Stable Isotope Analysis of Modern Human Hair Collected From Asia (China, India, Mongolia, and Pakistan)

A.H. Thompson,[1,2]* L.A. Chesson,[1,2] D.W. Podlesak,[1,2] G.J. Bowen,[3] T.E. Cerling,[1,2,4] and J.R. Ehleringer[1,2]

[1]*IsoForensics Inc., Salt Lake City, UT 84108*
[2]*Department of Biology, University of Utah, Salt Lake City, UT 84112*
[3]*Department of Earth and Atmospheric Sciences, Purdue University, West Lafayette, IN 47907*
[4]*Department of Geology and Geophysics, University of Utah, Salt Lake City, UT 84112*

KEY WORDS hair keratin; stable isotope; Asia

ABSTRACT We report isotopic data (δ^2H, $\delta^{18}O$ n = 196; $\delta^{13}C$, $\delta^{15}N$ n = 142; $\delta^{34}S$ n = 85) from human hair and drinking water (δ^2H, $\delta^{18}O$ n = 67) collected across China, India, Mongolia, and Pakistan. Hair isotope ratios reflected the large environmental isotopic gradients and dietary differences. Geographic information was recorded in H and O and to a lesser extent, S isotopes. H and O data were entered into a recently developed model describing the relationship between the H and O isotope composition of human hair and drinking water in modern USA and pre-globalized populations. This has anthropological and forensic applications including reconstructing environment and diet in modern and ancient human hair. However, it has not been applied to a modern population outside of the USA, where we expect different diet. Relationships between H and O isotope ratios in drinking water and hair of modern human populations in Asia were different to both modern USA and pre-globalized populations. However, the Asian dataset was closer to the modern USA than to pre-globalized populations. Model parameters suggested slightly higher consumption of locally produced foods in our sampled population than modern USA residents, but lower than pre-globalized populations. The degree of in vivo amino acid synthesis was comparable to both the modern USA and pre-globalized populations. C isotope ratios reflected the predominantly C_3-based regional agriculture and C_4 consumption in northern China. C, N, and S isotope ratios supported marine food consumption in some coastal locales. N isotope ratios suggested a relatively low consumption of animal-derived products compared to western populations. Am J Phys Anthropol 141:440–451, 2010.

Hair samples were collected from locations in China, India, Mongolia, and Pakistan (see Fig. 1) and analyzed for stable isotope ratios (δ^2H, $\delta^{18}O$, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$). The purpose of the study was twofold. First, we expected that the wide variations in diet and environment across the region would be recorded in the stable isotope ratios of human hair keratin. Most reported stable isotope data from modern human keratins have been measured from samples collected in Western Europe (e.g. Bol and Pfleiger, 2002; Petzke et al., 2005a; Bol et al., 2007) and the Americas (e.g. Schoeller et al., 1986; Nardoto et al., 2006; Ehleringer et al., 2008), with a few exceptions from Asia and Australasia (Nakamura et al., 1982; Minagawa, 1992; Yoshinaga et al., 1996). Our Asian survey provided an opportunity to expand the modern human global datasets available and to explore isotopic variation in hair from individuals spanning very large climatic and dietary gradients. Across the region, the high altitude, dry steppe lands of Mongolia contrast with the tropical climates across much of India. China has many ecological zones including tropical, temperate, and desert, whereas Pakistan is more arid and consists of shrub land and desert. As urbanization increases across the region the consumption of a more western-style diet is becoming common, although a wide variety of dietary habits still exist (Pingali, 2004). Mongolian climate and geography do not allow for extensive agriculture and therefore much of Mongolia is dominated by a pastoralist economy. Recent reports indicate that the typical Mongolian diet remains rooted in the traditional nomadic dependence on animal products. Per capita, Mongolians consume the largest quantities of meat and milk of any of the Asian countries studied here (Sheehy, 1996; Speedy, 2003; FAO, 2006c). In contrast, large sections of both the Indian and Chinese populations are engaged in agriculture and these countries produce and consume large quantities of rice and wheat (FAO, 2006a,b). Traditionally, a large part of India's population is vegetarian due to both religious and economic constraints. Although dairy products are widely consumed, Indians consume the least meat of the four countries on a per capita basis (USDA, 2002; Speedy, 2003). The Chinese diet typically does not include significant amounts of dairy products

Grant sponsor: IsoForensics Inc., 423 Wakara Way, Suite 205, Salt Lake City, UT 84108.

*Correspondence to: Alexandra Thompson, IsoForensics Inc., 423 Wakara Way, Suite 205, Salt Lake City, UT 84108.
E-mail: athompson@biology.utah.edu

Received 2 February 2009; accepted 14 July 2009

DOI 10.1002/ajpa.21162
Published online 16 November 2009 in Wiley InterScience (www.interscience.wiley.com).

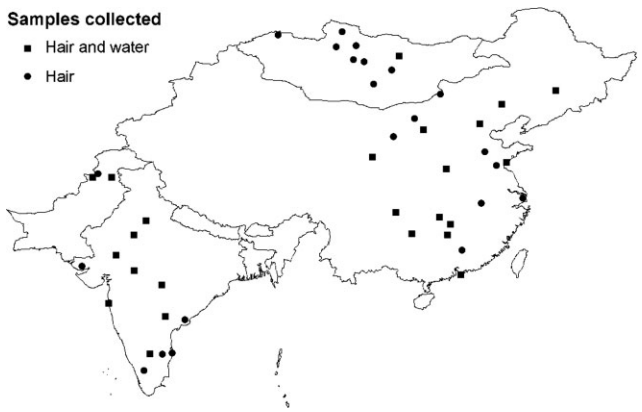

Fig. 1. Sites where human hair and tap water samples analyzed in this study were collected.

and relatively little meat is eaten, although these trends are changing (Leppman, 2005). On a per capita basis, the Chinese consume about half as much meat as the Mongolian population (Speedy, 2003) and only slightly less meat than the Pakistani population (FAO, 2006d). In terms of rice and wheat consumption, the typical individual diet in China is similar to India (Speedy, 2003; FAO, 2006b).

Second, this was an opportunity to compare our hydrogen (H) and oxygen (O) isotope ratio data with other published data. The relationships between H and O isotopes in hair and drinking water are not expected to be the same in all human populations due to differences in subsistence and diet. Establishing these differences is important in terms of applying such models to archaeological or forensic samples to reconstruct environment. Two comparable datasets have been used to develop a semimechanistic model predicting H and O isotope ratios in hair keratin, modern American human hair samples (Ehleringer et al., 2008) and pre-globalized populations (Bowen et al., 2009). However, this model has not been applied to modern non-USA samples. Differences in the diet of our sampled Asian populations, described above, compared to USA residents are expected that are likely to influence the isotopic relationships that are observed.

The model incorporates the isotopic contributions of both food and drinking water to human hair keratin. Briefly, it considers that H and O within ingested proteins undergo exchange with H and O in body water pools which contain a mixture of H and O from drinking water, from food water, from metabolized food, and an O contribution from diatomic oxygen as food is broken into individual amino acids. For O, this occurs as a near-complete exchange of carboxyl-bound O during peptide hydrolysis. For H, only a subset of H atoms in functional groups is freely exchanged between the point of amino acid hydrolysis and the ultimate protein condensation. As most nonessential amino acids not supplied by the diet are synthesized from the carbon skeletons of metabolic intermediaries, it is assumed that all H and O atoms within amino acids synthesized in vivo are derived from body water. Because H atoms from drinking water have a direct influence on the H atoms within amino acids only through exchange with body water, the more nonessential amino acids that are biosynthesized, the more the H isotope signal is predicted to reflect drinking water. In contrast, the near-complete exchange of amino acid O with body water for both essential and nonessential amino acids implies that the relationship between O isotope ratios in keratin and of drinking water should be relatively insensitive to the extent of in vivo synthesis.

We hypothesized that our samples would have characteristics that lay in between the modern USA residents and the pre-globalized populations and that this would be reflected in two model parameters required to reproduce the observed data. First, the degree to which isotopically "local" versus "imported" foods were consumed. Here, "locally" produced food is defined as being food produced within the same isotopic region as the consumer. This is in contrast to the concept of food that is imported into the consumer's region and consumed as a significant part of diet as in the case of the "supermarket" diet proposed in societies such as the modern USA. The relationships between both H and O isotopes in drinking water and hair are predicted to become steeper as more locally produced food is consumed, within boundaries prescribed by physiological processes. The analysis of hair samples collected in the mid-20th Century from indigenous populations around the world by Bowen et al. (2009) supported this prediction. The local dietary component was added to the Ehleringer et al. (2008) model that estimated the contribution of local diet, which strongly influenced the slope produced of modeled data. Although food habits across Asia are rapidly moving towards a more western "supermarket" diet (Pingali, 2004), we thought it likely that the study population consumed more local foods compared to modern USA residents, but less than the pre-globalized populations who had little access to world food markets. Second, differences in diet meant that the level of in vivo amino acid synthesis was likely to be higher than in the modern USA residents. On average, although this is rapidly changing, considerably less animal protein is consumed in Asia compared to the modern USA (Speedy, 2003).

Stable isotope systematics

The mechanisms for isotopic variation in water isotopes are well understood and are driven by factors such as latitude, longitude, and elevation during phase changes of water in the hydrological cycle (Gat, 1996). The H and O isotope ratios of meteoric water covary strongly along geographic gradients and tend to be lower at high latitudes and altitudes and higher towards lower latitudes and coastal regions (Bowen and Revenaugh, 2003). H and O stable isotope ratios of local water propagate throughout an ecosystem. They are incorporated into plant tissues, the tissues of herbivores that consume plants and drink local water, and predators, carrying the initial water-derived signal throughout all levels of a food chain. H and O isotope ratio analysis has been applied to questions of origin or migration in nonhuman keratin tissues such as bird feathers (Hobson et al., 1999a, 2004) and butterfly wing keratin (Hobson et al., 1999b). However, data from human hair (Sharp et al., 2003; O'Brien and Wooller, 2007; Ehleringer et al., 2008) and nail keratin (Fraser et al., 2006; Fraser and Meier-Augenstein, 2007) have recently been published.

While one focus of this study is on hydrogen and oxygen isotopes in hair as they relate to geography, it is impor-

tant to note that carbon, nitrogen, and sulfur stable isotope ratios are useful in reconstructing the dietary choices of consumers. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios from human hair and fingernail keratins have been extensively reported to give insight into both modern and ancient human diets (Nakamura et al., 1982; White, 1993; Macko et al., 1999; O'Connell and Hedges, 1999; White et al., 1999; O'Connell et al., 2001; Bol and Pfleiger, 2002; Petzke et al., 2005a,b; Roy et al., 2005; Nardoto et al., 2006; Bol et al., 2007; Buchardt et al., 2007). In the context of human diet carbon isotope ratios broadly indicate the source of C being consumed, reflecting ^{13}C-depleted values in C_3 plants (e.g. wheat, temperate grasses) or ^{13}C-enriched values in C_4 plants (e.g. maize, sorghum). Nitrogen isotope ratios in hair may broadly reflect a consumer's trophic level, as a consumer's tissues are generally ^{15}N-enriched compared to dietary protein (Post, 2002). Thus, N isotope ratios in human tissues such as hair can potentially discriminate between omnivores and vegans (O'Connell and Hedges, 1999; Bol and Pfleiger, 2002; Petzke et al., 2005a,b; Nardoto et al., 2006) and those consuming large quantities of marine foods (Buchardt et al., 2007). Other studies have found further subtle discrimination distinguishing ovo-lacto-vegetarians from omnivores (Bol and Pfleiger, 2002; Petzke et al., 2005a,b), although one found no difference (O'Connell and Hedges, 1999). However, the degree to which this tropic enrichment occurs varies widely even within identically maintained experimental animals (Sponheimer et al., 2003). The variation is thought to be related to dietary protein adequacy, although there is conflicting experimental evidence that suggests that increased protein quality is related to either increased (Sponheimer et al., 2003) or decreased (Robbins et al., 2005) fractionation. N isotope values in human hair are also affected by relatively short-term changes in health status and metabolism. This was demonstrated in recent studies showing ^{15}N enrichment in the hair of anorexics suffering from extreme protein malnutrition (Hatch et al., 2006; Mekota et al., 2006) and pregnant women who were considered to be under nutritional stress (Fuller et al., 2005). Broad relationships between aridity and N isotope ratios in plant (Heaton, 1987) and animal tissues (Gröcke et al., 1997) have long been observed. Environmentally-driven changes in N isotope values that are expressed may be natural, such seasonal aridity (Cerling et al., 2006) or due to anthropogenic changes such as the use of manufactured fertilizers (Vitoria et al., 2004). N isotope ratios in human hair therefore represent a complex integration of trophic level, physiological and environmental baseline influences.

Sulfur isotope ratios $\delta^{34}S$ from various human tissues including hair and nail keratin have been reported in both extant (Krouse and Levinson, 1984; Krouse et al., 1987; Katzenberg and Krouse, 1989; Vitoria et al., 2004; Bol et al., 2007; Buchardt et al., 2007) and archaeological populations (Richards et al., 2001; Wilson et al., 2007). Variations in $\delta^{34}S$ reflect regional differences in sulfur in the human food chain contributed from the pedosphere and biosphere and anthropogenic sources such as ammonium sulfate fertilizer (Thode, 1991; Mizota and Saaki, 1996; Vitoria et al., 2004). Apart from the large fractionations associated with bacterial dissimilatory sulfate reduction, there is little biological fractionation of S and so $\delta^{34}S$ is generally conserved along food chains (Krouse, 1980; Krouse et al., 1991; Thode, 1991; McCutchan, et al., 2003). Coastal regions have elevated $\delta^{34}S$ signals driven by the high $\delta^{34}S$ value of marine sulfate +21‰, deposited by the "sea-spray effect," which may also be observed at inland locations where ancient seas existed (Krouse et al., 1991). Similarly, the consumption of marine foods produces elevated $\delta^{34}S$ values. Inland regions typically have lower values and may even be negative depending upon location, so terrestrial diets tend to have lower $\delta^{34}S$ values.

METHODS

We collected hair samples in paper envelopes from anonymous volunteers and barbershops in China, India, Mongolia, and Pakistan. No donor information was collected as this was not permitted under the IRB approval that was issued. Samples were washed twice for ~10 min in a 2:1 chloroform/methanol solvent mixture to remove surface contamination and allowed to air dry. Samples were then ground to a fine powder using a ball mill and stored in vials until they were weighed for analysis to ±10% of the stated target weight. Measurement of nonexchangeable $\delta^{2}H$ and total $\delta^{18}O$ values required 150 μg of sample, weighed in duplicate into silver capsules treated to remove oxides. Samples for H and O isotope ratio analysis were equilibrated following established methods (Bowen et al., 2005) and stored under vacuum for at least 5 days prior to analysis to remove adsorbed water. Laboratory reference materials for which $\delta^{2}H$ of the nonexchangeable hydrogen fraction and total $\delta^{18}O$ were known were also loaded. For $\delta^{13}C$ and $\delta^{15}N$ analysis, 500 μg of sample was required and $\delta^{34}S$ analysis required 900 μg of sample, weighed into pressed tin capsules. Laboratory reference materials of known isotopic values, defined by measurement against international standards, were included at predefined intervals. Tap water samples were collected in glass vials, capped, then sealed with parafilm, and refrigerated to prevent evaporation prior to analysis. A 400-μl sub-sample of each water sample was loaded into sealed GC vials for analysis of two replicate injections. Reference waters of known isotopic values, calibrated against international standards, were included at defined intervals.

The H and O isotope ratios of hair and water samples were measured using an isotope ratio mass spectrometer (IRMS; ThermoFinnigan, Delta Plus XL, Bremen, Germany) operated in continuous-flow (CF) mode. Samples were pyrolyzed in a temperature conversion elemental analyzer (TC/EA; ThermoFinnigan, Bremen, Germany). Solid samples were introduced to the pyrolysis column via a zero-blank autosampler (Costech Analytical, Valencia, CA). Replicate water samples were injected into the TC/EA by a PAL autosampler (LEAP Technologies, Carrboro, NC). Carbon/nitrogen and sulfur were measured using separate CF-IRMS instruments (Finnigan MAT delta S, Bremen, Germany). Samples were loaded onto a zeroblank autosampler and flash combusted in an elemental analyzer (EA; Carlo Erba 1108, Milan, Italy).

Stable isotope values are reported using standard delta δ notation in units of per mil ‰, calculated as follows: $\delta X = [R_{standard}/R_{sample} - 1] \times 1{,}000$, where R is the molar ratio of the heavy to light isotope e.g. $^{2}H/^{1}H$. Data are reported against international standards $\delta^{2}H$ and $\delta^{18}O$, VSMOW; $\delta^{13}C$, VPDB; $\delta^{15}N$, AIR; $\delta^{34}S$, VCDT. Analytical precisions based on long-term measurements of laboratory reference materials were as follows for solid organic materials: $\delta^{13}C \pm 0.1$ ‰, $\delta^{15}N \pm 0.2$‰, $\delta^{34}S$

TABLE 1. Summary of hair isotope ratio data collected in this study by country

Country		δ^2H (‰)	δ^{18}O (‰)	δ^{13}C (‰)	δ^{15}N (‰)	δ^{34}S (‰)
China	Mean	−98 ± 10 (n = 112)	13.1 ±1.0 (n = 112)	−20.3 ± 1.5 (n = 74)	8.1 ± 1.1 (n = 74)	6.7 ± 2.1 (n = 28)[a]
	Min	−122	10.4	−23.8	5.2	3.3
	Max	−71	15.3	−17.4	10.6	11.8
	Range	51	4.9	6.4	5.4	8.5
India	Mean	−80 ± 11 (n = 48)	15.9 ± 2.0 (n = 48)	−19.8 ± 1.2 (n = 38)	8.1 ± 1.7 (n = 38)	10.1 ± 1.7 (n = 26)[b]
	Min	−109	10.9	−22.6	5.8	5.3
	Max	−63	20.0	−17.2	12.2	13.8
	Range	47	9.1	5.3	6.2	8.5
Mongolia	Mean	−117 ± 12 (n = 18)	9.8 ± 2.1 (n = 18)	−20 ± 1.0 (n = 20)	9.6 ± 1.4 (n = 20)	6.0 ± 1.0 (n = 19)[b]
	Min	−140	4.7	−21.7	7.3	3.7
	Max	−89	13.5	−17.4	13.3	7.2
	Range	52	8.9	4.3	6.0	3.5
Pakistan	Mean	−93 ± 14 (n = 12)	13.6 ± 1.3 (n = 12)	−20.6 ± 0.7 (n = 11)	8.2 ± 0.7 (n = 11)	4.0 ± 0.6 (n = 12)[c]
	Min	−121	11.4	−21.7	6.7	3.2
	Max	−78	15.1	−19.6	9.2	5.3
	Range	43	3.7	2.1	2.5	2.1

With results of Tukey-Kramer Honestly Significant Difference test for δ^{34}S.
[a–c] Mean values in columns with a different letter are significantly different ($P = < 0.0001$).

± 0.3‰, δ^2H ± 4‰, and δ^{18}O ± 0.4‰. For water analyses, analytical precision was ±1.55‰ for δ^2H and ±0.17‰ for δ^{18}O.

The analyses comparing the relationship between hair and water isotope ratios only used locations where data from a minimum of two hair and water samples were available. The Data Analysis Toolpak in Microsoft Excel for Windows and JMP 7.0 software (SAS Institute, Cary, NC), were used for statistical analyses.

RESULTS

Hair samples were collected from 46 separate locations within China, India, Mongolia, and Pakistan (see Fig. 1). Water samples were collected from 38 locations. Hair isotope ratio data are summarized by country (Table 1) and all data are given by location (Table 2).

Hydrogen and oxygen isotope ratios

The H and O isotope data measured from drinking water and hair samples are shown in Figure 2. Drinking water samples spanned a range of −114‰ to +4‰ and −15.1‰ to +17‰ for δ^2H and δ^{18}O, respectively. The water isotope ratios followed a similar geographic pattern to those from hair. The highest isotope ratios came from India and those with the lowest ratios from Mongolia which bracketed samples from China and Pakistan. The calculated local meteoric water line was $\delta^2H = 6.9 \times \delta^{18}O - 5.5‰$ [Eq. (1), $r^2 = 0.92$, $P < 0.01$]. Their position to the right of the local meteoric water line indicated that some of the drinking water samples from India and Mongolia were ^{18}O-enriched and appeared to have been affected by evaporation at their source (see Fig. 2). Some variation in water values within locations was observed and standard deviations within a sampling location ranged from 0 to 6‰ for H and 0 to 1.4‰ for O isotope ratio values. In all cases, the data measured reflected the water supply available to an inhabitant. As hair was treated as being isotopically homogenous, all the available water data were used to calculate a mean value per location.

The most ^{2}H- and ^{18}O-enriched hair samples originated from Indian individuals, while the most depleted values were measured in hair of individuals from Ulaanbaatar in Mongolia, bracketing samples from China and Pakistan. The H isotope ratios from hair ranged from −141 to −63‰ and the O data ranged from +4.7 to +20.0‰. The standard deviations for hair δ^2H and δ^{18}O values varied per collection site 1.7–17.5‰ and 0.2–2.5‰, respectively. The δ^2H and δ^{18}O data were strongly correlated and a linear regression through the data gave a relationship of $\delta^2H = 5.64 \times \delta^{18}O - 172‰$ [Eq. (2), $r^2 = 0.80$, $P < 0.01$]. The variations observed in H and O isotope ratios from hair suggested a strong geographical pattern (see Fig. 2). To further investigate this, a subsample of data from 23 cities/towns where a minimum of two of both tap water and hair samples were collected was analyzed (Figs. 3 and 4). Data from Ulaanbaatar in Mongolia were excluded from the H isotope ratio regression as a Mahalanobis distance test indicated the data point was an outlier. Although there was some scatter among the data, strong, significant correlations existed between the H and O isotope ratio values of tap water (δ^2H_w and $\delta^{18}O_w$) and H and O isotope ratio values of hair (δ^2H_h and $\delta^{18}O_h$). Least squared regression analyses produced the following relationships: $\delta^2H_h = 0.42 \times \delta^2H_w - 75‰$ (Eq. (3), $r^2 = 0.75$, $P < 0.01$) and $\delta^{18}O_h = 0.39 \times \delta^{18}O_w + 16.3‰$ (Eq. (4), $r^2 = 0.79$, $P < 0.01$).

To compare the results of this study with the isotope ratio data previously measured from modern USA inhabitants (Ehleringer et al., 2008) and globally collected indigenous populations (Bowen et al., 2009), the data were entered into the modified Ehleringer et al. model (Bowen et al., 2009). This model has adjustable parameters, including the amount of locally produced food that is consumed l and the average rate of amino acid synthesis represented by f_s, the fraction of nonexchangeable H atoms fixed in vivo. These values give information about general dietary patterns, giving insight into population-level characteristics. The parameters were adjusted for our dataset until the best fit between the predicted and measured results was produced (Table 3).

Carbon and nitrogen isotope ratios

A subset of 142 hair samples was analyzed for δ^{13}C and δ^{15}N values (Fig. 5, Table 1). Overall, the data showed no significant relationship between these two parameters ($r^2 = 0.00$, $P > 0.01$). However, when the data were examined on a country-by-country basis, a positive relationship between C and N isotope ratios was observed for hair samples from India ($r^2 = 0.29$, $P <$

TABLE 2. Isotope ratio data from collection locations across the Asian transect

		Hair															Water					
		δ^2H			$\delta^{18}O$			$\delta^{13}C$			$\delta^{15}N$			$\delta^{34}S$			δ^2H			$\delta^{18}O$		
Country	City	Mean (‰)	SD	*n*	Mean (‰)	SD	*n*	Mean (‰)	SD	*n*	Mean (‰)	SD	*n*	Mean (‰)	SD	*n*	Mean (‰)	SD	*n*	Mean (‰)	SD	*n*
China	Beijing	−105	4.8	13	12.7	0.9	13	−19.8	1.0	12	7.4	0.7	12	8.6	0.4	5	−59	5	7	−7.4	0.9	7
China	Chang de	−96	5.0	5	13.8	0.3	5	−23.3	0.4	5	8.9	0.6	5	4.5	–	2	−39	–	2	−5.7	–	2
China	Chang sha	−92	4.1	5	13.9	0.8	5	−20.7	0.4	4	8.5	0.3	4	–	–	–	−30	–	2	−4.8	–	2
China	Changchun	−110	7.6	5	11.9	0.9	5	−19.5	1.3	4	8.4	0.3	4	7.8	–	1	−60	–	2	−7.0	–	2
China	Chifeng	−108	5.0	5	11.9	0.5	5	−18.4	0.3	5	7.5	0.2	5	7.4	–	2	−81	–	2	−10.5	–	2
China	Chong qing	−98	4.9	5	14.0	0.9	5	−21.3	0.7	5	8.5	0.7	5	6.3	/	2	−43	–	2	−6.1	–	2
China	Duguitala	−99	5.3	3	12.9	0.5	3	−19.3	–	1	8.6	/	1	7.9	/	1	−75	–	1	−9.3	–	1
China	Erenhot	−105	–	1	10.5	–	1	–	–	–	–	–	–	–	–	–	−89	–	1	−10.8	–	1
China	Heng yang	−95	4.9	5	13.6	0.6	5	−21.5	0.5	5	9.0	0.6	5	6.6	0.3	3	−37	–	2	−5.5	–	2
China	Jinan	−94	–	1	12.7	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
China	Lanzhou	−92	4.8	5	13.1	0.7	5	−21.5	0.5	5	7.3	0.5	5	5.3	0.4	5	−50	–	2	−7.8	–	2
China	Linyi	−107	–	1	13.9	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
China	Luoyang	−103	8.6	5	12.6	0.4	5	−20.8	0.5	5	5.6	0.4	5	11.2	0.7	3	−70	–	2	−9.6	–	2
China	Nan Xiong	−101	–	2	14.0	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
China	Qian Shan	−101	1.7	3	13.4	0.4	3	–	–	–	–	–	–	–	–	–	−45	–	1	−6.4	–	1
China	Ri zhao	−100	11.4	4	13.4	0.4	4	−20.2	0.7	4	7.6	0.8	4	7.2	1.3	4	−49	–	2	−6.7	–	2
China	Shanghai	−82	–	1	13.8	–	1	–	–	–	–	–	–	–	–	–	−31	–	1	4.0	–	1
China	Wanchai	−89	7.0	28	13.6	0.6	28	−18.8	0.7	11	8.8	0.3	11	7.1	0.6	3	−44	–	2	−6.7	–	2
China	Xinjie	−102	–	2	14.3	–	2	−18.5	–	1	7.9	–	1	–	–	–	−76	–	2	−10.0	–	2
China	Yinchuan	−108	8.1	10	12.3	0.9	10	−19.9	0.6	3	8.8	1.5	3	6.9	0.3	3	−69	–	1	−8.6	–	1
China	Zhenyuan	−104	4.4	5	12.7	0.7	5	−22.2	0.5	3	9.4	1.0	3	3.9	0.6	3	−37	–	2	−5.7	–	1
India	Bangalore	−72	2.8	4	16.0	0.9	4	−20.6	/	1	7.4	–	1	10.7	–	1	−9	–	2	−1.7	–	2
India	Bhuj	−75	–	1	15.5	–	1	–	–	–	–	–	–	–	–	–	−35	–	1	−4.3	–	1
India	Chennai	−80	2.4	4	16.8	0.6	4	−19.5	0.6	4	11.1	1.1	4	12.0	0.7	4	–	–	–	–	–	–
India	Coimbatore	−85	11.7	6	15.0	1.8	6	−19.8	0.9	5	7.9	0.8	5	9.5	1.0	3	−22	–	1	−4.0	–	1
India	Hyderabad	−74	8.2	4	17.0	1.6	4	−19.8	1.4	4	7.4	0.9	4	9.9	0.7	4	17	–	2	4.2	–	2
India	Indore	−89	12.4	5	13.8	2.5	5	−20.9	0.9	4	6.8	0.8	4	10.4	–	2	−23	–	2	−2.7	–	2
India	Jaipur	−73	2.4	3	16.8	2.3	3	−19.5	0.7	3	8.0	1.6	3	9.7	1.2	3	−34	–	2	−3.7	–	2
India	Mumbai	−75	17.5	3	15.9	1.8	3	−19.4	1.3	3	7.6	0.7	3	8.9	–	1	−2	5	3	−0.4	0.2	3
India	Nagpur	−85	13.4	5	15.5	2.5	5	−21.0	1.2	4	6.5	0.3	4	10.0	–	1	−27	6	3	−2.9	0.6	3
India	New Delhi	−98	13.4	3	13.1	2.1	3	−18.9	–	2	8.2	–	2	8.7	–	2	−50	4	3	−7.1	0.9	3
India	Rajahmundry	−78	–	1	15.5	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
India	Udaipur	−75	7.8	5	18.2	1.2	5	−20.4	1.0	4	6.8	0.7	4	6.8	–	2	4	5	5	3.5	1.4	5
India	Vellore	−74	9.5	4	16.5	0.4	4	−18.3	1.0	4	10.4	0.5	4	11.3	1.8	4	–	–	–	–	–	–
Mongolia	Dund Shandny	−117	–	1	11.4	–	1	−19.7	–	1	12.6	–	1	7.0	–	1	−88	/	1	−11.4	–	1
Mongolia	Erdenet	−112	–	1	9.5	–	1	−20.3	–	1	8.7	–	1	5.1	–	1	–	–	–	–	–	–
Mongolia	Kharkhorin	−118	–	1	10.3	–	1	−20.2	–	1	10.4	–	1	5.4	–	1	−95	–	1	−12.2	–	1
Mongolia	Mandal Ovoo	−114	–	1	11.8	–	1	−19.5	–	1	13.3	–	1	6.2	–	1	−91	–	1	−11.2	–	1
Mongolia	Toilogt	−135	–	2	6.9	–	2	−20.5	–	2	8.0	–	2	6.7	–	2	−76	–	1	−7.5	–	1
Mongolia	Tsegeen Nuur	−129	–	1	5.2	–	1	−20.7	–	1	8.4	–	1	6.5	–	1	−33	–	1	−0.2	–	1
Mongolia	Tsenkher Sum	−127	–	1	10.6	–	1	−21.7	–	1	8.5	–	1	6.6	–	1	−101	–	1	−13.1	–	1
Mongolia	Ulaanbaatar	−112	10.2	11	10.3	1.4	11	−19.8	1.0	11	9.5	0.4	11	5.8	1.1	10	−114	2	3	−15.1	0.1	3
Mongolia	Uus	−88	–	1	11.7	–	1	−18.4	–	1	8.8	–	1	7.2	/	1	–	–	–	–	–	–
Pakistan	Islamabad	−82	4.3	3	14.8	0.2	3	−20.2	0.6	3	7.9	0.8	3	3.7	0.7	3	−18	5	9	−3.2	1.4	9
Pakistan	Mazar	−108	9.5	5	12.0	0.6	4	−21.4	0.2	4	8.2	0.3	4	4.2	0.1	4	−63	–	2	−9.6	–	2
Pakistan	Peshawar	−84	4.4	5	14.2	0.6	5	−20.1	0.3	4	8.2	1.1	4	4.0	0.8	5	−50	–	1	−8.1	–	1

SD = standard deviation; *n* = number of samples.

0.01), although there were no statistically significant patterns in China (r^2 = 0.00, $P > 0.1$), Mongolia (r^2 = 0.06, $P > 0.1$), or Pakistan (r^2 = 0.01, $P > 0.1$). The mean $\delta^{13}C$ values for all four countries were relatively low and there was no significant difference in the carbon isotope values between countries, reflecting the predominantly wheat and rice C_3-based agriculture of the region. Except for Pakistan, where C isotope ratios for all individuals were lower, a small number of individuals with more enriched ^{13}C-enriched isotope values indicative of some C_4 dietary input were observed in all the populations. In the samples collected from China, there was a positive correlation between carbon isotopic composition and latitude (r^2 = 0.52, $P < 0.01$). No significant relationships between $\delta^{13}C$ values and latitude or longitude were observed elsewhere. The N isotope values in hair spanned a large total range of 8.0‰. India, Pakistan, and China had almost identical means values, whilst the mean value observed in Mongolia was more than 1‰ higher than those seen in the other locations, with two very ^{15}N-enriched samples measured, both from rural regions. No correlation between N and H isotope ratios or N and "residual" δ^2H_{res} (here $\delta^2H_{res} = \delta^2H - \delta^{18}O \times 5.64 - 172$, from Eq. (2)), was observed (see Fig. 6).

Sulfur isotope ratios

A subset of 85 hair samples was analyzed for $\delta^{34}S$ values. Individuals from India had the highest $\delta^{34}S$ values and those from Pakistan the lowest (Table 1). A one-way ANOVA and post hoc Tukey-Kramer Honestly Significant Difference Test (THSD) found that whilst S isotope ratios of individuals from China and Mongolia were not

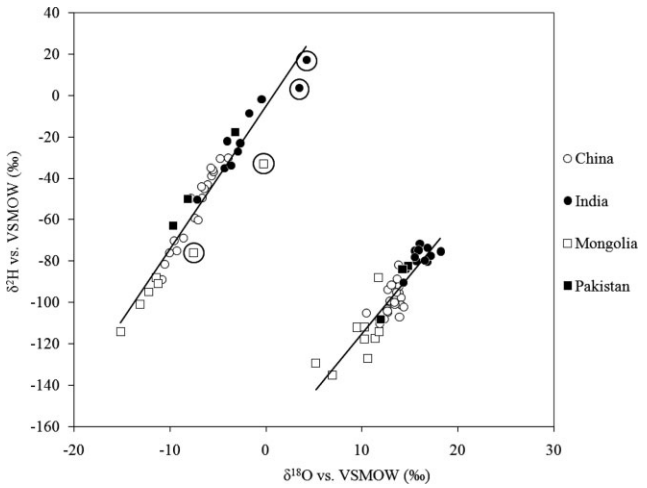

Fig. 2. Mean δ^2H v. $\delta^{18}O$ values measured from water (left, $\delta^2H = 6.90 \times \delta^{18}O - 5.5$, $r^2 = 0.97$, $P < 0.01$) and hair (right, $\delta^2H = 5.64 \times \delta^{18}O - 177$, $r^2 = 0.80$, $P < 0.01$) samples collected from China, India, Mongolia, and Pakistan. (Circled water data points indicate isotopically enriched samples, discussed in text).

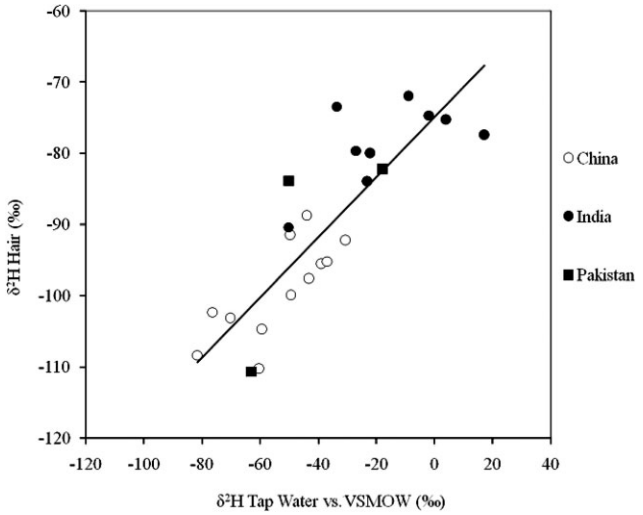

Fig. 3. Mean δ^2H values measured from paired hair (δ^2H_h) and tap water samples (δ^2H_w) ($\delta^2H_h = 0.42 \times \delta^2H_w - 75$ ‰, $r^2 = 0.75$, $P < 0.01$). Data points are mean per site and the δ^2H value for hair is for nonexchangeable H atoms.

significantly different from each other, sulfur isotope ratios of individuals from both Pakistan and India were significantly different from both the other populations and each other. The highest $\delta^{34}S$ values in India were measured in samples from coastal Chennai and nearby Vellore. Even if the data from these two locations were excluded from the analyses, the mean $\delta^{34}S$ value for India was 9.3‰, higher than the mean values of between 4.0 and 6.7‰ measured from other countries. A one-way ANOVA showed that the Indian populations were significantly different from other sampled populations. Equally elevated $\delta^{34}S$ values were observed in inland regions of China, which was also where the lowest $\delta^{15}N$ values were measured.

The $\delta^{34}S$ data appeared to have value as a geographical marker and were treated together with the H and O isotope values also measured for this subset. A principal component analysis was used to determine the extent to which $\delta^{34}S$ values enhanced the geographic signal measured from the H and O isotope data (Fig. 7, Table 4). A plot of the first two principal components indicated that the four national populations sampled could be further separated based on the combination of these isotopic parameters. The first principal component was correlated with the H and O isotope data, whereas the second principal component was correlated to the S isotope ratio data.

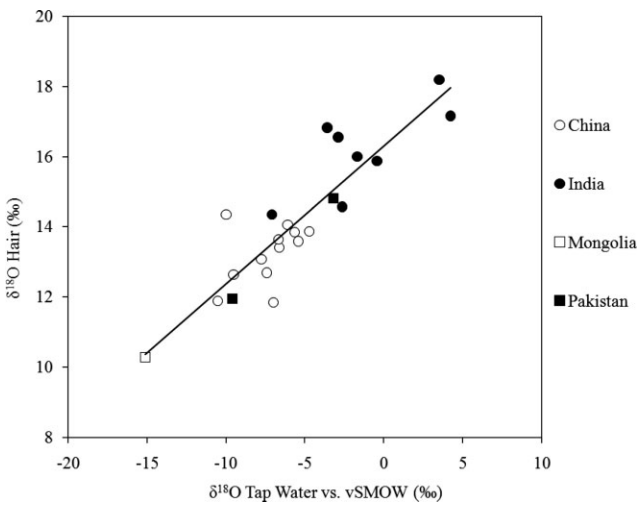

Fig. 4. Mean $\delta^{18}O$ values measured from paired hair ($\delta^{18}O_h$) and tap water ($\delta^{18}O_w$) samples ($\delta^{18}O_h = 0.40 \times \delta^{18}O_w + 16.4$‰, $r^2 = 0.79$, $P < 0.01$). Data points are mean per site.

DISCUSSION

Patterns in hydrogen and oxygen isotope ratios

Water samples. In general, the most ^2H- and ^{18}O-depleted water samples originated from Mongolia where high altitude and inland locations accounted for the isotopic depletion observed and the most ^2H- and ^{18}O-enriched samples were from inland regions of India. Regression analysis of the water samples produced a slope of 6.9, slightly lower than the global meteoric water line slope of 8 (Craig, 1961; GMWL). This was due to the inclusion of samples from India and Mongolia in the dataset that contained an excess of ^{18}O due to evaporation (circled in Fig. 2) and hence fell off to the right of the local meteoric water line. The Mongolian water samples from Toilogt and Tsegeen Nuur were collected from lakes that were clearly subject to evaporation. The evaporatively enriched samples from India were collected from Hyderabad and Udaipur where local water sources originate from reservoir systems and lakes, respectively, both sources subject to evaporation.

Hair samples. The Ehleringer et al. (2008) study of modern Americans found a strong relationship between the isotope ratios of hair and local drinking waters, despite no knowledge of the individuals involved. This phenomenon was also observed in indigenous historical populations by Bowen et al. (2009). Here, for the Asian survey with its significant gradient in water isotope values, we also expected a significant relationship between these parameters. In fact, the hair samples correlated

TABLE 3. Regression coefficients (a) and y-intercept values (b) of the relationships between H and O isotope ratios in human hair and drinking water and best-fit derived model parameters from Ehleringer et al. (2008) and Bowen et al. (2009) models

		Hair		Hair vs. drinking water				Best fit model parameters	
		δ^2H vs. $\delta^{18}O$		δ^2H_h vs. δ^2H_w		$\delta^{18}O_h$ vs. $\delta^{18}O_w$			
Study	Population	*a*	*b* (‰)	*a*	*b* (‰)	*a*	*b* (‰)	f_s	*l*
Ehleringer et al. 2008	Modern USA inhabitants	5.73	−166	0.27	−79	0.35	+15.2	0.30	0.00[a]
Bowen et al. 2009	Globally collected historic indigenous populations	8.86	−203	0.78	−49	0.70	+19.2	0.37	0.67
This study	Modern Asian populations	5.64	−172	0.42	−75	0.40	+16.4	0.35	0.15

f_s = fraction of H atoms fixed during *in vivo* amino acid synthesis, *l* = fraction of dietary H and O derived from locally produced diet.
[a] *l* = 0 is a product of the assumed isotopically-homogenous "supermarket" diet of modern USA inhabitants.

TABLE 4. Correlation coefficients, explained variances of first two principal components, correlations, and eigenvectors values of each contributing dataset

Variable	First component	Second component
Explained variance		
Eigenvalues	2.16	0.71
Variance %	72.1	23.8
Cumulative variance	72.1	95.9
Eigenvectors		
δ^2H	0.630	−0.335
$\delta^{18}O$	0.636	−0.293
$\delta^{34}S$	0.445	0.895

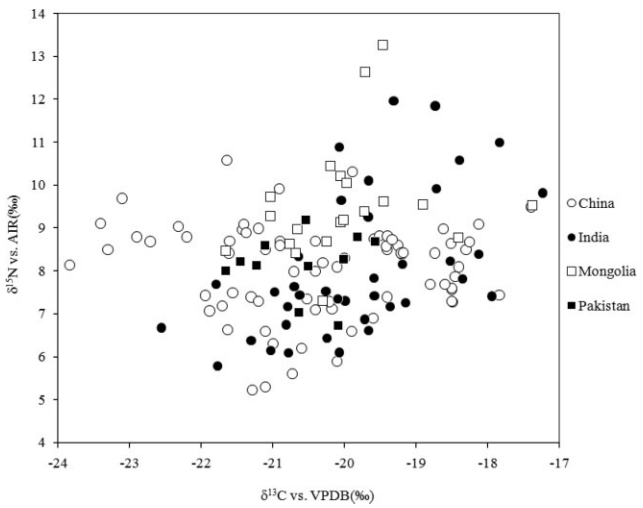

Fig. 5. $\delta^{13}C$ and $\delta^{15}N$ values measured from a sub-set of human hair samples.

well with the geographic pattern observed in the water samples in the Asian survey (see Fig. 2). The highest H and O isotope ratios were measured from Indian hair samples from Udaipur and Bangalore, where highly isotopically enriched water samples were collected. The lowest H and O isotope ratios were generally measured from Mongolian hair samples. The most depleted values were from Toilogt, where evaporatively enriched water samples were noted. This confirmed that in locations with only single samples available, the observed relationships may not be typical.

The relationships between H and O isotope ratios measured from hair were compared to those previously reported from human hair (USA; $\delta^2H = 5.73 \times \delta^{18}O - 166‰$; (Ehleringer et al., 2008) and pre-globalized populations; $\delta^2H = 8.86 \times \delta^{18}O - 203‰$; (Bowen et al., 2009) (Table 3)). We had no a priori reason to expect that the relationships observed in the study population should be similar to the patterns observed elsewhere. Yet, the relationships between H and O isotope ratios in local drinking waters and hair in the Asian dataset were closer to those observed in the USA (Ehleringer et al., 2008) than the historic populations analyzed by Bowen et al. (2009). The dietary and physiological factors that drive these relationships were therefore also likely to be closer in our sampled Asian population and the modern USA residents than in our dataset and the pre-globalized populations.

The relationships between the H and O isotope ratios of hair and water collected from the same locations were analyzed (Figs. 3 and 4, Table 3). These further supported the similarity between our study population and the modern USA residents (Ehleringer et al., 2008) versus the difference to the historic "global transect" analyzed by Bowen et al. (2009). The slope of the δ^2H_h versus δ^2H_w relationship in the study population was slightly higher than the USA population, but much shallower than the steep slope observed in the historic indigenous population. The intercept of the same relationship was almost identical for the Asian survey and the USA population and lower than the value for the population analyzed by Bowen et al. (2009). The differences in slopes in these relationships reflect the differences in the amount of H in hair that is directly related to local drinking water. This value was 42% for the Asian survey. By contrast, 27% of H atoms in hair were directly related to drinking water in the modern USA survey (Ehleringer et al., 2008), in close agreement with the previously published value of 31% calculated using data from a single individual (Sharp et al., 2003). A value of 78% was observed in Bowen et al.'s historical indigenous populations (2009). The slopes and intercepts of the regression relationships between $\delta^{18}O_h$ and $\delta^{18}O_w$ also showed our population to be more similar to the modern USA population than the historic global indigenous populations (Fig. 4, Table 3). The values of the intercepts and slopes of the Asian survey and USA transect relationships were close, the latter reflecting the respective 39% and 35% of O in hair that could be directly related to drinking water and were significantly lower than the 70% observed by Bowen et al. (2009).

To help interpret these observations, the modified version of the Ehleringer et al. (2008) model developed

TABLE 5. $\delta^{13}C$ and $\delta^{15}N$ values measured from human hair and fingernail keratins in modern humans

Location	Description[a]	n[b]	Tissue analyzed	Mean $\delta^{15}N$ vs. AIR (‰)[c]	Mean $\delta^{13}C$ vs. VPDB (‰)	Study
United States	O	9	Hair	9.6 ± 0.5	−16.6 ± 0.6	Schoeller et al., 1986
United States	O	455	Fingernail	9.7 ± 0.7[d]	−19.8 ± 0.9	Nardoto et al., 2006
United States	OLV	35	Fingernail	8.1 ± 0.6[d]	−18.8 ± 0.8	Nardoto et al., 2006
England	O	14	Hair	8.8 ± 0.5	−20.2 ± 0.7	O'Connell and Hedges, 1999
England	OLV	6	Hair	8.7 ± 0.5	−21.0 ± 0.3	O'Connell and Hedges, 1999
England	V	8	Hair	6.9 ± 0.5	−20.9 ± 0.8	O'Connell and Hedges, 1999
England	O	27	Hair	9.8 ± 0.5	−20.8 ± 0.4	Bol and Pfleiger, 2002
England	OLV	6	Hair	8.5 ± 0.6	−21.2 ± 0.3	Bol and Pfleiger, 2002
England	V	3	Hair	6.7 ± 0.7	−21.7 ± 0.1	Bol and Pfleiger, 2002
Germany	O	99	Hair	9.9 ± 0.6	−19.6 ± 0.4	Petzke et al., 2005a
Germany	OLV	15	Hair	7.7 ± 0.5	−20.2 ± 0.3	Petzke et al., 2005a
Germany	V	6	Hair	6.2 ± 0.4	−20.9 ± 0.3	Petzke et al., 2005a
Brazil	O	155	Fingernail	9.3 ± 0.7[d]	−15.4 ± 1.0	Nardoto et al., 2006
Brazil	OLV	118	Fingernail	9.3 ± 0.7[d]	−16.9 ± 1.4	Nardoto et al., 2006
Denmark	O	29	Fingernail	9.7 ± 0.6[d]	−21.0 ± 0.5	Buchardt et al., 2007
Denmark	OLV	8	Fingernail	9.0 ± 0.3[d]	−21.1 ± 0.3	Buchardt et al., 2007
Greenland	O	75	Fingernail	16.0 ± 1.5[d]	−18.2 ± 0.6	Buchardt et al., 2007
China	unknown	73	Hair	8.1 ± 1.1	−20.3 ± 1.5	This study
India	unknown	38	Hair	8.1 ± 1.7	−19.8 ± 1.2	This study
Mongolia	unknown	20	Hair	9.6 ± 1.4	−20.0 ± 1.0	This study
Pakistan	unknown	11	Hair	8.2 ± 0.7	−20.6 ± 0.7	This study

Values are given ± 1σ.
[a] O = omnivore, OLV = Ovo-lacto-vegetarian, V = vegan.
[b] n = number of individuals sampled.
[c] Fingernail $\delta^{15}N$ values were decreased by 0.7‰ following O'Connell et al. (2001) to make values comparable with hair data.

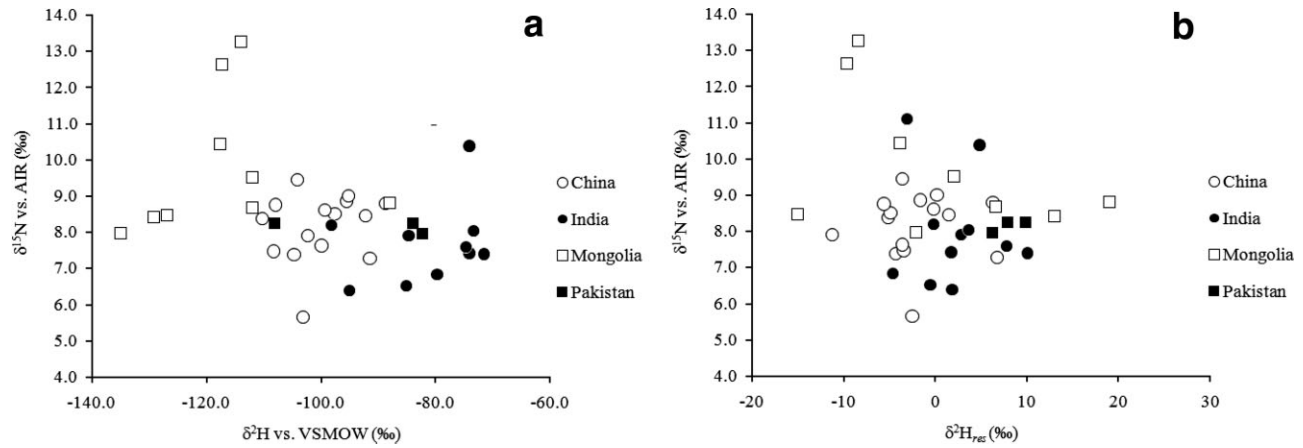

Fig. 6. (**a**) Mean δ^2H and $\delta^{15}N$ values per location; (**b**) Mean $\delta^{15}N$ and δ^2H_{res} values per location.

by Bowen et al. (2009) was applied to the Asian dataset and the values required to closely reproduce the observed results in modeled data were summarized (Table 3). This model has a number of assumptions including: 1) H and O in hair keratin are fixed before keratin synthesis, 2) hair samples are from individuals in a steady state that are resident in the location from which a sample was collected, 3) isotope ratios of drinking water samples are representative of a local water supply, and 4) an average, fixed isotope ratio food based on foods analyzed from the USA can be used to describe non-local foods. Mean tap water isotope ratios were used as the input for drinking water for locations where two or more data points were available. The H and O isotope ratios for diet in the model of −115‰ and +26‰, respectively were the same as previously used by Ehleringer et al. (2008). These represented approximate mean values for a modern American "supermarket" diet, assumed to be globally-derived and isotopically diverse and were also used by Bowen et al. (2009) to represent all "non-local" food sources. Here the agreements between the intercepts of modeled and measured H and O isotope data were improved where H and O isotope ratios of food were changed to −130‰ and +28‰, respectively, suggesting that mean dietary values for this region were slightly different to the "supermarket" values.

Observations of mean national dietary intakes across the region suggest that substantially less animal derived complete protein is consumed across China, India, and Pakistan compared to the USA, with the exception of Mongolia (Speedy, 2003). Based upon these dietary differences, we hypothesized that rates of de novo amino acid synthesis would be higher in the Asian survey population than in the USA population. The best agreement

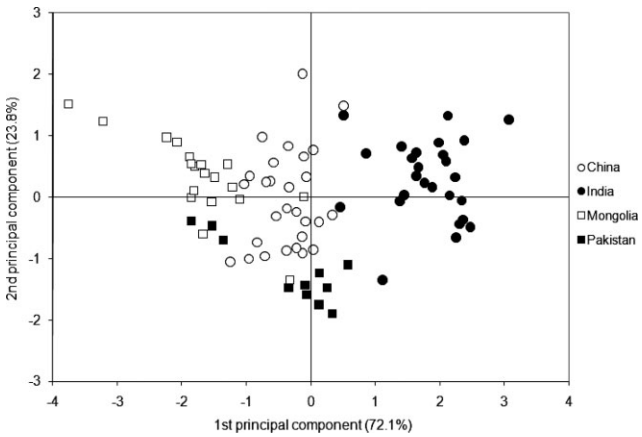

Fig. 7. Scores from the first two principal components of the subset of δ^2H, $\delta^{18}O$, and $\delta^{34}S$ data.

between the observed and modeled H isotope ratio data was achieved where the fraction of nonexchangeable H atoms that was fixed in vivo f_s was 0.35, slightly higher than the USA population value (Ehleringer et al., 2008) and closer to that observed in the historic indigenous population (Bowen et al., 2009) (Table 3). Although these differences were small, they were consistent with the expectation that the reported differences in dietary protein intake between the Asian and USA populations would be reflected in the H isotope data measured from hair.

In the countries where samples were collected, relatively small percentages of food are imported compared to the USA (FAO, 2006a,b,c,d,e). We hypothesized that this would be reflected in the relationships between H and O isotopes in water and hair. As expected, a difference was observed in the value describing the "local" fraction of the diet l (Table 3). The value of 15% locally-derived diet in the Asian population reflects a larger contribution of locally produced food in the average diet compared to the USA but much less than that observed in the historical indigenous populations analyzed by Bowen et al. (2009). The majority of the Asian survey hair samples were collected in urban environments where there is increased access to globally derived foods compared to rural dwellers, and a different pattern could be expected (Pingali, 2004).

Patterns of carbon, nitrogen, and sulfur isotope ratios in hair

The ranges of C and N isotope ratios suggested a diversity of diets across the sample population as a whole (see Fig. 5), although there were no discernable differences between the countries in terms of mean C isotope ratios as has been observed elsewhere (Nardoto et al., 2006). The ranges of N isotope values were similar across all of the populations except Pakistan (where the sample size was smaller), and also did not distinguish populations except for Mongolia, which had a slightly higher mean value. Although we did not have local food items that were isotopically representative of diet, we were able to compare data with other analyzed populations (Table 5) and reports of average food intakes. Nothing was known about the sample donors in terms of age or sex. Previous studies on modern populations that have collected this information have found either no difference in C or N isotope ratios caused by these factors (Nardoto et al., 2006) or very small differences (Petzke et al. 2005b). Therefore in the context of the population ranges we examined we did not consider this to be crucial in interpreting our data. As mentioned in the introduction, health status (e.g. severe protein malnutrition) can have an impact on isotope ratios. This was uncontrolled so the chances of including such a sample were unknown but were considered to be small.

The mean C isotope ratios observed were similar to those observed in Western Europe and suggested a mainly C_3-based diet. This was not surprising given the largely rice and wheat-based nature of agriculture in these regions. However, the range of values indicated some degree of C_4 input to human diet in all countries except Pakistan, where diet is predominantly wheat-based although some maize is consumed (FAO, 2006d). Whereas there was no relationship between C and N isotope ratios in the samples from China, Mongolia, or Pakistan, a significant relationship existed in India. The highest C isotope values observed in India were from coastal Chennai and nearby Vellore and correlated with elevated N and S isotope ratios, suggesting the consumption of marine foods by these individuals. A smaller number of Indian samples had high C isotope ratios that did not correlate with high N isotope ratios. Maize and sorghum make up a small part of per capita diet in India and consumption of C_4 grains may explain these isotope ratios (FAO, 2006b). In China, C isotope ratios in hair showed a significant positive correlation with latitude and were likely related to the traditional consumption of a C_4 millet-based diet in northern China and the C_3 rice-based diet favored in southern China (Leppman, 2005). However, a similar range of C isotope ratios was previously observed within southern China between different ethnic Chinese groups resident in the same region who favored rice- and maize-based diets (Tokui et al., 2000).

The mean N isotope ratios from the Chinese, Indian, and Pakistani hair samples were all below the means reported for omnivorous populations in North America and Europe and were closer to the values reported for ovo-lacto-vegetarians (Table 5). The complexity of environmental and trophic level factors influencing N isotope ratios that are integrated into the data measured from human hair means that interpretation of data can be problematic. However, the modeled H isotope data discussed earlier supported a slightly higher rate of in vivo amino acid synthesis than in the modern USA population, supporting lower animal product consumption. Furthermore, data from the Food and Agriculture Organization of the United Nations (FAO) indicate that average diet in all of these locations contains less animal-derived protein than in the USA and Western Europe (Speedy, 2003). The lowest N isotope ratios observed in China and India were below values published for vegans in western populations and are the lowest published values for human hair keratin that we are aware of. These data may reflect vegan or largely vegetarian individuals or individuals consuming plant materials with unusually low $\delta^{15}N$ isotope values, such as legumes. The high consumption of soy protein that is documented in China is a further possible explanation for such low N isotope ratios in the Chinese samples (Simoons, 1991). Nitrogen-fixing leguminous plants, such as soy, have relatively low N isotope values compared to other non nitrogen-fixing plants, a signal that would be passed onto a consumer (Shearer and Kohl, 1986). The two most ^{15}N-enriched samples observed were from rural

Mongolia, possibly reflecting the high consumption of animal products (Speedy, 2003; FAO, 2006c) coupled with an arid climate. Arid environments are known to contribute to elevated baseline $\delta^{15}N$ values due to inhibition of N-fixing bacteria and increased N recycling within the soil.

No significant correlations between N and H isotope ratios in either individuals (F-test, $P > 0.1$) or group means (F-test, $P > 0.1$) were seen in the population measured in this study (Fig. 6a). Correlations between N and H isotope ratios have previously been observed in animal and human bone collagen and interpreted as a trophic level effect where the influence of climate was minimized by sample selection (Birchall et al., 2005; Reynard and Hedges, 2008). In this study the dominant influence on H isotope ratios across the population was the large gradient in H isotope ratios of precipitation, reflected in the drinking water samples that were analyzed (see Fig. 2) and this is likely to have masked any trophic effect. To mitigate this effect, Bowen et al. (2009) examined "residual H," which describes the degree to which samples are enriched in ^{2}H relative to ^{18}O. The positive correlation between N and residual H observed in pre-globalized populations was not observed here either in individuals (F-test, $P > 0.1$) or group means (F-test, $P > 0.1$, Fig. 6b). The reasons for this are unclear, although the population did not contain individuals with the very large range of N isotope values and diets that were observed in pre-globalized populations.

The difference between mean $\delta^{34}S$ values measured from the sampled populations appeared to have relevance as a geographical signal. We combined these data with H and O isotope ratio values in a principal component analysis. The result was that this further distinguished these geographically distinct populations more than using H and O isotope ratios alone (Fig. 7, Table 4). Although some of the high S isotope ratio values in India appeared to be due to proximity to the coast and potentially the consumption of marine foods in these locations, S isotope values were generally elevated across the entire Indian sample set, possibly due to local pedogenic or anthropogenic inputs. The lowest S isotope ratio values observed were in inland Pakistan. Similar coastal versus inland patterns in S isotopes have been reported in bird feather keratins (Farmer et al., 2003; Lott et al., 2003). In contrast, elevated S isotope ratio values were also observed in China but only inland locations, suggesting that a ^{34}S-enriched source of sulfur was present in human diet, possibly derived from an anthropogenic source such as fertilizer rather than directly from a marine one.

CONCLUSIONS

Stable isotope ratios measured from human hair collected in a survey of Asian countries reflected the climatic and dietary gradients seen across the sampled countries and demonstrated the utility of stable isotope analysis to characterize populations at a relatively broad geographical level. H, O and to some extent S isotope ratio data provided a geographic signal. Analysis of a subset of samples that had paired tap water collected from the same locations suggested that 42% and 39%, respectively of the H and O in hair were directly related to drinking water sources. These data supported the modified version of the Ehleringer et al. model (Ehleringer et al., 2008; Bowen et al., 2009) developed to predict H and O isotope values in hair and suggested that it is broadly applicable to human populations. They also underlined the importance of considering dietary habits in the application of models predicting region of origin from human hair keratins using H and O isotope data. The H and O isotope ratio data measured from the modern Asian survey population reflected a sample set with characteristics lying between the two extremes represented by the previously analyzed USA and global indigenous populations, but closer to the modern USA transect population. The best-fit model parameter values supported the assumption that whilst more locally produced foods are consumed in Asia compared to the USA, where a globalized diet is assumed to be the norm, less of these foods are consumed compared to historic indigenous populations that were previously analyzed. The rates of amino acid synthesis were slightly higher in the Asian populations that were surveyed compared to the USA population, which agreed with published reports of diet and N isotope ratios.

In general, the C and N isotope ratios agreed well with both data collected on national food consumption habits and with previously published data from human keratin from known dietary groups. Whilst the $\delta^{15}N$ values were indicative of a range of trophic positions for diets across the surveyed populations, mean values suggested relatively low animal protein consumption in China, India, and Pakistan. When the Mongolian data were compared to data published from Western Europe and North and South America, an omnivorous diet was suggested, though perhaps not as ^{15}N-enriched as might be expected given the arid climate. The mean C isotope values reflected the predominantly C_3-based regional agriculture. However, some C_4 and marine dietary input was indicated, particularly when S isotope ratio data were included into the analysis. A subtle but significant change in C isotope values with latitude in China suggested that a regional isotopic signal from carbon isotope data was present.

ACKNOWLEDGMENTS

The authors thank many of their colleagues for their assistance in the collections of hair samples. Analyses were conducted at the Stable Isotope Ratio Facility for Environmental Research (SIRFER) at the University of Utah. They also thank C. Cook, M. Lott, A. May, C. Kitty, J. Barleycorn, and W. Ike for analytical assistance.

LITERATURE CITED

Birchall J, O'Connell TC, Heaton THE, Hedges REM. 2005. Hydrogen isotope ratios in animal body protein reflect trophic level. J Anim Ecol 74:877–881.

Bol R, Marsh J, Heaton THE. 2007. Multiple stable isotope (^{18}O, ^{13}C, ^{15}N, and ^{34}S) analysis of human hair to identify the recent migrants in a rural community in SW England. Rapid Commun Mass Spectrom 21:2951–2954.

Bol R, Pfleiger C. 2002. Stable isotope (^{13}C, ^{15}N, and ^{34}S) analysis of the hair of modern humans and their domestic animals. Rapid Commun Mass Spectrom 16:2195–2200.

Bowen GJ, Chesson L, Nielson K, Cerling TE, Ehleringer JR. 2005. Treatment methods for the determination of $\delta^{2}H$ and $\delta^{18}O$ of hair keratin by continuous-flow isotope-ratio mass spectrometry. Rapid Commun Mass Spectrom 19:2371–2378.

Bowen GJ, Ehleringer JR, Chesson LA, Thompson AH, Podlesak DW, Cerling TE. 2009. Dietary and physiological controls on the hydrogen and oxygen isotope ratios of hair from mid-20th century indigenous populations. Am J Phys Anthropol 139:494–504.

Bowen GJ, Revenaugh J. 2003. Interpolating the isotopic composition of modern meteoric precipitation. Water Resources Res 39 doi:10.1029/2003WR002086.

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

Cerling TE, Wittemyer G, Rasmussen HB, Vollrath F, Cerling CE, Robinson TJ, Douglas-Hamilton I. 2006. Stable isotopes in elephant hair document migration patterns and diet changes. Proc Natl Acad Sci USA 103:371–373.

Craig H. 1961. Isotopic variations in meteoric waters. Science 133:1702–1703.

Ehleringer JR, Bowen GJ, Chesson LA, West AG, Podlesak DW, Cerling TE. 2008. Hydrogen and oxygen isotope ratios in human hair are related to geography. Proc Natl Acad Sci USA 105:2788–2793.

FAO. 2006a. China. FAO statistical yearbook. Rome: Food and Agriculture Organization.

FAO. 2006b. India. FAO statistical yearbook. Rome: Food and Agriculture Organization.

FAO. 2006c. Mongolia. FAO statistical yearbook. Rome: Food and Agriculture Organization.

FAO. 2006d. Pakistan. FAO statistical yearbook. Rome: Food and Agriculture Organization.

FAO. 2006e. USA. FAO statistical yearbook. Rome: Food and Agriculture Organization.

Farmer A, Rye R, Landis G, Bern C, Kester C, Ridley I. 2003. Tracing the pathways of neotropical migratory shorebirds using stable isotopes: a pilot study. Isotopes Environ Health Stud 39:169–177.

Fraser I, Meier-Augenstein W. 2007. Stable ^{2}H isotope analysis of modern-day human hair and nails can aid forensic human identification. Rapid Commun Mass Spectrom 21: 3279–3285.

Fraser I, Meier-Augenstein W, Kalin RM. 2006. The role of stable isotopes in human identification: a longitudinal study into the variability of isotopic signals in human hair and nails. Rapid Commun Mass Spectrom 20:1109–1116.

Fuller BT, Fuller JL, Sage NE, Harris DA, O'Connell TC, Hedges REM. 2005. Nitrogen balance and $\delta^{15}N$: why you're not what you eat during nutritional stress. Rapid Commun Mass Spectrom 19:2497–2506.

Gat JR. 1996. Oxygen and hydrogen isotopes in the hydrologic cycle. Ann Rev Earth Planet Sci 24:225–262.

Gröcke GR, Bocherens H, Mariotti A. 1997. Annual rainfall and nitrogen-isotope correlation in macropod collagen: application as a palaeoprecipitation indicator. Earth Planet Sci Lett 153:279–285.

Hatch KA, Crawford MA, Kunz AW, Thomsen SR, Eggett DL, Nelson ST, Roeder BL. 2006. An objective means of diagnosing anorexia nervosa and bulimia using $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios in hair. Rapid Commun Mass Spectrom 20:3367–3373.

Heaton THE. 1987. The $^{15}N/^{14}N$ ratios of plants in South Africa and Namibia: relationship to climate and coastal/saline environments. Oecologia 74:236–246.

Hobson KA, Atwell L, Wassenaar LI. 1999a. Influence of drinking water and diet on the stable-hydrogen isotope ratios of animal tissues. Proc Natl Acad Sci USA 96:8003–8006.

Hobson KA, Bowen GJ, Wassenaar LI, Ferrand Y, Lormee H. 2004. Using stable hydrogen and oxygen isotope measurements of feathers to infer geographical origins of migrating European birds. Oecologia 141:477–488.

Hobson KA, Wassenaar LI, Taylor OR. 1999b. Stable isotopes (δD and $\delta^{13}C$) are geographic indicators of natal origins of monarch butterflies in eastern North America. Oecologia 120:397–404.

Katzenberg MA, Krouse HR. 1989. Application of stable isotope variation in human tissues to problems in identification. Can J Forensic Sci 22:7–19.

Krouse HR. 1980. Sulphur isotopes in our environment. In: Fritz P, Fontes JC, editors. Handbook of environmental isotope geochemistry. Amsterdam: Elsevier. p 435–471.

Krouse HR, Levinson AA. 1984. Geographical trends of carbon and sulphur isotope abundances in human kidney stones. Geochim Cosmochim Acta 48:187–191.

Krouse HR, Levinson AA, Piggott D, Ueda A. 1987. Further stable isotope investigations of human urinary stones: comparison with other body components. Appl Geochem 2:205–211.

Krouse HR, Stewart JWB, Grinenko VA. 1991. Pedosphere and biosphere. In: Krouse HR, Grinenko VA, editors. Stable isotopes in the assessment of natural and anthropogenic sulphur in the environment. Chichester: Wiley. p 267–304.

Leppman EJ. 2005. Changing rice bowl. Economic development and diet in China. Hong Kong: Hong Kong University Press.

Lott CA, Meehan TD, Heath JA. 2003. Estimating the latitudinal origins of migratory birds using hydrogen and sulfur stable isotopes in feathers: influence of marine prey base. Oecologia 134:505–510.

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

McCutchan JH Jr, Lewis WM, Kendall C, McGrath CC. 2003. Variation in trophic level shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102:378–390.

Mekota AM, Grupe G, Ufer S, Cuntz U. 2006. Serial analysis of stable nitrogen and carbon isotopes in hair: monitoring starvation and recovery phases of patients suffering from anorexia nervosa. Rapid Commun Mass Spectrom 20:1604–1610.

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

Mizota C, Saaki A. 1996. Sulfur isotope composition of soils and fertilizers: differences between Northern and Southern Hemispheres. Geoderma 71:77–93.

Nakamura K, Schoeller DA, Winkler FJ, Schmidt HL. 1982. Geographical variations in the carbon isotope composition of the diet and hair in contemporary man. Biomed Mass Spectrom 9:390–394.

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

O'Brien DM, Wooller MJ. 2007. Tracking human travel using stable oxygen and hydrogen isotope analyses of hair and urine. Rapid Commun Mass Spectrom 21:2422–2430.

O'Connell TC, Hedges REM. 1999. Investigations into the effect of diet on modern human hair isotopic values. Am J Phys Anthropol 108:409–425.

O'Connell TC, Hedges REM, Healey MA, Simpson AHRW. 2001. Isotopic comparison of hair. Nail and bone: modern analyses. J Archaeol Sci 28:1247–1255.

Petzke K-J, Boeing H, Klaus S, Metges CC. 2005b. Carbon and nitrogen stable isotopic composition of hair protein and amino acids can be used as biomarkers for animal-derived dietary protein intake in humans. J Nutr 135:1515–1520.

Petzke K-J, Boeing H, Metges CC. 2005a. Choice of dietary proteins in vegetarians and omnivores is reflected in their hair protein ^{13}C and ^{15}N abundance. Rapid Commun Mass Spectrom 19:1392–1400.

Pingali P. 2004. Westernization of Asian diets and the transformation of food systems: implications for research and policy. Rome: Food and Agriculture Organization.

Post DM. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718.

Reynard LM, Hedges REM. 2008. Stable hydrogen isotopes of bone collagen in palaeodietary and palaeoenvironmental reconstruction. J Archaeol Sci 35:1934–1942.

Richards MP, Fuller BT, Hedges REM. 2001. Sulphur isotopic variation in ancient bone collagen from Europe: implications for human palaeodiet, residence mobility and modern pollutant studies. Earth Planet Sci Lett 191:185–190.

Robbins CT, Felicetti LA, Sponheimer M. 2005. The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. Oecologia 144:534–540.

Roy DM, Hall R, Mix AC, Bonnichsen R. 2005. Using stable isotope analysis to obtain dietary profiles from old hair: a case study from plains Indians. Am J Phys Anthropol 128:442–452.

Schoeller DA, Minagawa M, Slater R, Kaplan IR. 1986. Stable isotopes of carbon, nitrogen and hydrogen in the contemporary North American food web. Ecol Food Nutr 18:159–170.

Sharp ZD, Atudorei V, Panarello HO, Fernandez J, Douthitt C. 2003. Hydrogen isotope systematics of hair: archeological and forensic applications. J Archaeol Sci 30:1709–1716.

Shearer G, Kohl DH. 1986. N_2-fixation in field settings: estimations based on natural ^{15}N abundance. Aust J Plant Physiol 13:699–756.

Sheehy DP. 1996. Sustainable livestock use of pastoral resources. In: Bruun O, Odgaard O, editors. Mongolia in transition. Richmond: Curzon Press. p 42–64.

Simoons FJ. 1991. Food in China: a cultural and historical inquiry. Boca Raton: CRC Press.

Speedy AW. 2003. Global production and consumption of animal source foods. J Clin Nutr 133:4048S–4053S.

Sponheimer M, Robinson T, Ayliffe L, Roeder B, Hammer J, Passey B, West A, Cerling T, Dearing D, Ehleringer J. 2003. Nitrogen isotopes in mammalian herbivores: hair $\delta^{15}N$ values from a controlled feeding study. Int J Osteoarchaeol 13:80–87.

Thode HG. 1991. Sulphur isotopes in nature and the environment: an overview. In: Krouse HR, Grinenko VA, editors. Stable isotopes in the assessment of natural and anthropogenic sulphur in the environment. Chichester: Wiley. p 1–26.

Tokui N, Minari Y, Kusunoki K, Yoshimura T, Yamamoto T, Minagawa M. 2000. Evaluation of dietary intake using carbon and nitrogen isotope analysis of human hair of Chinese living in the southern part of China. J Univ Occup Environ Health Sci 22:219–228.

USDA. 2002. Passage to India. Country profile foreign agricultural service horticultural and tropical products division. Washington, DC: United States Department of Agriculture.

Vitoria L, Otero N, Soler A, Canals A. 2004. Fertilizer characterization: isotopic data (N. S, O, C, and Sr). Environ Sci Technol 38:3254–3262.

White CD. 1993. Isotopic determination of seasonality in diet and death from Nubian mummy hair. J Archaeol Sci 20:657–666.

White CD, Longstaffe FJ, Law KR. 1999. Seasonal stability and variation in diet as reflected in human mummy tissues from the Kharga Oasis and the Nile Valley. Palaeogeog Palaeoclimatol Palaeoecol 147:209–222.

Wilson AS, Taylor T, Ceruti MC, Chavez JA, Reinharde J, Grimes V, Meier-Augenstein W, Cartmell L, Stern B, Richards MP, Worobey M, Barnes I, Gilbert MTP. 2007. Stable isotope and DNA evidence for ritual sequences in Inca child sacrifice. Proc Natl Acad Sci USA 104:16456–16461.

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