endocuticle may be subject to both chemical and microbial attack in preference to the overlying exocuticle, with the A-layer most resistant to alteration. Given that cuticle condition largely defines the optical properties of the fiber, it is perhaps unsurprising that many archaeological samples appear dull and sometimes faded.

The cortex also exhibits a similar differential degradation with separation of cortical cells beginning along the intercellular δ-layer of the cell membrane complex. The breakdown of the intracellular matrix and nuclear remnants releases individual macrofibrils. Different chemical conditions define the differential decomposition of the macrofibril keratin intermediate filaments and matrix proteins. Significantly, the pigment granules appear to be resistant to much of the enzymatic damage inflicted on the majority of the fiber components during biodegradation. With all hair (including even the blackest Asiatic hair) there is a mixture of eumelanin (responsible for brown-black coloration) and phaeomelanin (responsible for red-yellow coloration). Of these two biopolymers, eumelanin is the less stable. As a consequence, chemical alteration of melanin (under both extreme oxidizing and extreme reducing conditions) will commonly result in breakdown of the eumelanin to enhance the red-yellow coloration of the phaeomelanin (Wilson et al., 2001).

Assessing morphological condition using histological techniques is frequently regarded as a fundamental approach with bone and teeth. Much of the research investigating the susceptibility of hair to biodegradation has focused on the ability to recover ancient DNA from archaeological specimens (e.g., Gilbert et al., 2004, 2006). Given the heterogeneity of the hair ultrastructure, histology is therefore a valuable tool to examine the extent to which fibers have undergone degradation. Attention has been paid to methods that can offer an assessment of the outward appearance/condition as well as assessing change to the internal structures. As such, an attempt to devise histological scoring methods has been proposed for use with degraded hair (Wilson et al., 2004, 2010) and has been applied to assess the levels of amplifiable mtDNA from hair (Gilbert et al., 2006). MtDNA was successfully amplified from bison hair dating to ~64,000 BP by Gilbert et al. (2004). Data on the carbon and nitrogen composition of the same hair showed that the atomic C:N ratio was consistent with modern samples. This tentatively hinted that, under the correct circumstances, the limits of hair survival could be similar to other archaeological proteins such as bone collagen.

Contrasting environments are known to affect protein survival at a chemical level. For instance, both DNA and bone collagen are known to have poor survival in hot, arid conditions. With bone, collagen yield becomes an important criterion for condition assessment given the intimate association of the protein with the mineral structures. Hair, however, as a largely protein structure can usually be considered for isotopic analysis where it survives, with bulk amino acids surviving largely unaltered. A study by Macko et al. (1999a) analyzed the amino acid profiles of both well-preserved archaeological hair from mummified remains excavated at various locations and modern hair that was artificially aged by exposure to radiation and compared them with modern human hair. This analysis determined that in samples that dated to between approximately 5200 and 800 BP only very small changes in
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amino acid composition occurred. The largest changes occurred in the oldest samples, with greater loss of some of the more unstable amino acids such as serine and threonine, but overall profiles of amino acids were very similar to modern hair. Convention has to date favored the use of the carbon to nitrogen atomic ratio for assessment of the chemical integrity of hair fibers as this relates directly to the sample as combusted. However, its adoption for hair is largely a reflection of the existing usage of this approach with other tissues and currently the accepted range (2.9–3.8) has not been subject to rigorous scrutiny. Percent yields of carbon and nitrogen from non-altered hair are in the order of 44–46% carbon and 13–14% nitrogen by weight (Roy et al., 2005).

Vibrational spectroscopy has been used with various archaeological and forensic hair samples to illustrate some of the structural changes that can occur, particularly in relation to the initial breakdown of disulfide bonds and amide groups. These methods can therefore be used to discern degrees of change, but interpretation needs to be mindful that without careful sample preparation the data largely relate to the outer structures. However, it remains a useful screening approach to consider in the context of wider taphonomic issues concerning the depositional environment (e.g., contaminants on the fiber surface and the nature of putative inorganic/organic residues) (Wilson et al., 1999).

Given the likely association of archaeological and forensic hair samples with soil and other external contaminants, sample preparation is key. Adherent soil and exogenous organic deposits can usually be removed from the fiber surface by overnight soaking/gentle agitation in organic solvent (e.g., 2:1 methanol:chloroform), followed by sonication (3 × 15 min). The organic solvent is then removed and the hair sample itself is then rinsed in deionized water (×3 separate washes, each with sonication). The final wash is decanted off and the cleaned sample is then frozen and lyophilized. It is important that samples are then preconditioned for weighing, particularly in advance of oxygen and hydrogen isotope analysis (Bowen et al., 2005a), especially given the nontrivial nature of measuring the nonexchangeable fraction of hydrogen isotope ratios (Chesson et al., 2009). Given that in some circumstances it may be valuable to know the nature of any organic residues removed from the hair (whether applied during life, derived from the depositional environment, or applied during curation), the solvent extract can be concentrated by blow-down under nitrogen gas and submitted for detailed separation and analysis by gas chromatography mass–spectrometry (GC–MS).

14.23.3 Studies of Isotope Ratios in Animal Hair

Before considering the work that has been done using ancient and modern human hair, the large amount of work done on modern animal hair should be mentioned. Unlike in human subjects, the ability to manipulate the diets and environments of experimental animals with less stringent ethical and financial considerations has led to the establishment of fractionation factors, growth rates, and models of hair growth that have subsequently been applied to or stimulated work in humans. Collecting hair from both wild and domestic animals is clearly attractive from the standpoint of a quick, noninvasive sampling technique that can be incorporated in sampling schemes directed at collecting other tissues. Hair from a wide variety species of modern wild and domestic animals together with other excretory by-products have been analyzed for isotope ratios to investigate topics including ecology, migration, dietary habits, and hair growth rates. First discussion centers on some studies involving hair from experimental animals in controlled settings, which have largely focused on diet-switching experiments to investigate turnover times. Then we will review some work that considers animals as samplers of their environment.

14.23.3.1 Animal Hair from Controlled, Experimental Situations

Many studies using isotope ratios from animal hair have been part of diet-switching experiments in large, domestic herbivores as part of controlled feeding studies. Depending upon the species being studied, tail hair has been of particular importance in these longitudinal studies due to its ability to record isotopic changes over significant periods of time (years). Partly as a product of the ease of controlling the carbon source in such diets by switching grass sources, the majority of data collected have been carbon isotope ratios, but some nitrogen isotope data have also been published, along with sulfur, hydrogen, and oxygen data.

The first published stable isotope data from animal hair were carbon isotope ratios measured in cattle from different environments (Minson et al., 1975). A second study where cattle hay diet was switched from C₄ to C₃ and then back to C₄ (Jones et al., 1981) defined that a 74-day period was required for a new carbon isotope signal to be expressed in steer hair as the body carbon pool equilibrated with diet. More recently, numerous studies have been conducted such as that by West et al. (2004) who used horse tail hairs to demonstrate the high resolution of the dietary record present in hair. Horses on a baseline C₃ diet that was spiked with C₄ grass for periods of 1, 3, and 7 days and analysis of the data demonstrated the sensitivity with which changes in carbon isotope ratios can be detected as even the 1-day spike was clearly seen recorded in tail hair (see Figure 3). Ayliffe et al. (2004) analyzed tail hair from horses whose diet was switched between C₃ and C₄ grasses. Longitudinal analysis of the high-resolution isotopic data from the hair, combined with the use of an exponential data model, revealed that multiple pools of carbon exist within the body, each with different turnover times. These data were later reexamined by Cerling et al. (2007), who used a reaction progress variable previously used in experiments using tritium as a marker for metabolic activity. This approach identified three pools of carbon; short (0.5 day; 41%), medium (4.3 days; 15%), and long (140 days; 44%) pools, the meaning of which are debated. However, the application of such approaches has begun to give both a more sophisticated understanding of the period when tissues approach equilibrium with a new isotopic value and candidate sources for the multiple carbon pools that contribute to hair formation. The advantages include the ability to compare isotopic data across experiments even where the initial and final values differ. Longitudinal data from cattle tail hair were collected in a similar diet-switching experiment to Ayliffe et al.’s (Zazzo et al., 2007). The same
approach to modeling data suggested that three carbon pools were also present in cattle with slightly different half-lives and contributions, likely due to physiological differences between cattle and horses. Sheep wool has also been used as a temporally resolved record of diet to assess differences in the rate at which new carbon is incorporated into hair due to age and growth rate (Zazzo et al. 2008). It was observed that younger, actively growing lambs equilibrated with the carbon isotope ratio of their new diet faster compared to older, mature animals. Analyses have also been conducted on cattle where multiple tissues (hair, blood plasma, liver, kidney muscle, fat, ruminal contents) have been examined for intertissue carbon isotope comparison (e.g., De Smet et al., 2004) and swine (Nardoto et al., 2006), with the aim of reconstructing the diet of animals producing human food products.

Relatively few experimental studies have focused exclusively on nitrogen isotope ratios in animal hair. Sponheimer et al. (2003) studied hair from multiple species of herbivores (llama, alpaca, goat, cattle, horse, and rabbit) to assess differences in the diet–hair fractionation of nitrogen isotopes due to physiology, as well as for any effects caused by high versus low protein diets. Differences in diet–hair fractionation were indeed observed, not only between fore- and hind-gut fermenters and between individuals of the same species on high versus low protein diets, but also between individuals of the same species consuming the same diet (up to 3.6%), illustrating the complexities of interpreting nitrogen isotope data from animal tissues. There are even fewer examples of sulfur isotopes in animal hair. Richards et al. (2003) performed longitudinal analysis of horse tail hair from two horses in a controlled feeding experiment, where diet was switched from a C3 to a C4 source that also had a different sulfur isotope ratio. The data showed that the sulfur isotope shift was expressed in tail hair and that there was very little fractionation between diet and hair (c. 1‰).

Whilst most work done on hair from wild animals has been done on hair collected from study populations in the field, some diet-switching experiments have been performed on captive wild animals. One example is Voigt et al. (2003) who assessed differences in metabolic rates between bat species with different feeding habits using carbon isotope turnover in their hair after a switch in dietary carbon source. Darr and Hewitt (2008) conducted a diet-switching experiment using captive white-tailed deer where hair was compared to other tissues including antler. Podlesak et al. (2008) performed an experiment using two woodrat species where the isotope ratios of their drinking water source were switched, whereas diet remained constant. This study represents a rare published example of hydrogen and oxygen isotope ratios measured from animal hair to establish turnover times.

### 14.23.3.2 Animal Hair as an Environmental Sampling Tool

The second application of isotopic analyses to animal hair involves collecting hair samples, amongst other tissues, as part of either local or regional surveys. In this case animals are effectively sampling the environment without experimental manipulation of their diet. Hair collected from both wild and domestic species has been analyzed for environmental monitoring, dietary reconstruction through diachronic analysis, or for conferring the origin of an animal. The interpretations of isotopic data from animal hair have often been based on previously discussed work in domestic animals that has elucidated how tissues turnover and record isotopic signatures. In the case of domestic animals there has recently been intense interest in developing an independent means of assigning an origin to an animal and hence any food products derived from it for food traceability purposes (Kelly et al., 2005).

Sheep wool was investigated in an early, limited study as a tool for effectively monitoring the extremely large tracts of rangeland that are used for grazing in Australia and South Africa (Witt et al., 1998). The authors sampled a single,
longhairy sheep from Australia on the premise that carbon isotope ratios along the length of the wool would reflect the proportion of C₃ versus C₄ grass species available to the sheep, and thus sampled, per season. They also analyzed historically collected wool from South African rangeland sheep dating back to 1916 that illustrated a shift through time in the proportions of C₃ and C₄ grasses over a period of almost eight decades. A decrease in C₄ species in the 1920s, attributed to increased stocking of the land and hence increased grazing pressure, was observed as generally lower carbon isotope ratios during this period. This pressure was subsequently relieved, C₄ grasses recovered and higher carbon isotope ratios were observed in wool samples that were collected later on. Other, more recent studies using cattle have investigated sampling methodologies for collecting hair for high resolution, time sequence analysis. For example, Schwertl et al. (2003) exploited a seasonal diet change that existed as part of winter housing and feeding versus summer grazing. Carbon isotope ratios were further investigated as an environmental recorder in cattle tail switch hair that was collected as part of a study investigating climatic conditions in a temperate, agricultural, C₃ pasture setting (Schnyder et al., 2006). Cattle hair not only recorded seasonal foddering shifts, but also reflected small-scale geographic differences between paddocks that were linked to the two underlying soil types.

Recently, the assignation of the geographic origin of an animal through carbon and nitrogen isotope analysis was investigated by sampling cattle hair across four locations in China (Guo et al., 2010). As animal husbandry and foddering practices differ regionally, it was possible to assign animals with a reasonably high degree of confidence to a known region. Alternatively, within a discrete geographic region where multiple farming systems are employed such as Upper Bavaria, cattle tail hair combined with isotopic analysis of cattle fodder, enabled the isotopic distinction of confined versus pasture foddering systems, dairy, suckling, and preslaughter fattening systems (Schwertl et al., 2005).

Field-collected hairs from many wild mammal species, including terrestrial and marine mammals, have been analyzed for isotope ratios. These studies have been largely aimed at elucidating dietary preferences and movements across the landscape. (Extensive and important wildlife work has also been done on isotope ratios in bird feather keratin to investigate migration (e.g., Hobson, 2005; Hobson et al., 2004, 2009, but for the purposes of this review we have focused solely on hair keratin). Large African mammals have been the focus of multiple longitudinal studies using isotope ratios of their hair to quantitatively describe dietary preferences, movement through the landscape, and competition of these animals with human activities. Bulk hair studies have also been conducted to determine predator–prey relationships in the savannah ecosystem using hair (Codron et al., 2007). African elephants (Loxodonta africana) represent one of the best-studied animals in terms of isotope ratios in hair (Erlich et al., 2004, 2006, 2009; Wittemyer et al., 2009). These studies have supported conservation efforts by quantifying the use of various resources by elephant populations, including their impact on human activities such as agriculture, and conversely human impacts on elephant populations. High resolution carbon, nitrogen, and hydrogen isotope ratio records in elephant tail hairs combined with GPS collar data of actual movements and normalized vegetation index (NDVI) captured fast seasonal shifts in elephant diet between C₃ browse and C₄ grasses. Keratin revealed that C₄ grass consumption became a large part of diet shortly after C₄ species’ productivity increased following rainfall events. This was subsequently followed by a peak in elephant fertility (Figure 4), showing the power of isotope ratio data when combined with other data. These data also showed faithful tracking of hydrogen isotope ratios from water sources in elephant keratin, as the new water sources of the rainy season were reflected in keratin analyses (Figures 4 and 5). This work further showed that competition for grazing between livestock and elephants was likely at certain locations (Cerling et al., 2009). Analysis of carbon isotope ratios along a single hair from a hippopotamus (Hippopotamus amphibious), in conjunction with analysis of tooth enamel, revealed that diet differed from what was assumed based upon observations of feeding and analysis of the contents of the digestive tract, showing the utility of a long-term integrated isotopic record (Cerling et al., 2008). Diet was not, as was typically assumed, almost exclusively C₄ graze but moved between complete C₃ browse to C₄ graze over a period assumed to be months. Large animal hair has also been collected from other regions for similar survey purposes, for example multiple mammal species from Argentina (Panarello and Fernandez, 2002).

Relatively few field studies have been conducted on hair from omnivorous and carnivorous animals. Terrestrially, a considerable amount of work has been done using carbon and nitrogen isotope ratios of hair collected from bears (Hilderbrand et al., 1996; Jones et al., 2006; Mizukami et al., 2005a,b; Mowat and Heard, 2006), arctic fox (Roth, 2002), and wolves. One of the wolf studies investigated the dietary habits of wolves resident in British Columbia to establish adaptive, seasonal consumption of salmon by segmental analysis of hair (Darimont and Reimchen, 2002). In another case, the trophic relationship of wolves to other species was examined by analyzing hair collected from multiple species, including prey species, and modeling wolf diet using these data inputs (Urton and Hobson, 2005). Most recently, Fox-Dobbs et al. (2007) investigated isotopic fractionations between wolf tissues, including hair and bone collagen, with the aim of applying these to ancient wolf populations to investigate foraging ecology. Marine mammals are another example of predators where carbon and nitrogen isotope analysis has been applied to vibrissae, which record medium-term isotopic records. Sea lion (Eumetopias jubatus) vibrissae roots were analyzed to establish diet–tissue fractionation in pups and juveniles (Stegall et al., 2008). Southern sea otter (Enhydra lutris nereis) vibrissae were also analyzed to investigate dietary heterogeneity within a population and seasonal dietary shifts between individuals (Newcombe et al., 2009).

While the bulk of the work on animal hair has used carbon and nitrogen isotope ratios, there is increasing interest in the application of hydrogen isotope ratios to hair from migratory mammalian species to determine their regions of origin. Much of this work is based upon studies of feather keratin in migratory birds, but examples include work done on bat hair. Hydrogen isotope ratios measured in hair from migrant New World bats provided evidence of a single, seasonal molt and...
Figure 4  Isotopic chronologies derived from elephant tail hairs demonstrate that isotopic shifts related to water resources and diet (C₃ browse versus C₄ grass) were synchronized with seasonal rainfall pulses and their impact on vegetation (here measured as NDVI). The average timing of the initiation (beginning of shaded area), maximum increase (%), peak values (black circle), maximum decrease (*), and end of each peak (end of shaded area) for: mean (5-day average) daily rainfall (a); median NDVI (b); median δD (c); median δ¹³C (d); and conceptions (e). All are referenced to the maximum NDVI value, and dashed lines show the 25th and 75th percentiles. Reproduced from Cerling TE, Wittemyer G, Ehleringer JR, Remein CH, Douglas-Hamilton I (2009) History of Animals using Isotope Records (HAIR): A 6-year dietary history of one family of African elephants. Proceedings of the National Academy of Sciences of the United States of America 106: 8093–8100, with permission.

Figure 5  Relationships between the isotope composition of local water and hair. δD values for elephant hair and for water collected from the Ewaso N’giro at Samburu Reserve for 18 months from October 2001 through March 2003. The value for the elephant hair is a ≈15-day average (3-point mean) centered on the day of the water collection (#3 days). Reproduced from Cerling TE, Wittemyer G, Ehleringer JR, Remein CH, Douglas-Hamilton I (2009) History of Animals using Isotope Records (HAIR): A 6-year dietary history of one family of African elephants. Proceedings of the National Academy of Sciences of the United States of America 106: 8093–8100, with permission.
confirmation of the long distances that they travel annually, evidence that had previously been impossible to collect through conventional tracking methodologies (Cryan, 2004). More recent work on bats in the eastern United States demonstrated significant differences in the relationships between environmental water and bat hair keratin between species and within groups, suggesting caution will be required to interpret regions of origin as this approach becomes more widespread (Britzke et al., 2009).

14.23.4 Anthropological Studies on Modern and Historically Collected Hair

Physical anthropologists have studied isotope ratios in hair collected from both New and Old World primates to examine dietary ecology, habitat utilization, and health status. Species where hair has been collected in the field have included arboreal galagos, prosimians resident in Madagascar and Kenya (Schoeninger et al., 1998), ring-tailed lemurs (Lemur catta) in Madagascar (Loudon et al., 2007), and chimpanzees in Kenya and the Democratic Republic of Congo (Schoeninger et al., 1999). Other primate studies have used museum collections as sources of hair for carbon and nitrogen isotope ratio studies examining variations in diet and habitat preferences in primate species, including New World monkeys from central and South America (Schoeninger et al., 1997) and Asian macaques (Macaca mulatta) (O’Regan et al., 2008). Studies on modern primate hair such as these provide valuable data for the interpretation of isotope ratio data from early hominin tooth enamel specimens. Despite the fact that isotope ratios from hair and tooth enamel cannot be directly compared due to isotopic fractionation, once this is accounted for an understanding of the modern isotope ecology of savannah versus forest systems may add valuable context to the debate over the ecological conditions that drove early hominin evolution.

A recently published example of carbon and oxygen isotope ratios from tooth enamel of Ardipithecus ramidus led to a vigorous debate. The authors argued that the data suggested a closed woodland environment (White et al., 2009b), which was subsequently refuted by another group, who argued that the same data, along with modern soil data from the same locations, supported previous accounts of a more open, tree, or bush-savannah type of habitat (Cerling et al., 2010). Isotope ratio data from the hair of modern primates resident in similar ecological settings may further inform this debate.

Multiple anthropological studies have been published on the isotopic composition of hair collected from human populations, both modern and historic. This work has largely used hair where degradation has not been an issue and has been focused on three main areas. First, establishing isotopic relationships between hair and diet in the case where both have been available for sampling and analysis. Second, and most recently, investigating relationships between the isotopic composition of drinking water or environmental water, diet, and hair. Finally, hair has also been used to establish diet in historically collected hair as an independent means of corroborating written records.

Many anthropological applications using hair have concentrated on carbon and nitrogen isotopes as dietary indicators.

Early observations that the carbon isotope composition of human diet is reflected in hair (Webb et al., 1980) were quickly widely applied to modern human populations. Nakamura et al. (1982) analyzed human hair and food items collected from the United States (Chicago), Germany (Munich), and Japan (Tokyo) and observed differences in carbon isotope ratios due to broad geographic differences in diet. Populations in Japan and the United States tended to be enriched in $^{13}$C compared to Germany due the consumption of larger amounts of marine and C$_4$-derived carbon, respectively. Nakamura et al. (1982) also collected beard hair from traveling individuals and observed that carbon isotope ratios shifted to reflect the new carbon isotope composition of the regional diet. A study that concentrated on a small population from the United States was published by Schoeller et al. (1986), who measured carbon, nitrogen, and, for the first time, hydrogen isotope ratios in human hair and an extensive collection of supermarket purchased foods to investigate the North American food web and the relationship between diet, hair, and blood plasma isotope ratios. Differences in hair carbon isotope composition between human populations resident in the United States and in western Europe have subsequently been reported in a number of publications. McCullagh et al. (2005) analyzed British and North American hair and again observed that the hair from the United States was isotopically heavier than that from the resident British population and that individuals who moved to the United States from the United Kingdom and vice versa reached equilibrium with their new diets after approximately 4 months (Figure 6). The largest sample of hair analyzed to

![Figure 6](image-url)
date from the United States and western Europe was recently published and again showed that while there was little difference between the populations’ nitrogen isotope composition, they were distinctly separated in carbon and sulfur isotope compositions (Valenzuela et al., 2012; Figure 7). Geographically related gradients in sulfur isotope ratios have also been observed within the United States (Valenzuela et al., 2011) and as more data become available similar relationships will doubtless be observed in other regions.

Samples collected from indigenous people, such as the Gidra-speaking people of Papua New Guinea, have been used in isotopic studies (Yoshinaga et al., 1996). Hair was collected and analyzed for carbon and nitrogen isotope ratios, whilst the isotopic composition of diet was estimated to establish the range of diet–hair fractionations. In this case the authors sought a population whose diet was effectively confined to local foods, unlike the previously analyzed populations that were assumed to consume ‘supermarket’ diets. In contrast, a study on hair collected from industrialized populations across the United States examined hydrogen and oxygen isotope ratios in hair keratin and compared these data with drinking water isotope ratios to establish their relationship (Ehleringer et al., 2008). The strong link was explained by a semi-mechanistic model linking the sources of hydrogen and oxygen available to consumers (through diet, drinking water, and air in the case of oxygen) to their mixing into body pools and subsequent incorporation into new keratin synthesis. Using the well-characterized variation in hydrogen and oxygen isotope ratios in environmental water, hydrogen, and oxygen isotope ratios could therefore be linked to geography and thus predictions could be made about what might be expected at a given location. Incidentally, recent research into the hydrogen and oxygen isotopic variability of the human food supply has helped to inform this work and will be increasingly important in the future (e.g., Chesson et al., 2008, 2010). Further survey work using historic hair collected from indigenous people around the globe during the twentieth century developed this model using samples from people assumed to consume a largely locally derived diet (Bowen et al., 2009). This work used museum-archived hair and expanded the range of isotopic analyses from homogenized hair to include hydrogen and oxygen isotope ratios as well as the more commonly measured carbon and nitrogen. A ‘local component’ was included in the original model that describes the relationship between hydrogen and oxygen isotope ratios in hair and drinking water. This model was subsequently tested using modern human hair samples collected in a broad survey of four Asian countries (Thompson et al., 2010) and was found to largely describe the variation that was observed.

Between the large-scale surveys that have been carried out on the modern, ‘supermarket diet’ consuming population of the United States (Ehleringer et al., 2008), the modern largely local food consumers of Asia (Thompson et al., 2010), and indigenous groups from around the globe (Bowen et al., 2009), an interesting phenomenon was observed. Each of these groups had a different isotopic relationship to their drinking water sources, with the highest slope seen in the indigenous groups, an intermediate slope in the modern Asian sample, and the lowest slope in the modern US population (Figure 8). These differences could be accounted for by the prevailing diet consumed by each group; the most local diet in the indigenous groups contributing to the higher slope, a mixed ‘local–supermarket’ diet in modern Asians, and the most nonlocal ‘supermarket’ diet in modern Americans. The larger quantity of

**Figure 7** Figure showing cumulative frequencies of (a) carbon, (b) nitrogen, and (c) sulfur isotope ratios in human hair from two surveys collected across the US and western Europe. Reproduced from Valenzuela LO, Chesson LA, Bowen GJ, Cerling TE, Ehleringer JR (2012). Dietary heterogeneity among western industrialized countries reflected in the stable isotopes of human hair. *PLoS ONE* 7: e34234.
animal protein typically consumed by modern Americans compared to many Asian populations was also reflected in the smaller fraction of hydrogen in hair that was fixed in vivo in modern Americans versus Asians. This was also supported by generally higher nitrogen isotope ratios in the American population as opposed to the Asian groups. These observations highlighted the critical importance of having some broad knowledge of typical diets when investigating the origins of hair, and vice versa, as well as demonstrating the use of hair as an isotopic recorder of the globalization of diet in the developed world, versus other cultures.

Lastly, human hair has been isotopically analyzed to examine indigenous people who are no longer alive but about whom written records survive. An excellent example is the study on historically collected hair from two groups of North American Plains Indians in the late 1800s to early 1900s by Roy et al. (2005). The museum-archived hair was sampled and analyzed longitudinally for carbon and nitrogen isotope ratios and revealed distinct differences in dietary profile between the Lower Brule and the Blackfoot. These data were interpreted in the context of historic and ethnographic accounts of the groups’ diets after resettlement into reservations and provided insight into inter- and intragroup dietary differences as well as seasonal dietary variations.

**14.23.5 Health and Medical Applications of Hair Analysis**

Studies have been conducted to investigate the possible clinical uses of isotope ratios of human hair as markers for a number of diseases and conditions related to nutrition and metabolic status. Clinical studies often rely upon measurements of disease markers in conjunction with detailed self-reporting of food intakes by participants. However, this reporting process can produce inaccurate, unreliable dietary records. Stable isotope ratios potentially provide objective markers for disease that remove both the burdens of the reporting process for participants and its associated problems for the clinician and the technique has recently been reported in the clinical literature (e.g., Petzke et al., 2010). Further advantages are again presented in the medium-term, time-resolved record of hair, which can potentially record three significant periods in study candidates: the time prior to the start of a disease, the disease period itself, and the recovery.

An individual’s body pool of amino acids, which are ultimately derived from diet, is the origin of all carbon and nitrogen in their hair keratin. This is the underlying basis for using isotope ratios in hair as biomarkers for diet, diseases, and conditions with associated metabolic changes. During anabolism in normal individuals dietary proteins are incorporated into growing hairs. Although the exact amount varies, nitrogen isotope fractionation ultimately results from these metabolic processes as \(^{14}\text{N}\) is preferentially excreted as urea, producing \(^{15}\text{N}\) enrichment of all consumer body tissues, including hair. However, individuals in starvation will enter negative nitrogen balance, where the breakdown of body tissues provides the source of energy and protein not being provided through dietary intake through gluconeogenesis. This leads to a recycling of nitrogen within the individual, who has effectively increased a tropic level, which will ultimately increase the \(^{15}\text{N}\) enrichment of tissues biosynthesized from this recycled nitrogen, including hair. Carbon isotope ratios may be affected to a smaller degree, but be inversely related to changes in nitrogen isotope ratios for reasons that are less clear but relate to changes in dietary composition.

**14.23.5.1 Human Dietary Preferences and Stable Isotope Ratios**

Observations on the relationships between human diet and isotope ratios in hair had long been made based upon the
results of animal studies. However, until relatively recently only a small amount of isotopic data has been published from hair collected from individuals whose diet was well known. Due to the expense and ethical constraints of conducting human studies where subjects only consume a controlled diet, individuals who are already part of other ongoing medical studies or other volunteers have been recruited for such efforts. One of the first studies using modern human hair to investigate diet analyzed a small number of individuals resident in the UK whose diet was recorded (O’Connell and Hedges, 1999b). This study used homogenized hair samples and determined that isotopic differences could be detected between ovo–lacto vegetarian, vegan, and omnivorous individuals. Nitrogen isotope ratios were positively related to the amount of animal protein consumed. This was followed by a study that used hair collected from samples of UK individuals, domestic animals, and a few immigrant individuals whose diet was recorded (Bol and Pfleiger, 2002; Bol et al., 2007). This work showed that it was possible to discriminate between the different groups based upon carbon, nitrogen, and sulfur isotope ratios to determine geographic origin as well as dietary preferences. Data collected from a much larger sample size of hair from German individuals who were part of a wider dietary study also showed this trend, confirming the positive relationship between animal protein consumption and bulk and specific amino acid nitrogen isotope ratios (Petzke et al., 2005a,b). This work also indicated a positive relationship between carbon isotope ratios and the amount of animal protein consumption, reflecting the lower 13C content of plant derived versus animal derived foods.

Most recently, nitrogen isotope ratios in hair were used as an indicator of fatty acid consumption in members of a Yup’ik Eskimo population (Nash et al., 2009). Elevated levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in blood are markers of the consumption of the traditional Eskimo diet which is very high in marine foods. This approach exploited the elevated nitrogen isotope signature of this diet, which can be measured in consumers’ blood but less invasively in hair. Hair was proposed as a reliable indicator of fatty acid consumption in this population after a strongly correlated positive relationship was observed between nitrogen isotope ratios and the amount of EPA and DHA in red blood cells.

14.23.5.2 Use of Isotope Ratios to Investigate Nitrogen Balance during Pregnancy

One of the first investigations using serial analysis of isotope ratios in hair for a medical study examined nitrogen balance in pregnancy (Fuller et al., 2004). Hair was collected that was long enough to have grown during conception, through pregnancy, and after birth. The authors discovered that even though no major changes in diet were reported, nitrogen isotope ratios decreased (by 0.3–1.1‰) during pregnancy as weight increased. During pregnancy the body is in an anabolic state; that is, positive nitrogen balance, and this decrease in nitrogen isotope ratios was attributed to progressive nitrogen retention throughout pregnancy as new tissues are formed. No changes in carbon isotope ratios through time were reported. This study was followed by an investigation of hair from subjects who suffered nutritional stress due to severe morning sickness during pregnancy (Fuller et al., 2005). In this case the opposite trend was seen, as during periods of weight loss nitrogen isotope ratios were observed to increase as the subjects entered negative nitrogen balance. However, when weight was gained nitrogen isotope ratios tended to decrease.

14.23.5.3 Investigation of Stable Isotope Ratios as a Means of Diagnosing Eating Disorders

Pathological eating disorders have been the subject of recent studies where hair was collected from consenting participants undergoing treatment in clinical settings and analyzed for carbon and nitrogen isotopes along its length (e.g., Hatch et al., 2006; Mekota et al., 2006, 2009). The disorders investigated to date involve severe calorie restriction by the sufferer resulting in a number of physical maladies (anorexia nervosa) and excessive eating followed by purging (bulimia nervosa). The authors also noted that those suffering from eating disorders were also likely to have modified their diets to minimize fat and carbohydrate intakes, often becoming vegetarian. This would have a separate, reverse impact on isotope ratios, causing a decrease in nitrogen isotope ratios in the period leading up to the full, catabolic manifestation of the disease when an increase would be expected. These studies therefore have further implications in forensic medicine where starvation as part of neglect is presented during casework and independent evidence is required (Mekota et al., 2009).

The first study examined hair collected from hospitalized anorexia nervosa patients that represented periods of starvation and recovery from the disease (Mekota et al., 2006). It was observed that isotope ratios in hair would shift, increasing for carbon and decreasing for nitrogen as body mass index (BMI) increased and metabolism returned to normal as the individual recovered from their state of starvation (Figure 9). This was followed up by another study to validate the earlier work and include a control (Mekota et al., 2009). Another blind study using carbon and nitrogen isotope ratios in hair investigated sufferers of both anorexia and bulimia nervosa. The aim was to determine if they could be statistically distinguished from a control group of clinically normal individuals and from each other. The algorithm developed by the authors had an 80% success rate in identifying anorexic individuals and those suffering from anorexia and bulimia (Hatch et al., 2006). As previously observed by Fuller et al. (2005), these studies demonstrated 15N enrichment in hair from patients who had undergone prolonged nutritional stress, which decreased as normal dietary regimes were followed and body mass increased (Hatch et al., 2006; Mekota et al., 2006, 2009). A potentially confounding factor was noted by Mekota et al. (2006) as a patient who changed to an omnivorous diet during recovery actually had an increase in hair nitrogen isotope ratios during recovery due to their increase in animal protein consumption rather than a change in metabolic status. However, unlike the results from nutritionally stressed pregnant individuals, all of the individuals suffering from eating disorders showed an increase in carbon isotope ratios as their weight increased. This was ascribed to a change in diet rather than metabolism alone as many individuals increased their protein and lipid consumption once they entered treatment. The diet to tissue fractionation differs for proteins compared to
carbohydrates and lipids and this was postulated as being a cause for the change in carbon isotope ratios (Mekota et al., 2006).

14.23.5.4 Trace Element Analysis of Human Hair as a Tool for Health Investigation

Trace element analysis of hair has been explored for medical applications such as establishing nutritional deficiencies, disease states, or exposure to pollutants. Indeed, both the US Environmental Protection Agency and International Atomic Energy Agency collect and analyze human hair as a useful biological material for global environmental monitoring. Trace metals are incorporated into hair during growth, largely bound to cysteine and thus, if exogenous sources can be successfully removed from a sample without affecting endogenous metals, hair can provide a record of exposure that could also be resolved temporally (Morton et al., 2002). High concentrations of certain trace metals (e.g., Cd, As, and Pb) and deficiencies in others (e.g., Se, Zn) are known to contribute to morbidity and mortality in affected populations and the ability to link this with environment and diet is an advantage when trying to tackle these causes. However, measuring trace elements in human hair can be problematic, largely due to uncertainties as to the endogenous versus exogenous origins of trace elements in hair, which were noted early in the medical literature (Manson and Zlotkin, 1985) and have remained a concern (e.g., Morton et al., 2002). An early study that made high-resolution measurements of trace element profiles across the diameter of human hairs showed that many elements, such as lead, arsenic, and copper, were concentrated at the hair surface and were likely present as a result of exposures to exogenous sources rather than through biosynthetic incorporation into the hair body (Cookson and Pilling, 1975). Other work has shown that it is extremely difficult to remove certain elements, such as mercury, from the exterior of hair despite using multiple washing techniques, so occupational exposure on the exterior of hair may easily confound any biologically integrated signal (Li et al., 2008).

Multiple analytical techniques may be used to measure trace elements in hair including high-resolution inductively coupled plasma mass spectrometry (HR–ICPMS), standard ICPMS, instrumental neutron activation analysis (INAA), and X-ray techniques such as particle-induced X-ray emission (PIXE). Trace element studies typically treat hair as a homogenous sample, but some studies have attempted to make longitudinal measurements along hair (e.g., Gellein et al., 2008; Sera et al., 2002). In the case of environmental toxicity studies, hair is typically collected from populations that consume a contaminated diet or live near point pollution sources that can then be compared to a negative control population without the same exposure.

Although the relationships between certain types of pollution and the quantities of given trace elements in hair have been established empirically, global baseline trace elements levels and inter- and intrapopulation variations due to diet and habits such as smoking have not been widely established. However, there are some exceptions to this as human ecology studies have been conducted that have attempted to link variations in suites of trace elements in hair from nonurban human populations to geography and diet (e.g., Batzevich, 1995; Vance et al., 1988). An example of a generally accepted approach is the measurement of methyl mercury (MeHg) in hair, which is strongly linked to the consumption of large amounts of fish and is known to be well correlated with MeHg in other tissues and blood. Methyl mercury is particularly concentrated through aquatic food chains (although terrestrial bioaccumulation also occurs) and this technique has been applied to both modern (Barbosa et al., 2001) and ancient, preindustrial populations not exposed to modern industrial pollution (Egeland et al., 2009; Gerlach et al., 2006). A clear advantage of analyzing the latter class of samples is the ability to determine preindustrial environmental baselines for comparison with modern ecosystems. Pollution detected in human hair may originate from either anthropogenic sources such as industrial facilities or urban emissions (e.g., Salameh et al., 2008; Sera et al., 2002) or natural sources, such as volcanoes (Amaral et al., 2008). Thus trace element analysis of human hair in such cases clearly has a forensic aspect, especially if liability for industrial pollution is established.

14.23.6 Archaeological Hair

Archaeological remains often require sophisticated research questions in order to justify both destructive sampling and

![Figure 9](https://example.com/figure9.png)
the use of expensive interrogation methods such as geochemical analysis. There have therefore been relatively few published studies of isotope ratios in archaeological human hair compared to studies on extant human populations. Each individual hair fiber embodies a detailed record and provides evidence of changes that are time-resolved to the order of weeks or months, presenting insight into short-term dietary changes that would be otherwise invisible to archaeology. This information is particularly valuable when used in combination with other lines of evidence. These may include isotopic evidence from bone collagen, a long-term, integrated signal, archaeobotanical, and archaeozoological evidence of human diet, paleopathological evidence from skeletal and dental remains, and/or material culture associated with an excavation site. Where chronological information is required, segmental analysis using defined fiber lengths can build a detailed picture of change. Current research questions have focused on major themes including variation in diet, status change, seasonality, weaning, and geographic variation. Given that hair will only survive under certain environmental conditions, it is hardly surprising that much of the published research utilizing archaeological hair relates to assemblages from either hot dry conditions as in the west coast of South America and frozen contexts as with high altitude mummies and human and animal remains recovered from other regions of permafrost. These hair analyses were largely conducted using human hair but data from some animal hair are included. These locations are, as expected, in desert or tundra settings where the preservation of biological remains is favored.

An early survey of ancient hair samples collected from geographically dispersed archaeological sites included isotopic data from mummified human remains from the Nile Valley, the Atacama Desert, and the Oetztaler Alps (Macko et al., 1999a,b). Hairs were analyzed to provide single isotope values per individual, as the study’s focus was on exploring hair as an alternative recorder of isotope values to bone collagen, rather than as a detailed recorder of short-term changes. Using carbon, nitrogen, and, unusually, sulfur isotope ratios from hair and associated archaeological food remains, the wide varieties of diets consumed at these sites were reconstructed. Hair from the Nile Valley, dated to the Late Middle Kingdom and Coptic periods, showed dietary differences between periods. The ranges of carbon and nitrogen isotope ratios were both larger in the Coptic samples (which were more numerous than the Middle Kingdom samples) and also showed heavier carbon from C4 sources. Isotope ratios from Chinchorro hair recovered from three sites in Chile reflected the availability of marine foods at two coastal sites versus the inland site in the availability and consumption of C3 and C4 foods and marine foods.

14.23.6.1 The Americas

The Americas are an important test-bed for examining the exploitation of C4 versus C3 type plants in dietary studies because of the widespread cultivation of maize. Throughout the Americas many different varieties of maize are grown. As such some of the earliest applications of geochemical analysis in archaeology have examined the exploitation of maize using surviving hair and bone. One of the earliest geochemical studies to utilize hair examined domesticated dog hair from the site of Ancon in Peru and concluded that maize formed a significant component of their diet (Burleigh and Brothwell, 1978). Subsequent geochemical studies have used human scalp hair to examine how early highland migrants had adapted to a coastal existence (Aufderheide et al., 1994). Diachronic studies have also examined seasonal variation in the consumption of marine products and C4 plants such as maize (Knudson et al., 2007) and the seasonality of diet in relation to timing of death (Williams and Katzenberg, 2008).

Significantly, the use and distribution of maize was controlled at various timeframes within the Americas. For instance, across the Inca Empire maize was considered an elite food. An interesting illustration of this is evidenced by the diachronic picture of diet for the frozen body of the 15-year-old Llullailaco Maiden discovered in 1999 concealed at a mountain-top shrine in northwest Argentina (Wilson et al., 2007b). Analysis of her hair showed a diet that changed markedly from C3 to C4 dominant foods, with a concurrent increase in protein intake, all occurring roughly 12 months prior to her death. This finding mirrors noncontemporary Spanish accounts that describe the practice of selecting young girls to live apart from society, under the guardianship of priestesses, before being sacrificed as ‘capacocha’ to the gods. Another major question sparked by such mountain-top finds is where they could have originated from. The archaeology, coupled with many of the surviving textiles and associated artifactual evidence, is suggestive of an Imperial rite that would have required significant logistical involvement. Geochemical data from the hair of a 10-year-old male capacocha from Volcan Aconcagua in Argentina provide compelling evidence for the altitude at which this individual had been resident before the trek to the mountains. Hydrogen isotope data from hair and tissue samples from this mummy were related to IAEA datasets by regression analysis to offer an estimate of elevation (Sharp et al., 2003).

More recently carbon and nitrogen isotope data from archaeological hair from Pacatnamu, Peru, have been published where changes in diet through time were interpreted as also being a reflection of human movement across the landscape (White et al., 2009a). This suggests that in situations where dietary resources, the environment, and cultural context are well understood, the ‘dietary isotopes’ can be used beyond simpler interpretations of diet to infer mobility. The hairs dated from two periods, the Moche (AD 450–750) and the Lambayeque (AD 900–1100). Isotopic analysis not only revealed that marine resources were consumed more heavily in the Lambayeque, but also that sequential analysis of months of growth in eight hairs showed intraindividual variability in both carbon and nitrogen isotopes. This was most pronounced in the adult males analyzed and was ascribed to frequent and irregular movement between locations such as the coast and the highlands where different dietary resources would have been available.

The remains of frozen individuals evoke strong emotions amongst the wider public, because very often these are in fact the most intact surviving bodies. As such there is much that can potentially be learned from their remains with, for instance, optimum chances of DNA survival. Where an extant cultural group attaches particular significance to such remains there has to be due care and sensitivity to the sampling and use of biological tissues – again hair is particularly helpful in this
Hair as a Geochemical Recorder: Ancient to Modern

14.23.6.2 The Nile Valley

The arid environment of the Nile Valley creates conditions for excellent organic preservation and this has enabled human hair from multiple sites to be analyzed for isotope ratios. The first study conducted by White (1993) investigated seasonality in food consumption, and by inference food production in X-group (AD 350–550) and Christian (AD 550–1300) period people. The author measured carbon isotope ratios along the length of single hairs at low resolution (2 month increments) and determined that seasonal differences existed that mirrored modern crop scheduling. During the summer drought tolerant C₄ crops (millet and sorghum) are grown, whereas during the wetter, milder winter cultivation of C₃ crops (wheat, barley, vegetables) takes place. This seasonal change in the carbon isotope ratios of food available for consumption was reflected in the hairs that were analyzed. Changes in carbon isotope ratios along hairs of up to 4% suggested that dietary carbon sources shifted through time between C₄ and C₃ derived carbon. Moreover, as the hairs were sampled from mummies the proximal end was known to reflect the period immediately prior to death and the carbon isotope values here tended to be heavier. This was interpreted as reflecting greater mortality in the human population during the physiologically stressful summer months.

A further study used the C isotope composition of the hair along with other human tissues excavated from the same region (White and Schwarz, 1994). Bone collagen, (a long-term, integrated recorder of dietary isotope ratios) and skin and muscle tissue (both relatively short-term, integrated recorders) were used to examine changes over the time period from the Merotic (350 BC–AD 350) to the Christian periods. The hair data provided temporally resolved data that augmented the interpretation of the other datasets. C isotopes in bone collagen, skin, and muscle showed differences in C₄ consumption between the three time periods, whereas hair C isotope composition showed the seasonal changes within these periods which otherwise would not have been detected.

14.23.6.3 Other Regions

Further examples of geochemical analyses of hair come from other regions which tend to be cold, including examples from Greenland (Raghaven et al., 2010; Rasmussen et al., 2010). The oldest surviving archaeological hair comes not from humans, but from other animals preserved within permafrost. Examples most widely studied include mammoth and bison. Mammoth samples have been used to provide short-term climatic records from the Arctic from variations in carbon and nitrogen isotope ratios along the length of the hair. The detailed resolution for these seasonal records relates to the fiber thickness (Iacumin et al., 2005).

Examples of the most recent surviving archaeological hair come from eighteenth/nineteenth century contexts. For this timeframe, hair survival is most apparent amongst assemblages that have either been subject to waterlogged site conditions, or where the air-void within the coffin has survived, as with remains recovered from crypts such as at Christ Church, Spitalfields, London. This assemblage was amongst the first to undergo detailed geochemical analysis for dietary analysis and provided a template for studies relating bone collagen and hair keratin (O’Connell and Hedges, 1999a).

14.23.7 Applications to Forensic Investigations

14.23.7.1 Unidentified Human Remains

The use of isotope ratios to investigate the region of origin of unidentified human remains was first proposed decades ago (Katzenberg and Krouse, 1989). Hair and nails from individuals with known regions of residence were analyzed longitudinally for carbon, sulfur, hydrogen, and oxygen isotope ratios and differences were seen between all populations. Additionally, hair and urine from individuals with known travel histories were analyzed to determine if there were observable changes through time, which were indeed detectable. Similar efforts aimed at identifying unknown individual samples by collecting samples from known populations and applying the observed patterns have continued (e.g., Fraser & Meier-Augenstein, 2007; Fraser et al., 2006; Mützel et al., 2008). Research into longitudinal, high-resolution sulfur isotope ratios may offer a further geographically linked signature that might be useful in the attribution of human hair samples, either for reconstruction approaches or for where only very small samples are available for analysis (Santamaria-Fernandez et al., 2009). A number of examples of casework using isotope ratios have also been published, usually in conjunction with ‘cold cases’ where standard, modern forensic techniques such as DNA analysis cannot provide answers for completely unknown individuals for whom no claim is made by the public. These studies have included cases from Europe (Meier-Augenstein and Fraser, 2008; Rauch et al., 2007), as well as the United States (Ehleringer et al., 2009). This work has primarily used hydrogen and oxygen isotope ratios from human hair to reconstruct the geographic region associated with a victim’s place of residence prior to death and whether any movement took place in the period recorded in the available hair. Whilst some of these studies have claimed to establish extremely specific points of origin, more cautious interpretations have established regions of origin based upon isotopically consistent areas identified using Geographic Information Systems (GIS) techniques (see Chapter 14.19 for further examples of forensic casework involving unidentified homicide victims).

This GIS-based approach is predicated on a good understanding of the human drinking water supply, the factor driving hydrogen and particularly oxygen isotope variation in human tissues. In order to interpret human regions of origin, in the United States drinking water has been studied well enough to allow the production of a GIS map that predicts isotope ratios (Bowen et al., 2007). Recently a more sophisticated understanding of the variation in human drinking waters based upon regional variations in water supplies due to both natural seasonal variations and anthropogenic influences (e.g., importation of water from nonlocal sources) has been developed. This has allowed the reinterpretation of some original
14.23.7.2 Wildlife Forensics and Isotopic Analysis of Hair

Isotope ratio analyses of animal hair also present useful opportunities for provenancing both legally manufactured valuable animal hair products such as wools in textiles as well as illegally produced products. However, they have been little applied and archaeological examples perhaps provide the best illustration of the potential for this technique. In the case of valuable antique textiles, as well as modern fabrics manufactured from animal hair, opportunities for economic fraud clearly exist where provenance is a key factor in determining the value of the item. A pilot study applied carbon, nitrogen, and sulfur isotope ratios in wool collected from sheep resident in known carpet producing regions of Turkey with the aim of determining the provenance of carpets (Hedges et al., 2005). This work revealed that many sites could not be distinguished from one another and that an unexpectedly complicated isotopic system existed. However, larger production centers could possibly be identified through such an approach. One method might be to combine the results with an orthogonal isotope system such as Sr. The usefulness of Sr in animal hair attribution is illustrated in the application of the system in a pilot study of modern sheep wool, with the goal of provenancing ancient textiles (Frei et al., 2009a). Unlike the other light isotope systems previously discussed, Sr isotope ratios are largely related to the age of the bedrock in a region and thus impart a geographic signature. Small amounts of local bioavailable Sr are taken in through food and water, incorporated into biological tissues, and eventually excreted into hair (for a review see Beard and Johnson, 2000). Consequently, a relatively large quantity of hair is required for Sr analysis compared to light isotope measurements. In Frei et al.’s study wool and soils from a variety of sites in northern Europe and New Zealand with different, and in some cases highly complex, geological backgrounds were collected, treated to remove Sr-containing contaminants and analyzed. It was observed that Sr ratios in wool and soils generally covaried and that they reflected the expected Sr isotope ratios given the known bedrock. The authors also analyzed some archaeological Scandinavian textiles and concluded that the wool was likely produced locally to the regions of excavation. This approach was subsequently applied to an archaeological wool garment recovered from the Huldremose bog in Denmark by the same group, which suggested that the wool was sourced from three geologically distinct regions, further suggesting the forensic applications of this system (Frei et al., 2009b).

14.23.8 Geography and Temporal Dynamics in Hair Oxygen Isotope Ratios

From the discussion thus far, it is expected that the oxygen isotope ratios of hair should be correlated with the oxygen isotope ratios of body water, which in turn should closely parallel the oxygen isotope ratios of local meteoric water as modified by metabolic isotope fractionation events. Several semimechanistic isotope models of the environmental and physiological processes influencing body water have been developed and evaluated (Kohn 1996; Podlesak et al., 2008, 2012), with each establishing that environmental water is the foundation for variations in oxygen isotope ratios of hair. These dynamics in meteoric water isotope values could come about primarily as a result of two factors that influence isotope ratios of water: (1) interannual changes in precipitation (i.e., summer versus winter precipitation in temperate climates) or (2) geographical changes reflecting regional differences in precipitation associated with continental-scale rainout effects (Bowen and Revenaugh, 2003; Bowen et al., 2007). While it is not possible to distinguish between these two possibilities based on a single isotope, it is practical to suggest that these two possibilities can be distinguished on the basis of how humans and animals consume water. For grazing vertebrates that might derive the majority of their water as leaf water from grasses supplemented by temporary pools of water, one might expect that seasonal differences in local precipitation would be recorded in the oxygen isotope ratios of hair. Unfortunately, there are limited, if any, data demonstrating seasonal dynamics on the oxygen isotope ratios of animal hair, although Podlesak et al. (2008) provided laboratory-based evidence of such relationships. However, there are supporting oxygen isotope ratio data for teeth of grazing mammals that require more than a year to mature; here teeth layers in grazing vertebrates reflect the interannual dynamics associated with changes in the isotope ratios of meteoric waters (Passey et al., 2005).

Examining the geographic based possibilities further, we see an extensive body of literature demonstrating that movement patterns, such as migration, are detectable in keratin (recall earlier C_3 versus C_4 discussion). These variations in keratin isotopes expressed in hair, feathers, and nails have been linked to geography using hydrogen or oxygen isotopes with the spatial modeling approaches initiated with studies by Bowen and Revenaugh (2003) and Bowen et al. (2005b, 2007). This growing literature reveals that the keratin in bird feathers recorded the region in which that feather had been produced (Chamberlain et al., 1997; Hobson, 1999; Hobson and Wassenaar, 1997). As bird feathers are produced over a very short time period, the isotopes in bird feathers represent a brief period in the animal’s life history. This same approach has been applied to animal and human hair (Ehleringer et al., 2008; Meier-Augenstein and Fraser, 2008; O’Brien and Wooller, 2007). In contrast to a seasonal signal in feathers, hair is a continuous linear recorder, allowing a more detailed and continuous reconstruction of the organism’s travel history.

Consider two examples to illustrate this point: the movements of a horse (Figure 10) and of a human (Figure 11) as revealed through oxygen isotope ratio analyses of the keratin in hair. In Figure 10, we present sequential analyses of segments of a horsetail hair. This particular horse was transported across the United States from Virginia to Utah. In Figure 11, we present sequential analyses of segments of a human scalp hair. This particular individual spent the summer in Utah in the United States before returning home to the United Kingdom at the end of the summer. In both cases, there was
a rapid movement from one location to the other, resulting in clear differences in the oxygen isotope ratios of hair that was produced in one region versus the other. It is also evident that the oxygen isotope ratios of horsetail hair and human scalp hair from the same geographical region (Utah) were not identical. The oxygen isotope ratios of horsetail hair were nearly 2% lower than those for human scalp hair from the same region. While the basis of this difference has not been quantified, it likely reflects a lower oxygen isotope ratio of bodywater pool in horses than in humans. It is less likely that the fraction factors between body water and protein are different in horses than in humans (Chesson et al., 2011).

The complete turnover of oxygen isotope ratios of hair produced in one region versus another was approximately 60 days for the horsetail hair (Figure 10) and 50 days for human scalp hair (Figure 11). Ehleringer et al. (2008) and Podlesak et al. (2012) reported a somewhat longer time period for human hair. Had the hair segments in the horsetail and human scalp of Figures 10 and 11 been collected at a greater frequency immediately after the movement to the new location, it would have been possible to apply the reaction progress model developed by Cerling et al. (2007) to determine how many metabolic pools are likely associated with temporal variation in hair oxygen isotope ratio values. However, with the data available, we are able only to examine the turnover rate of a single long-turnover pool.

Nevertheless it is possible to propose components of a model describing oxygen isotope ratio variations along the length of hair. First, it is important to note that in the movement of the horse and the human from one location to another, the response was not instantaneous even though the movement was essentially immediate. One component contributing to this lag must be a delay in the turnover of body water when moving from one environment to another. Podlesak et al. (2008, 2012) have shown that the turnover rates of isotopes in body water are not instantaneous but dependent on body size and drinking, urination, and evaporation rates. It is not uncommon for a lag period of 5–10 days before a new equilibrium is attained after moving to a new location. Yet still the body water lag is insufficient to account for the longer time lag required before oxygen isotope ratio equilibrium is attained in hair. A second component contributing to the isotope ratios of amino acids is required.

Decomposition of protein into the amino acids that are required for synthesis of keratin can occur in one of two primary locations: in muscle tissues, where actin and myosin are decomposed as these proteins turn over, or in the gut, where proteins from ingested food are digested. From previous studies, we know it is likely that the isotope ratio of cell water in metabolically active cells, such as in muscles, are elevated

![Figure 10](image1.png)  
**Figure 10** A time series of oxygen isotope ratios of horsetail hair over a nearly 300-day period. Approximately 150 days prior to the time the hair was cut, this horse was moved from Virginia to Utah in the United States. The horsetail hair was sectioned and analyzed for oxygen isotope ratios on an isotope ratio mass spectrometer using methods as described by West et al. (2004).

![Figure 11](image2.png)  
**Figure 11** A time series of oxygen isotope ratios of human scalp hair over a 17-month period. The individual lived in the central United Kingdom and occasionally traveled to the northern United Kingdom. Approximately 9 months prior to cutting the hair, the individual moved to Utah in the United States for a 3-month period. The scalp hair was sectioned and analyzed for oxygen isotope ratios on an isotope ratio mass spectrometer using methods as described by Ehleringer et al. (2008). We calculated that the hair of this individual grew at a rate of 0.38 mm day$^{-1}$. 

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above that of body water because of the additional water production during metabolism (Bowen et al., 2009; Kreuzer-Martin et al., 2005, 2006). In contrast, the isotope ratio of gut water is likely to be similar to that of body water. Thus, if there is isotopic exchange during these two different protein decomposition events, then it is likely that this could contribute an additional time lag before keratin oxygen isotope ratio equilibrium is attained.

The more extensive travels of the human in Figure 11 reveal that returning to the same original location (central United Kingdom) did result in similar oxygen isotope ratios. In addition, brief or protracted excursions to a colder and more northernly region in the United Kingdom also result in brief excursions in the oxygen isotope ratio of hair from the equilibrium central UK value.

For archaeological, ecological, and forensic studies, both hydrogen and oxygen isotope ratio analyses in keratin can be used. Yet it is simpler and more straightforward to apply only oxygen isotopes. This is because there is nearly a total isotope exchange of oxygen with all amino acids during synthesis and decomposition of proteins. In contrast, many of the hydrogen atoms in amino acids are attached to the primary carbon backbone or to attached moieties. These hydrogen atoms do not exchange during peptide synthesis or degradation so they reflect an historical water source, likely the leaf water environment when the amino acids were initially synthesized. While the correlations of hydrogen and oxygen isotopes of human hair are often high, that need not be the case if food sources among individuals are different (Bowen et al., 2009).

### 14.23.9 Future Directions

In many of the examples discussed above, samples of modern and historical human and animal hair derived from museum collections have provided invaluable insight into isotope ratio systems. These collections are unique, invaluable, biological repositories of the isotopic record prior to the globalization of human diets and the influence of human industrial activities on the environment. They provide the ability to sample globally without the need for field work, enhancing data from existing field studies. Their great usefulness in conducting future systematic work, particularly for hydrogen and oxygen isotope ratios, is contrasted by the difficulty in analyzing the small numbers of individuals curated in many private and museum collections where provenance and other metadata may be uncertain or even absent.

The relative ease and low cost of isotope ratio and elemental analysis of hair suggest that their prevalence will increase in the coming years. The studies reviewed here demonstrate two different approaches to analyzing hair, both as a bulk recorder of the environment in a population and, arguably more powerfully, as a time-resolvable, sequential recorder of changes in the environment or location of individuals. As more data are collected from hair, a better understanding of variation within and between populations is garnered, and better analytical techniques that allow meaningful measurement from smaller samples become commonplace, higher resolution, longitudinal studies on hair may become more common. The development of techniques that allow the high-resolution measurement of new isotope systems such as sulfur along the length of hair also opens up the possibility for information to be extracted from samples that was not previously accessible (e.g., Santamaria-Fernandez et al., 2009).

The ability to interpret these data meaningfully at high resolutions becomes key, and relies upon a solid foundation of understanding about the origin of hair keratin amino acids and their potential for isotopic variation. Adding a further dimension to the analysis of hair, particularly for light isotope systems, will be the measurement and understanding of compound-specific isotope ratios. The small amount of work that has been conducted to date using human hair samples (e.g., McCullagh et al., 2005; Petzke and Lemke, 2009) reflects the analytical challenges of this technique. However, this work has demonstrated that there are large maximum ranges in carbon and nitrogen isotope ratios within a single individual ($\delta^{13}N = 22\%o$; $\delta^{15}C = 31\%o$) and that these are characteristic of essential versus nonessential amino acids (Petzke and Lemke, 2009). As methods are developed to extend this technique into hydrogen isotope space, further effects due to physiology and diet should be observed according to current models. For example, differences between essential amino acids and those produced in vivo, as the latter should contain $H$ derived from the body pool and thus differ from those derived directly from diet (Ehleringer et al., 2008). This will extend the information that can be gleaned about diet in unknown samples, thus enabling more effective modeling of predicted source isotope ratios for provenance work.

As more isotopic data are gathered about ecological and hydrological systems and these systems are subsequently better characterized, improvements in the interpretation of isotope ratios in biological tissues such as hair will follow. For example, understanding where and why the human drinking water supply, which may also be used to supply agriculture, diverges isotopically from predicted precipitation due to ground water usage or water importation is key in the production of the large-scale isotopic models that enable predictions to be made about hair. Another key system that requires better characterization to interpret hair data is the variation in bioavailable strontium isotope ratios, both across the landscape and within hair from given populations. The use of such information, in conjunction with light isotope data, should significantly improve the resolving power of geochemical approaches in attribution cases. Along with ecological surveys and paleoclimatic reconstruction, forensic attribution is likely to be one of the most practical applications of this technology in the future, which could be applied as a tool to a range problems, from surveying victims of mass disasters where DNA is unavailable to provenancing illegally produced animal hair products such as elephant hair items.

### References


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