Dietary and Physiological Controls on the Hydrogen and Oxygen Isotope Ratios of Hair from Mid-20th Century Indigenous Populations

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ABSTRACT A semimechanistic model has recently been proposed to explain observed correlations between the H and O isotopic composition of hair from modern residents of the USA and the isotopic composition of drinking water, but the applicability of this model to hair from non-USA and preglobalization populations is unknown. Here we test the model against data from hair samples collected during the 1930s–1950s from populations of five continents. Although C and N isotopes confirm that the samples represent a much larger range of dietary "space" than the modern USA residents, the model is able to reproduce the observed δ^2H and δ^{18}O values given reasonable adjustments to 2 model parameters: the fraction of dietary intake derived from locally produced foods and the fraction of keratin H fixed during the in vivo synthesis of amino acids. The model is most sensitive to the local dietary intake, which appears to constitute between 60% and 80% of diet among the groups sampled. The isotopic data are consistent with a trophic-level effect on protein H isotopes, which we suggest primarily reflects mixing of ^{2}H-enriched water and ^{2}H-depleted food H in the body rather than fractionation during biosynthesis. Samples from Inuit groups suggest that humans with marine-dominated diets can be identified on the basis of coupled δ^2H and δ^{18}O values of hair. These results indicate a dual role for H and O isotopic measurements of keratin, including both biological (diet, physiology) and environmental (geographic movement, paleoclimate) reconstruction. Am J Phys Anthropol 139:494–504, 2009.

Although applications of stable isotopes of carbon and nitrogen in organic tissues are well established in paleodietary and anthropological studies, progress toward the application of organic hydrogen (δ^2H) and oxygen (δ^{18}O) isotopes has been limited. Early work on the δ^2H values of mammalian tissues focused on their use in food web studies (Estep and Dabrowski, 1980), but the interpretation of hydrogen isotope data from complex organic tissues quickly became clouded as it was recognized that a significant fraction of the H atoms in these tissues were subject to continuous exchange with ambient water and water vapor (DeNiro and Epstein, 1981; Schimmelmann et al., 1993). The reluctance to pursue organic oxygen in part reflects analytical challenges (Santrock and Hayes, 1987) and the view that interpretation of δ^{18}O would be more complex than for δ^2H (Hobson et al., 2004).

With improvements in instrumentation (Hilkert et al., 1999; Sharp et al., 2001) measurements of H and O isotopes in organic tissues have now become near-routine. Established methods also now exist for the routine correction of δ^2H data for H exchange in the laboratory (Wassenaar and Hobson, 2002; Bowen et al., 2005), largely eliminating methodological barriers to the development of large organic δ^2H and δ^{18}O datasets. These techniques have been applied to the δ^2H analysis of modern faunal (Birchall et al., 2005) and archaeological human and faunal bone collagen (Leyden et al., 2006; Reynard and Hedges, 2008). Concurrently, studies demonstrating that δ^2H and δ^{18}O values of tissue samples can be used as a tracer for the geographic movements of animals (Chamberlain et al., 1997; Hobson and Wassenaar, 1997) have motivated interest within the ecological, anthropological, and forensic science communities. The power of this method derives from strong, geographic variation in δ^2H and δ^{18}O values of water that is propagated thorough food webs to animals and humans. However, the majority of the isotopic signal in animal tissues, at least for H in proteinaceous tissues of humans, is derived from diet and not inherited directly from water (Sharp et al., 2003). Isotopic variation related to dietary composition represents a potentially confounding factor in the use of H and O isotopes for geographic tracking, but also a potential signal of interest for dietary research.

In an attempt to account for the influences of both diet and water on the δ^2H and δ^{18}O values of human hair,

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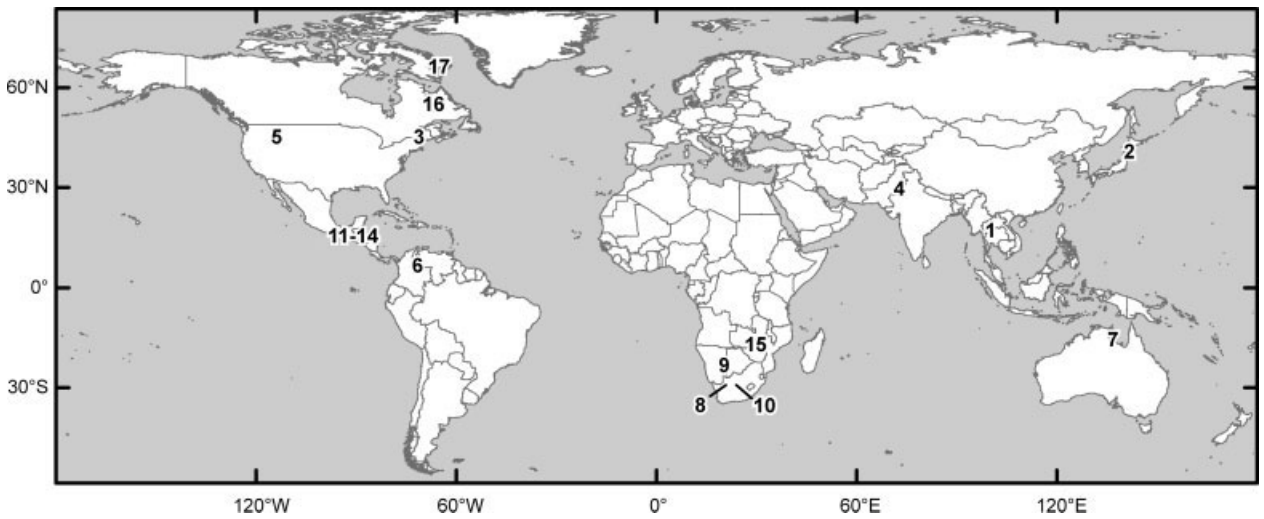

Fig. 1. Map of sites represented in the anthropological hair sample suite. In some cases the marked locations reflect the geographic center of several closely spaced collecting sites. Site numbers correspond to those in Table 1.

our group has developed a process-based model of H and O isotope incorporation in mammal keratins and tested it against data from the hair of modern residents of the USA (Ehleringer et al., 2008). The usefulness of that test derives from the fact that the population under study can be considered to have a relatively homogeneous diet, both in terms of isotopic and macronutrient composition, because of the centralized distribution and ready availability of foods provided in USA supermarkets. Here, we present a sharply contrasting test of the model based on H and O isotopic data from hair collections, amassed by the late Mildred Trotter, representing populations resident in largely undeveloped areas of the world during the 1930s through 1950s. Although no direct dietary documentation is available for the individuals represented in the collection, their diet can be assumed to have been much less homogeneous than those of modern USA residents and likely included a significant fraction of locally grown or gathered foods. We first confirm this assumption based on measurements of C and N isotopes in the hair samples, and attempt to identify diet-related influences on the δ^2H and δ^{18}O values of hair through analysis of the multi-isotope dataset. We then develop a modified version of the hair δ^2H and δ^{18}O model, which incorporates isotopic inputs from locally derived (as opposed to "supermarket") dietary items, and conduct sensitivity tests of the model using data from the anthropological samples. These tests confirm the generality of the model, elucidate the strength of the relationship between drinking water and hair isotopic composition, and provide insight into potential future uses of H and O in anthropological and archeological research.

MATERIALS AND METHODS

We obtained 123 hair samples from the Mildred Trotter collection held by the Department of Anthropology, Smithsonian Institution (SI). These samples were collected between ~1935 and 1966 for research on hair morphology (Trotter and Dawson, 1934; Trotter, 1936, 1956; Duggins and Trotter, 1959) and donated to the SI in 1967, where they have been stored in paper envelopes and housed in a climate-controlled, low-humidity room. The provenance information for these samples is incomplete and varied, but in most cases includes the cultural identity, sex, and age of the hair donor as well as the location and date of collection. Aliquots weighing several milligrams were taken from each sample and transferred to a clean coin envelope for storage. For the purpose of our data analysis, samples were grouped into 17 discrete local groups (Fig. 1, Table 1) based on the geographic locations and cultural identity of the hair donors, and for some analyses data were averaged within each group.

All samples were physically cleaned of debris in the laboratory and washed three times in a 2:1 mixture of chloroform:methanol to remove lipids. Samples were ground to a fine powder using a ball mill and duplicate 150 μg aliquots were weighed into precombusted silver foil capsules for H and O isotope ratio analysis. Because a small fraction of the structural H atoms in proteins undergo active exchange with H in atmospheric water vapor, all unknown samples were allowed to equilibrate alongside two powdered hair reference materials (for which the δ^2H value of nonexchangeable H had been established) for 7 days and then vacuum dehydrated prior to analysis (Bowen et al., 2005). Samples and reference materials were transferred to a Zero Blank Autosampler (Costech Analytical) interfaced with a Thermochemical Elemental Analyzer (Thermo Fisher Scientific). They were pyrolized at 1400°C in an oxygen-free environment to produce H_2 and CO gases, which were chromatographically separated and introduced sequentially to the source of a Delta Plus XP isotope ratio monitoring mass spectrometer (IRMS; Thermo Fisher Scientific). The data for the reference samples were used to calibrate raw measurements of the δ^{18}O and nonexchangeable δ^2H values for unknown samples relative to the VSMOW-VSLAP standard scale using the two point regression-based approach of Wassenaar and Hobson (2002). Analytical precision, based on the repeated analysis of a check reference keratin, was less than 2‰ and 0.3‰ (1 σ) for δ^2H and δ^{18}O, respectively.

TABLE 1. Isotope ratio data for local groups

Site	Country	Ethnic Group	δ^2H (Hair)		δ^{18}O (Hair)		δ^{13}C (Hair)		δ^{15}N (Hair)		δ^2H (Water)[a]		δ^{18}O (Water)[a]	
			Ave	SD	Ave	SD	Ave	SD	Ave	SD	Ave	CI	Ave	CI
1	Thailand	Thai	−84	3.8	13.2	0.52	−19.3	0.48	11.8	0.58	−43.0	6.0	−6.5	0.6
2	Japan	Ainu	−97	6.0	13.1	0.44	−19.1	0.89	9.6	0.55	−61.0	4.0	−8.6	0.4
3	Canada	French Canadian	−111	4.4	12.7	0.35	−18.7	0.29	10.2	0.50	−76.0	6.0	−11.2	0.7
4	Pakistan	n.a.	−80	3.7	14.1	0.74	−18.s6	0.76	10.2	0.78	−27.0	4.0	−4.8	0.3
5	USA	Blackfoot	−132	2.9	8.5	0.63	−18.0	0.37	10.8	0.35	−107.0	8.0	−14.2	1.0
6	Venezuela	Yupa	−83	6.2	15.7	0.44	−15.5	1.48	8.6	1.68	−51.0	4.0	−7.6	0.5
7	Australia	Aborigine	−58	8.4	15.9	0.49	−15.1	0.17	9.3	0.56	−30.0	4.0	−5.2	0.5
8	South Africa	Keimos Bushmen	−56	1.4	17.8	0.38	−15.1	1.06	12.9	0.29	−16.0	5.0	−3.8	0.4
9	Botswana	Kung Bushmen	−77	4.2	15.8	1.06	−14.2	1.88	9.2	1.02	−22.0	5.0	−4.3	0.4
10	South Africa	Zulu	−64	4.0	16.2	0.72	−12.2	3.27	11.4	0.49	−14.0	5.0	−3.4	0.4
11	Guatemala	n.a.	−103	3.8	13.0	0.64	−10.0	1.75	7.2	0.46	−63.0	7.0	−9.3	0.8
12	Mexico	n.a.	−72	3.2	15.6	0.43	−10.0	0.46	8.7	0.20	−26.0	4.0	−4.1	0.4
13	Guatemala	n.a.	−94	1.9	13.7	0.28	−10.0	0.52	6.6	0.89	−63.0	6.0	−9.2	0.7
14	Guatemala	n.a.	−97	3.3	13.2	0.29	−9.7	0.78	6.7	0.63	−69.0	8.0	−10.0	0.9
15	Zambia	Bantu	−78	5.8	14.4	0.96	−9.2	0.51	9.4	0.64	−29.0	4.0	−5.2	0.4
16	Canada	Inuit	−54	5.6	10.5	0.53	−14.7	0.29	17.8	0.31	−106.0	11.0	−14.8	1.4
17	Canada	Inuit	−83	27.1	9.8	1.22	−15.5	1.50	14.9	3.01	−146.0	10.0	−19.0	1.6

[a] Environmental water isotope ratios estimated using the online isotopes in precipitation calculator v. 2.1 (http://waterisotopes. org). Ave, Average; SD, Standard deviation; CI, 95% Confidence interval; n.a., Not available.

For analysis of C and N isotope ratios 250 μg aliquots of the washed, powdered samples were weighed into tin capsules and combusted in an elemental analyzer (Carlo Erba) to produce N_2 and CO_2 gas. The analyte gases were separated by chromatography and introduced sequentially to the source of a Delta S IRMS (Thermo Fisher Scientific). Samples were analyzed together with laboratory reference materials (cellulose for δ^{13}C, keratin for δ^{15}N) having known values relative to the VPDB standard for δ^{13}C or AIR for δ^{15}N, and raw data were linearly corrected relative to the known reference material values. Analytical precision, based on the repeated analysis of a check reference keratin, was ~0.1‰ and 0.2‰ (1 σ) for δ^{13}C and δ^{15}N, respectively.

Data analysis, modeling, and statistical calculations were conducted in Microsoft Excel for Windows using the Data Analysis toolpack, with the exception of reduced major axis regressions which were conducted using the RMA software package for Java (Bohonak and van der Linde, 2004).

RESULTS

The stable isotopic composition of the anthropological hair samples span a range of 93‰ for δ^2H, 10.6‰ for δ^{18}O, 12.0‰ for δ^{13}C, and 12.5‰ for δ^{15}N (Fig. 2A,B). Compared with data from modern human populations from the USA, the observed ranges for H and O isotope ratios of the anthropological samples are similar to but slightly larger than those for the modern samples (34% larger for δ^2H and 8% for δ^{18}O; Ehleringer et al., 2008). In contrast, the range of δ^{13}C and δ^{15}N values for the anthropological samples is 172% larger for δ^{13}C and 297% larger for δ^{15}N than that seen in the modern samples from the USA (Fig. 2B).

The range of isotope values observed within individual local groups is much lower than that of the entire sample suite (Fig. 2C,D; Table 1). Excluding data from Baffin Island samples, which are much more variable than those from other local groups, the average within-group standard deviation of hair isotopic compositions is 4.3‰ for δ^2H, 0.6‰ for δ^{18}O, 0.9‰ for δ^{13}C, and 0.6‰ for δ^{15}N. Compared with the equivalent statistics calculated for the entire modern USA sample suite (13.2‰, 2.2‰, 0.8‰, and 0.5‰, respectively), the isotopic variability within single anthropological local groups is somewhat less for the isotopes of H and O but very similar for C and N.

With the exception of samples from Inuit populations on Baffin Island and Labrador Bay, Canada, strong covariation exists between the H and O isotope ratios of samples or group means (Fig. 2A,C; R^2 = 0.75 and 0.88, respectively). The strength of these correlations is similar to that previously observed for modern USA residents, but the slopes of the ordinary least squares regression relationships between hair δ^2H and δ^{18}O values (8.25 for individuals, 8.86 for group means) is much steeper than for the modern data (5.73; Ehleringer et al., 2008). No significant correlation exists between the δ^{13}C and δ^{15}N values of the hair samples or between either of these isotopic values and δ^2H or δ^{18}O.

ANALYSIS AND DISCUSSION

The wide range of δ^{13}C and δ^{15}N values for hair from the anthropological sample set clearly demonstrates that the individuals sampled encompass a huge range of dietary diversity relative to modern residents of industrialized nations such as the USA. The δ^{15}N values, which integrate information on trophic level and δ^{15}N values at the base of the food chain, are particularly variable. The highest δ^{15}N values were measured in samples from Inuit communities in northern Canada. The only modern humans for which such high ^{15}N-enrichment has been reported are Uummannaq Inuits on Greenland (Buchardt et al., 2007) who obtain most of their dietary protein from high trophic-level animals feeding in high-^{15}N marine food webs (e.g., seal). Carbon isotope ratios suggest that diets of the sampled individuals ranged from those based in pure C3 to pure C4 ecosystems. The highest δ^{13}C values in our sample set lie outside of the range seen in the USA data set and are simi-

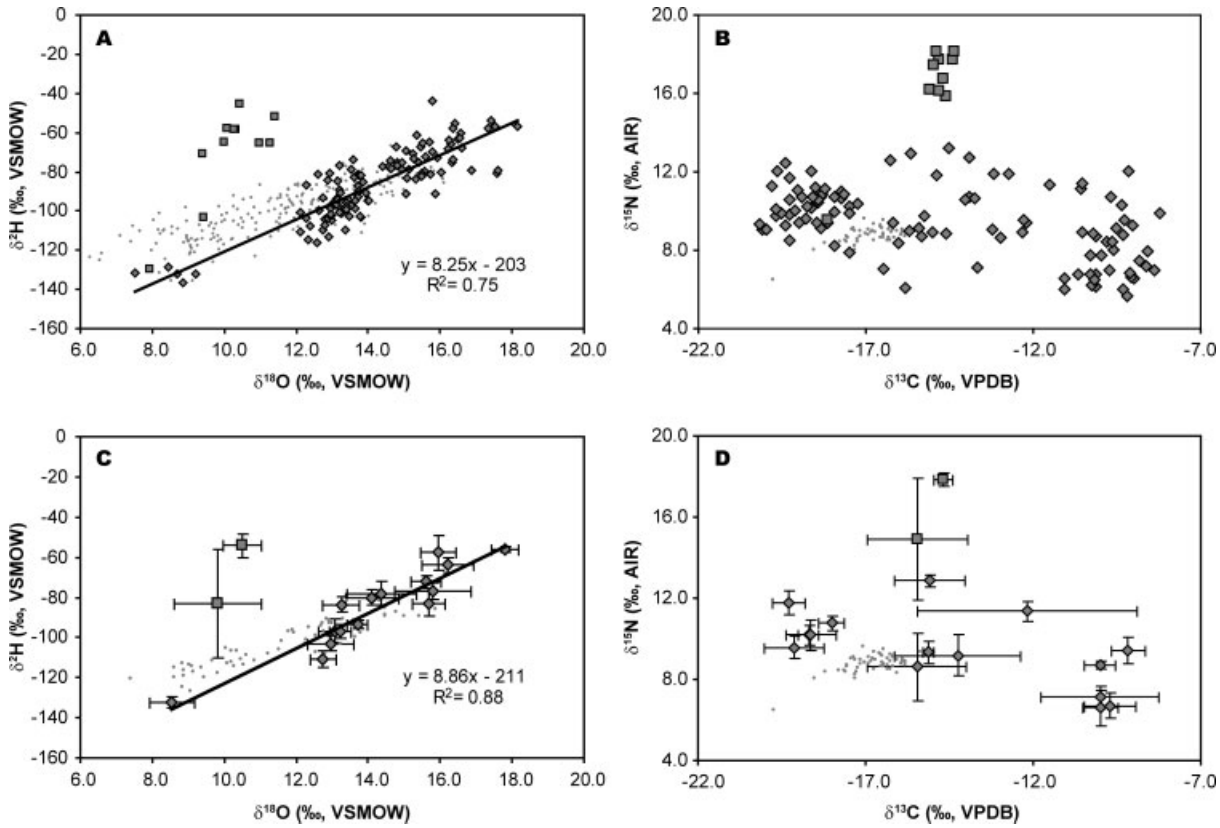

Fig. 2. Stable isotope data for anthropological hair samples. Hydrogen and oxygen isotope ratios (**A**, **C**) and carbon and nitrogen isotope ratios (**B**, **D**) are shown for individual hair samples (A, B) and for geographically defined local groups (C, D; average ±1 standard deviation). In all plots the large diamonds signify anthropological hair samples from mid- and low-latitude sites, the large squares indicate anthropological samples from Baffin Island and Labrador Bay, and the small gray dots show data from modern residents of the USA (Ehleringer et al., 2008; unpublished). Samples from Baffin Island and Labrador Bay were excluded during calculation of the linear regression lines describing the relationship between H and O isotopic values (A, C).

lar to those reported for bone collagen of indigenous Americans prior to European contact (Williams et al., 2005; Finucane et al., 2006; Schwarcz, 2006).

The lack of correlation between either of the "dietary" isotope systems (C, N) and δ^2H or δ^{18}O values of hair suggests that the dietary variation that controls δ^{13}C and δ^{15}N values is not a primary determinant of hair δ^2H or δ^{18}O values. Hair δ^2H and δ^{18}O values were strongly correlated across the sample suite (Fig. 2A,C), however, implying that controls on these isotopic systems were coupled and relatively consistent across the broad range of dietary variability sampled here (the Inuit samples being the one clear exception). We hypothesized that factors influencing the H and O isotope systems independently might be reflected in terms of deviations from this covariant relationship, which we term "residual ^{2}H" values [$\delta^2H_{res} = \delta^2H - (\delta^{18}O \cdot m + b)$, where δ^2H and $\delta^{18}O$ represent a individual hair sample or group mean and m and b are the corresponding regression parameters from Fig. 2A or C] (see Fig. 3). With Inuit samples excluded, δ^2H_{res} values are significantly, positively correlated with δ^{15}N values for individual hair samples (F-test, $P < 0.001$) but not for group means.

The correlation appears to be dominated by a positive covariant relationship for samples with δ^{15}N values greater than ~8 or 9‰. Although some of these high-δ^{15}N samples may reflect the influence of environments having high ^{15}N levels at the base of the food chain (e.g., in parts of South Africa; Codron et al., 2005), many of the samples originate from regions where this is unlikely (e.g., Montana, Canada) and in these cases elevated δ^{15}N values likely indicate significant consumption of animal protein. The correlation between residual ^{2}H and δ^{15}N may suggest some influence of dietary trophic level on the H/O isotope systematics of hair, perhaps reflecting previously observed trophic enrichment of ^{2}H (Birchall et al., 2005). When the data are treated as group averages this correlation is not significant, although there is some suggestion of a similar trend in the data. The Inuit samples have the highest δ^{15}N and residual ^{2}H values, but given their potentially unique diets their residual ^{2}H values may reflect different mechanisms than those of the non-Inuit samples (see discussion later).

Previous work has suggested that the isotopic composition of ingested water and food exerts a primary control on hair δ^2H and δ^{18}O values (Sharp et al., 2003; Ehleringer et al., 2008). Given that drinking water and food samples for the studied groups were not available, we estimated local water isotope compositions using the Online Isotopes in Precipitation Calculator (OIPC) version 2.1 (http://www.waterisotopes.org). Briefly, this tool allows estimation of long-term annually averaged precipitation isotope ratios at specified locations through spatial modeling of a large database of precipitation isotopic data covering the time period 1960–2004 (Bowen and

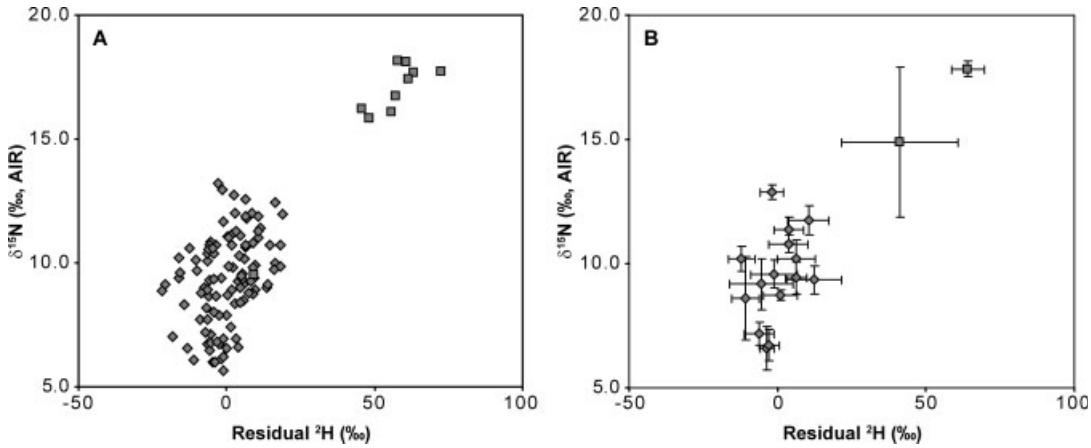

Fig. 3. Relationship between hair sample nitrogen isotope ratios and "residual ^{2}H", which describes the deviation of H and O isotopic values for an individual sample (**A**) or local group (**B**) from the corresponding best-fit regression lines shown in Figure 2. Symbols are as in Figure 2.

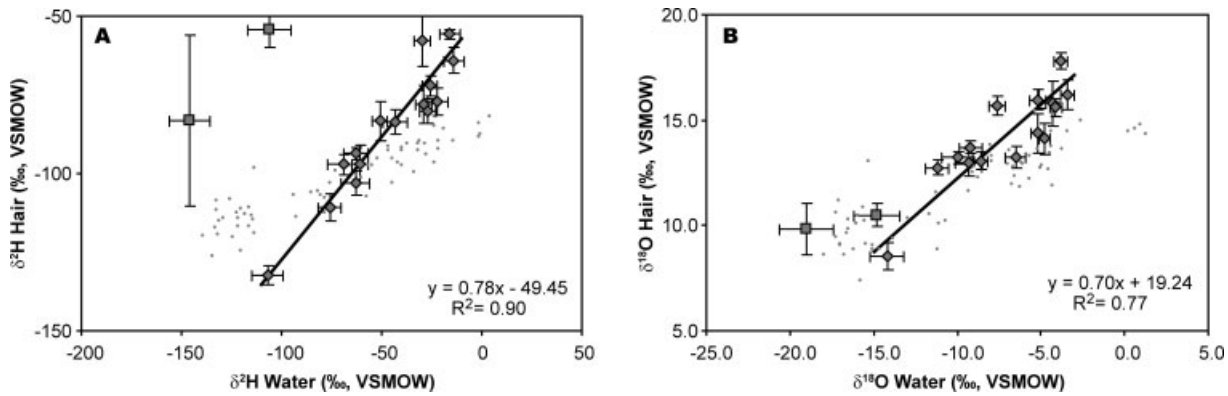

Fig. 4. Correlation between group-average H (**A**) or O (**B**) isotopic values of hair and estimated δ^2H or δ^{18}O values of local drinking water. Symbols are as in Figure 2. Data from Baffin Island and Labrador Bay were excluded in the calculation of the linear regression lines describing the relationship between hair and water isotope ratios.

Wilkinson, 2002; Bowen and Revenaugh, 2003). The primary source of isotopic variability in surface, ground, and soil waters, which are the likely sources of water ingested by the individuals sampled here, is variation in the δ^2H and δ^{18}O values of precipitation feeding these reservoirs. Although the OIPC estimate are an imperfect representation of drinking water isotopic values, they reflect large, robust, spatial gradients in water isotopic composition and should provide good first order estimates of isotopic variation among local water sources. Because the levels of variance or uncertainty for isotopic values of hair and environmental water are similar (e.g., Table 1), the relationship between these parameters was fitted using reduced major axis regression (which assumes similar levels of uncertainty in both variables) rather than ordinary least squares (which assumes that the independent variable is known without uncertainty).

Variation in the H- and O-isotope ratios of local environmental water explains 90% and 77% of the isotopic variation in δ^2H and δ^{18}O values (respectively) among groups (Fig. 4; excluding Inuit samples). This level of correlation is similar to that measured for modern, globalized populations (Ehleringer et al., 2008), implying that: (1) the OIPC-estimated precipitation isotopic compositions are reasonable proxies for the isotope ratios of ingested water, and (2) dietary heterogeneity in the preglobalization populations does not compromise the relationship between hair H- and O-isotope ratios and those of ingested water.

The isotopic values of hair samples from the Inuit groups, however, fall well off of the hair/water relationship for the rest of the samples, particularly for δ^2H and less so for δ^{18}O. Interestingly, the slopes of the hair/water relationships for the anthropological samples, which describe the damping of the geographically driven variation in H- and O-isotope ratios during the assimilation of these elements in hair keratin, are much higher than those observed for modern USA residents, but lower than the slope of ~1 commonly observed for δ^2H in migratory birds (Hobson and Wassenaar, 1997). We explore the implications of these observations using a model describing the isotope effects associated with the incorporation of H and O in hair proteins.

MODEL DESCRIPTION

The model for hair H and O isotopic composition used here (see Fig. 5) is a modified version of box model presented by Ehleringer et al., (2008). Based on consideration of the biochemistry of the amino acids in human

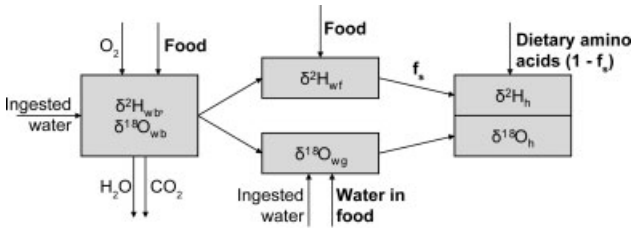

Fig. 5. Generalized structure of the mass-balance model for H and O isotopes in human hair proposed by Ehleringer et al., (2008). The model traces the elemental fluxes of H and O through body water (subscript *wb*), gut water (*wg*), and hair follicle water (*wf*) into bulk hair keratin (*h*). The fluxes in bold [isotopic composition of food and food water and the fraction of keratin H fixed during the in vivo synthesis of nonessential amino acids (f_s)] are modified in the modeling presented here. Other model parameters are as given in Ehleringer et al. (2008).

hair, the model assumes that the non-exchangeable (sensu Bowen et al., 2005) H atoms in hair amino acids are a mixture of H inherited from dietary amino acids and H fixed from intercellular water onto newly synthesized amino acids. Depending on the degree to which nonessential amino acids are assimilated directly from diet vs. synthesized in vivo, the fraction of H fixed in vivo (here designated f_s) can range from ~15% (no fixation; even in the absence of amino acid synthesis a small fraction of H exchanges freely with body water in vivo but is later stabilized during protein assembly) to 46% (no assimilation). H atoms inherited directly from diet are assumed to have the same δ^2H value as bulk dietary protein. To estimate the δ^2H value of H atoms fixed in vivo the model calculates an isotopic mass balance of H inputs and outputs to/from the body, including H intake as drinking water and food, and transcutaneous water loss. For calculation of bulk body water, the magnitude of each flux is estimated based on compilations of data for modern humans (Gretebeck et al., 1997), which suggest that ~81% of H intake is in the form of drinking water and 19% through the metabolism of food. To calculate the isotopic composition of intercellular water at the site of amino acid synthesis, we make the simplifying assumption that the pool of H within the cell is a mixture of 81% H from extracellular water and 19% H from metabolism within the cell (Ehleringer et al., 2008).

The O atoms in hair are assumed to be fixed primarily from intestinal water during hydrolysis of dietary protein in the gut, with no inheritance directly from diet. To estimate the δ^{18}O of the O fixed during hydrolysis, the model calculates the isotopic composition of gut water as a mixture of body water (in the form of digestive fluids; 57%) and water in food (43%). Estimates of the mixing ratios of these sources were tuned to fit data from modern USA residents (Ehleringer et al., 2008) but are within the range of constraints from studies of modern humans (Malagelada et al., 1976). Body water δ^{18}O values are calculated as a mass balance of inputs and outputs including O intake as drinking water, food, and metabolic uptake of atmospheric O_2 and O losses as CO_2 during respiration and transcutaneous water loss. Intake fluxs, based on Gretebeck et al., (1997), are 62% from water, 14% from food, and 24% from O_2.

In the model as originally applied, the isotopic compositions of dietary inputs, including organic H and O in food (δ^2H_d and $\delta^{18}O_d$) and oxygen in food water ($\delta^{18}O_{wd}$), were held fixed for all subjects. This was assumed to represent the "supermarket" diet of most Americans, where the geographic origin of foods was assumed to be essentially independent of the location of the consumer. To apply the model to the anthropological samples, we added a "local" food parameter l, which describes the fraction of dietary H and O intake that is derived from local foods, such that:

$$\delta_{\mathrm{i,t}} = \delta_{\mathrm{i,l}} \cdot l + \delta_{\mathrm{i,g}} \cdot (1 - l), \quad (1)$$

where δ_i is the isotopic composition of a dietary input and the subscripts t, l, and g refer to total intake, locally derived items, and nonlocal items, respectively.

For the purposes of modeling, two simplifying assumptions are made: (1) locally derived dietary items have an isotopic composition that is related to that of local environmental (drinking) water by a fixed offset, and (2) all nonlocal (e.g., trade-derived) items have a fixed isotopic composition equal to that inferred by Ehleringer et al., (2008) for the modern US supermarket diet. We take:

$$\delta^2H_{\mathrm{d,l}} = \delta^2H_{\mathrm{we}} - 50, \quad (2)$$

$$\delta^{18}O_{\mathrm{d,l}} = \delta^{18}O_{\mathrm{we}} + 35.4, \quad (3)$$

and

$$\delta^{18}O_{\mathrm{wd,l}} = \delta^{18}O_{\mathrm{d,l}} - 27.0, \quad (4)$$

where δ^2H_{we} and $\delta^{18}O_{\mathrm{we}}$ are the isotopic compositions of drinking water. The coefficients in Eqs. (2) and (3) are approximations based on comparing our own observations of US supermarket meats with the approximate δ^2H and δ^{18}O values of water in the parts of the US hosting major feedlot operations (e.g., central California, the Midwest and southern Great Plains). The coefficient in Eq. (4) is based on the average isotopic offset between carboxyl oxygen and source water in carbohydrates (Sternberg et al., 1986) and represents a reasonable average offset for a wide range of biomolecules (Schmidt et al., 2001). Taken together, Eqs. (3) and (4) approximately reproduce observed isotopic offsets between tissue water in beef samples from Germany and feedlot drinking water sources (Boner and Forstel 2004).

Neither assumption regarding the dietary isotopic composition is likely to be strictly true, but together they constitute a reasonable approach to defining endmember compositions for our modeling study. In the case of assumption no. 1, our constant offset approach does not account for several cultural and physiological factors that might lead to group specific variation in dietary isotopic composition about a general, geographic relationship, including isotopic effects associated with differences in macronutrient routing among groups with widely different dietary composition, strong seasonal variation in dietary composition for some groups, and variation in dietary δ^2H and δ^{18}O values due to selection of food types and cultivation methods (e.g., foods from plants using the CAM photosynthetic pathway, which are highly ^{2}H-enriched; Sternberg et al., 1984). In addition, our approach does not account for some factors that may lead to systematic variation in diet-water isotopic offsets

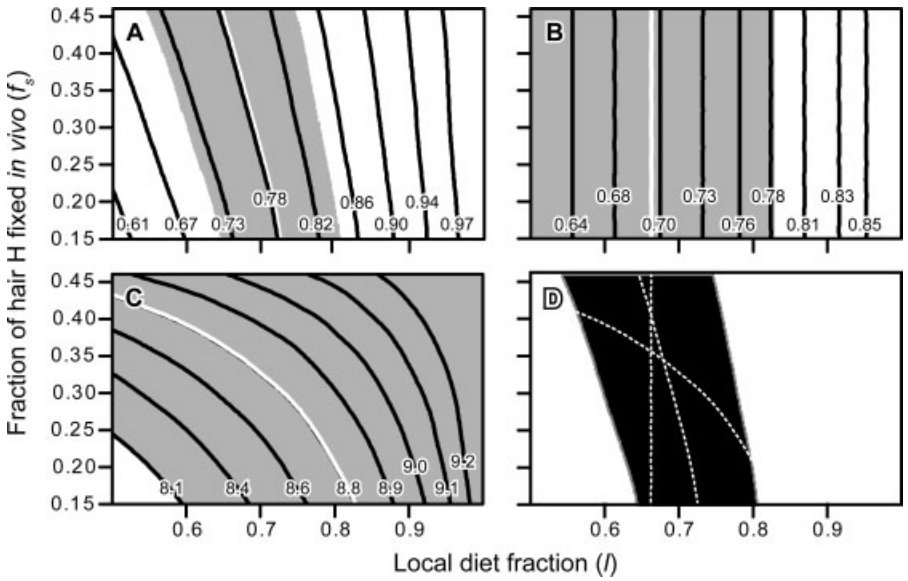

Fig. 6. Results of model sensitivity testing. Black contours in panels **A–C** show the slope of model-predicted relationships between δ^2H values of hair and local water (A), δ^{18}O values of hair and local water (**B**), or δ^2H values and δ^{18}O values of hair (C) for different levels of locally derived food consumption and in vivo fixation of H. The observed slopes for the anthropological data set are shown as white lines bounded by 1 standard error envelopes (gray). Panel D shows combinations of model parameter values that reproduce each of the three observed best-fit relationships (dashed white lines) and parameter values that are consistent with all 3 observed relationships within 1 standard error (black field).

across the range of samples studied, such as variation in evaporative fractionation, which would increase δ^2H and δ^{18}O values in regions of high heat and low humidity (Roden et al., 2000), or incorporation of metabolic O_2-derived oxygen in animal tissues, which would damp the geographic δ^{18}O variability of animal protein-rich diets. With respect to assumption no. 2, it is unlikely that the isotopic composition of food items obtained through trade was identical for all local groups, but it is reasonable to assume that trade-derived food items were more isotopically homogeneous than local foods across the range of populations sampled. Although improved representation of dietary endmembers would be critical for studies attempting to make quantitative inferences for specific individuals or populations, with a few exceptions noted here most of the potential confounding factors are likely to be somewhat randomized across our multicultural dataset and are unlikely to bias our results derived from analysis of the dataset as a whole.

MODEL SENSITIVITY TESTING

The new data set presented here documents strong relationships between the isotopic composition of hair and local water and between the H and O isotopic composition of hair across a globally distributed range of indigenous populations. These relationships provide a powerful test of the predictions of our model for H and O isotope incorporation in hair: if valid, this model should be able to reproduce the observed relationships given reasonable values of the model parameters. To test the model performance, we conducted sensitivity tests by varying the values of 2 model parameters (l, the fraction of locally derived dietary intake, and f_s, the fraction of H fixed in vivo) that were likely different for the preglobalization humans and modern USA residents, and predicting how hair isotopic values changed as dietary input isotope ratios (water and local food) changed. For predicting the relationship between hair δ^2H and δ^{18}O values, we assumed that drinking water isotopic values varied along the Global Meteoric Water Line, $\delta^2H_{we} = \delta^{18}O_{we} \cdot 8 + 10$ (Craig, 1961) characterizing most continental waters. We evaluated the model's ability to represent factors controlling variation in hair isotope ratios within the anthropological data set by comparing the model-predicted slope for relationships between hair δ^2H and water δ^2H, hair δ^{18}O and water δ^{18}O, and hair δ^2H and hair δ^{18}O to the observed relationships (see Fig. 6). To account for uncertainty in the slopes of the observed relationships, we considered that the model adequately matched the observed relationships if it reproduced the observed slope parameter to within one standard error.

As expected, local diet fraction has a strong influence on each of the relationships describing hair isotopic variation. As local dietary intake increases, an increasing fraction of the H and O atoms entering the body carry a "local" isotopic signature, as opposed to a homogeneous "global" signature. As a result, the predicted range of isotopic variation in hair across any given gradient of local water isotopic values increases. For H isotopes, where food and water are the only two sources of H to the body, the slope of the relationship between hair δ^2H and water δ^2H is in the range of 0.6–0.7 if 50% of the diet is locally derived and approaches one as l approaches 1 (Fig. 6A). For O isotopes, the hair/water relation has a similar slope of 0.6 if 50% of diet is local, but because body water oxygen includes a small amount of O derived from isotopically homogeneous atmospheric O_2 the maximum slope of the modeled $\delta^{18}O_{hair}/\delta^{18}O_{we}$ relationship is less than unity (0.87; Fig. 6B). Because the slopes of the hair/water relationships vary independently as local dietary intake increases, the slope of $\delta^2H_{hair}/\delta^{18}O_{hair}$ relationship is also sensitive to l, and

increases from as low as 8 with 50% local dietary intake to 9.2 if all food is locally derived (Fig. 6C).

The fraction of amino acid H fixed in vivo (f_s) may scale with the extent of nonessential amino acids synthesis in the body, and thus with the level and amino acid composition of dietary protein intake. Because f_s affects the fraction of hair H that is fixed from intercellular water, rather than routed from diet, this parameter affects the slope of the δ^2H hair/water relationship in cases where water and diet are out of "equilibrium" with each other (i.e. $l < 1$) and thus have different effects on hair δ^2H variation (Fig. 6A). At low values of l, higher levels of H fixation in vivo equate with greater incorporation of the local isotopic signal from drinking water into hair H, and the slope of the modeled $\delta^2H_{hair}/\delta^2H_{we}$ relationship increases from ~0.6 at low levels of f_s to 0.7 for maximum f_s values. At high values of l, diet and drinking water each impart a local isotopic signature to hair, and changes in f_s have little to no effect on the slope of the $\delta^2H_{hair}/\delta^2H_{we}$ relationship. Because f_s has no impact on the O isotope system (Fig. 6B), the modeled relationship between δ^2H and δ^{18}O values of hair samples is also sensitive to f_s under conditions where changes in dietary isotopic composition are damped relative to drinking water variability (Fig. 6C).

The revised model incorporating dietary intake of locally derived foods successfully reproduces the slopes the isotopic relationships observed in the anthropological hair sample suite within the range of l and f_s parameter values tested (Figs. 6D and 7). The best-fit values for all three relationships ($\delta^2H_{hair}/\delta^2H_{we}$, $\delta^{18}O_{hair}/\delta^{18}O_{we}$, and $\delta^2H_{hair}/\delta^{18}O_{hair}$) coincide for values of $l = 0.67$ and $f_s = 0.37$, and the model matches the observed relationships within the standard error for values of l between 0.6 and 0.8 and across all possible values of f_s. Our testing suggests that the diets of the individuals sampled here were dominated by locally derived foods, but likely included some items that were not locally derived (e.g., trade goods) that acted to "damp" the isotopic variability in hair relative to that in the local environment. In the absence of detailed data on these individuals, this model-constrained range of approximately 60% to 80% intake of locally derived items seems reasonable for a diverse collection of mid-20th century indigenous populations.

The model results suggest several implications for the use of protein H and O isotope ratios in anthropological research. Model sensitivity testing suggest that, particularly for individuals who consume 100% local diets, the relationship between hair isotope ratios and drinking water isotope ratios may be relatively constant. This supports the idea that hair isotope ratios could be measured as a proxy for environmental water isotope ratios and used for applications such as paleoclimate reconstruction or inference of travel history. This work would compliment similar research based on oxygen isotope ratios of skeletal remains (e.g., Prowse et al., 2007), but would offer the opportunity to develop temporally resolved reconstructions representing the time leading up to death. This application may be somewhat sensitive to changes in behavior and physiology not examined here (e.g., changes in metabolic rate or the fraction of water intake obtained from food vs. drinking water), but the consistency of our results representing individuals spanning a huge range of dietary, and likely physiological, variability, suggests that in most cases the influence of these factors may be relatively minor. The modeling further suggests that coupled measurements of hair δ^2H and δ^{18}O values may provide a screening tool for damping of hair isotopic variability relative to that in environmental water, e.g., because of the consumption of food items not in isotopic "equilibrium" with drinking water. As hair isotopic variability is damped, the model suggests that the slope of the covariant relationship between hair δ^2H and δ^{18}O values decreases predictably. Slope values near the model-predicted maximum of ~9.2, therefore, might provide a useful criterion for identifying samples or samples suites that preserve high-fidelity records of paleowater isotopic variation. Lastly, the possibility that hair isotope ratios might preserve information on levels of amino acid synthesis in ancient human populations is intriguing. The sensitivity testing conducted here suggests that it may be difficult to make such interpretations, however, particularly for individuals consuming local diets and in cases where independent constraints on factors such as dietary origin and composition are lacking.

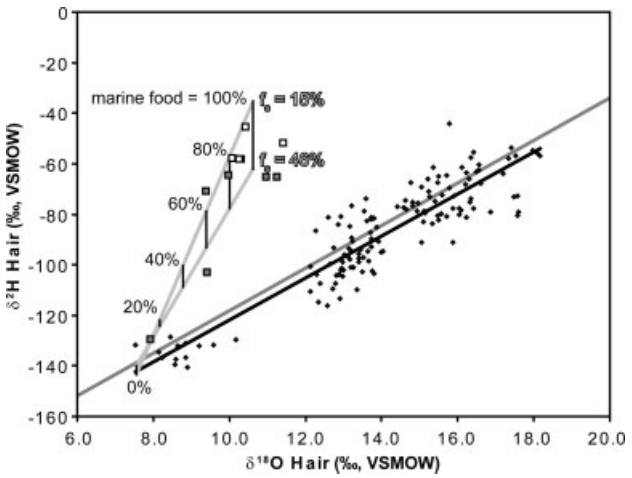

Fig. 7. Model predictions for the H and O isotopic values of anthropological hair samples. Data are from the mid- and low-latitude sites (small diamonds), Baffin Island (gray-filled squares) and Labrador Bay (open squares). The best-fit, model-predicted relationship between hair H and O isotope ratios for non-Inuit anthropological samples (thick gray line) closely matches the best-fit regression relationship (thick black line). Model-predicted isotope ratios for Inuit hair (light gray lines) reflect dietary intake of local freshwater and between 0% and 100% of marine-derived dietary protein. Predictions are given for levels of in vivo amino acid H fixation (f_s) ranging from 15% to 46%, with the vertical black lines indicating the range of possible values for a given marine dietary intake over the range of possible f_s values.

MODEL APPLICATION TO INUIT SAMPLES

Within the multi-isotope data set, a suite of 11 samples collected from Inuit communities in Labrador Bay and Baffin Island stand out as anomalous. These samples have the highest δ^{15}N values and, despite originating from a region characterized by very low environmental water isotope ratios, have high δ^2H values similar to those of individuals from tropical climates and modern residents of Florida and Texas (Figs. 2 and 4). Moreover, isotopic values of most of these samples deviate strongly from the tight relationship between δ^2H and δ^{18}O values observed for the rest of the data set (see Fig. 2). Although there was a large range of variation in isotope

TABLE 2. Data for individual Inuit hair samples

Site	Location	Sex	Age[a]	δ^2H	δ^{18}O	δ^{13}C	$\delta^{15}N$
16	Baffin Island	M	Middle	−65	10.0	−14.7	16.7
16	Baffin Island	M	Young	−65	11.0	−14.6	15.8
16	Baffin Island	M	Middle	−130	7.9	−18.1	9.5
16	Baffin Island	M	Young	−71	9.4	−14.8	16.1
16	Baffin Island	M	Young adult	−103	9.4	n.d.	n.d.
16	Baffin Island	F	Old	−66	11.3	−15.1	16.2
17	Labrador Bay	F	Old	−58	10.1	−14.8	17.7
17	Labrador Bay	M	n.a.	−45	10.4	−14.4	17.7
17	Labrador Bay	M	Old	−58	10.3	−14.9	18.1
17	Labrador Bay	M	n.a.	−58	10.3	−15.0	17.4
17	Labrador Bay	F	Young	−52	11.4	−14.4	18.2

[a] Relative age indicated on sample envelopes, where specified. n.d., not determined; n.a., not available.

values among the samples from Baffin Island, none of the isotope parameters measured is significantly correlated with sex or age group (Table 2; ANOVA).

High δ^{15}N values previously reported from Inuit fingernail samples have been associated with consumption of marine predators such as seals (Buchardt et al., 2007), which were also an important, though declining, component of the diet of early-20th century Inuits living along the Labrador Sea (Jenness, 1921). Within our dataset the δ^{15}N values of individual hair samples were closely related to the anomalous H and O isotope values (see Fig. 3). Based on these observations, we applied the hair H- and O-isotope model to understand whether intake of marine dietary items could explain the unusual δ^2H and δ^{18}O values of the Inuit hair samples. We estimated the isotopic composition of nonmarine dietary sources (−145‰ and +25.0‰ for δ^2H and δ^{18}O, respectively) by using the generalized anthropological sample model to fit the measured isotope ratios of one Inuit sample with "normal" δ^{15}N, δ^2H, and δ^{18}O values. There are few published measurements of δ^2H values for marine vertebrate proteins (Wassenaar and Hobson, 2000), and none (of which we are aware) documenting O isotopic composition. The available data are consistent with our own (unpublished) measurements of marine fish protein from supermarkets, which give values that are ~60‰ (δ^2H) and ~9‰ (δ^{18}O) higher than those of USA supermarket meats, on average. Given the lack of constraints, we experimented with a range of δ^2H and δ^{18}O values for marine diet and used our model to predict hair δ^2H and δ^{18}O values resulting from varying mixtures of local and marine dietary intake and values of f_s.

The model experiments suggest that the pattern of anomalous δ^2H and δ^{18}O values in Inuit hair samples is consistent with expectations for individuals consuming ^{2}H and ^{18}O-depleted drinking water and food that is relatively enriched in ^{2}H and ^{18}O (see Fig. 7). As the fraction of ^{2}H- and ^{18}O-enriched marine dietary intake increases so does the relative abundance of these isotopes in hair. Because dietary contributions to hair amino acid are more dilute for O than for H, the model-predicted impact of marine dietary intake is greater for hair δ^2H values than δ^{18}O values, mimicking the pattern observed in the Inuit samples. To reproduce the entire range of observed hair isotope ratios, however, we must assume δ^2H and δ^{18}O values of the marine dietary source that are somewhat higher and lower, respectively, than suggested by the limited measurements currently available (δ^2H ≈ −25‰, δ^{18}O ≈ +31.0‰). It is possible that these values are reasonable approximations for the isotopic composition of higher trophic-level marine mammal tissue, but additional data will be required to determine whether this is the case or if other sensitivities in the model need to be explored.

The Inuit hair model is also quite sensitive to adjustments in the level of in vivo fixation of H on amino acids (f_s). At low levels of f_s transfer of H from diet to hair is maximized, enhancing the effects of heavy marine-derived H on the isotopic composition of hair. Low levels of amino acid synthesis, which might be consistent with expectations for a population consuming large amounts of animal protein (Kromhout 2005), may thus contribute to the unusually high δ^2H values of hair from the Inuit samples. Other factors such as unique food handling practices (e.g., smoking or dry curing) and higher-than-average metabolic rates may also play a role: either of these factors might be expected to raise body water isotope ratios and increase the δ^2H and δ^{18}O values of hair.

Given the apparent influence of high trophic-level, marine protein consumption on δ^2H and residual ^{2}H values of the Inuit hair samples, it is interesting to consider whether the weak relationship between hair δ^{15}N and residual ^{2}H values within the non-Inuit anthropological samples might reflect marine dietary intake within these populations. Among the groups with the highest δ^{15}N and residual ^{2}H values, only one (Thailand) is likely to include individuals with a diet rich in marine proteins. Conversely, samples from some groups that might be expected to have significant marine dietary intake, notably the Ainu, are not characterized by high δ^{15}N and residual ^{2}H values (in fact the Ainu samples measured here have C and N isotope ratios indicating little, if any, intake of marine dietary protein; Schoeninger and Tauber, 1983). This suggests that the coupling of these parameters in the Inuit and non-Inuit sample suites may result from distinct processes. We believe that the pattern observed among the non-Inuit samples may largely reflect simple isotope mass balance effects that lead to apparent trophic-level effects in both H and O isotope systems. Where diet is in "equilibrium" with local water [Eqs. (2) and (3)], mixing of H and O derived from both drinking water and diet in consumer protein gives isotopic values that are similar to or enriched in ^{2}H relative to diet but depleted in ^{18}O relative to dietary inputs (e.g., Fig. 4). This pattern is consistent with and may largely explain recent observations suggesting a 10–20‰ trophic level enrichment of ^{2}H in consumers (Birchall et al., 2005; Reynard and Hedges, 2008), and has important implications for understanding the potential utility and complications associated with applying H isotopes in trophic studies. It implies that higher trophic-level consumers should be ingesting dietary protein with higher δ^2H values (and lower δ^{18}O), and provides a mechanism coupling of high residual ^{2}H and δ^{15}N values in human consumers of animal protein-rich diets.

IMPLICATIONS AND CONCLUSIONS

Stable isotope ratio data from hair samples collected in the mid-20th century confirm that first order trends observed in the H- and O-isotope ratios of modern residents of the USA also characterize communities with limited ties to global trade and an extremely diverse range of diets. These include strong relationships between the isotopic composition of hair and local water and covariation of hair δ^2H and δ^{18}O values. Quantitative differences between the parameters of these rela-

tionships for the anthropological sample set and the modern USA data can largely be attributed to the influence of locally derived foods on the isotopic composition of body tissues. No strong support was found for ubiquitous effects related to physiological differences among the sampled populations.

These results show that among populations consuming locally derived foods, variation in the δ^2H and δ^{18}O values of environmental water exerts the dominant control on human protein H and O isotope ratios, independent of broad range of dietary and physiological variation. They thus confirm that H- and O-isotope analysis of human tissues such as hair or collagen may be useful for applications such as reconstructing geographic movements of individuals, detecting the presence of immigrant individuals within populations, and paleoclimate reconstruction. Moreover, they support the general applicability of a previously proposed model for dietary and physiological controls on hair δ^2H and δ^{18}O values. This model should improve attempts to quantitatively relate protein isotope ratios to the δ^2H and δ^{18}O values of local water, for example through the development of constraints based on model-predicted covariation in hair H and O isotope ratios.

Although our data indicate that variation in water isotope ratios is the most ubiquitous control on hair δ^2H and δ^{18}O values, they also suggest some secondary, and in extreme cases profound, influences related to dietary composition. In the case of samples from Inuit communities, unusually high hair δ^2H and δ^{18}O values can be attributed to the strong isotopic "disequilibrium" between local drinking water and marine-based diet, potentially amplified by low rates of amino acid synthesis among these individuals. Within the larger data set, there is some suggestion that deviation from the mean relationship between δ^2H and δ^{18}O values may be related to "trophic level" or meat-rich vs. meat-poor diet. These factors suggest that a potential second level of dietary reconstruction may be supported by coupled analysis of δ^2H and δ^{18}O values in human and animal proteins, but significant work remains to characterize the precise origin and sensitivities of the observed signals.

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