Hydrogen and oxygen isotope ratios in human hair are related to geography

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We develop and test a model to predict the geographic region-of-origin of humans based on the stable isotope composition of their scalp hair. This model incorporates exchangeable and nonexchangeable hydrogen and oxygen atoms in amino acids to predict the δ^2H and δ^{18}O values of scalp hair (primarily keratin). We evaluated model predictions with stable isotope analyses of human hair from 65 cities across the United States. The model, which predicts hair isotopic composition as a function of drinking water, bulk diet, and dietary protein isotope ratios, explains >85% of the observed variation and reproduces the observed slopes relating the isotopic composition of hair samples to that of local drinking water. Based on the geographical distributions of the isotope ratios of tap waters and the assumption of a ''continental supermarket'' dietary input, we constructed maps of the expected average H and O isotope ratios in human hair across the contiguous 48 states. Applications of this model and these observations are extensive and include detection of dietary information, reconstruction of historic movements of individuals, and provision of region-of-origin information for unidentified human remains.

stable isotopes | water | anthropology | forensics | meteoric water

The carbon (δ^{13}C), nitrogen (δ^{15}N), and sulfur (δ^{34}S) isotope ratios of humans, other animals, and microbes are strongly correlated with the isotope ratios of their dietary inputs (1–5). The adage ''You are what you eat'' reflects the observation that there are limited differences ($\leq$1‰) between heterotrophic organisms and their diet in either the δ^{13}C or δ^{34}S values (6–8). These small isotopic differences arise because of fractionation events during metabolism; they also reflect that diet-derived carbon and sulfur are the only input sources into most heterotrophs. Although there are larger ''spacing'' differences in δ^{15}N values ($\approx$3‰) between an organism and its dietary source (9), the isotopic relationships between organism and diet persist. δ^{13}C, δ^{15}N, and δ^{34}S values provide limited geographic-based information about the origins of a food source. Hydrogen (δ^2H) and oxygen (δ^{18}O) isotope ratios of organic matter are more useful, because δ^2H and δ^{18}O values of precipitation and tap waters vary along geographic gradients (10, 11).

Although differences in the δ^2H and δ^{18}O values of scalp hair have been noted in humans (12), less is known about diet–organism patterns of δ^2H and δ^{18}O values. Four potential sources can be important: dietary organic molecules, dietary waters, drinking waters, and atmospheric diatomic oxygen. Hobson *et al.* (13) provided evidence that δ^2H values of drinking water were incorporated into different proteinaceous tissues of quail, although no mechanistic basis was proposed for this relationship. The δ^2H values of bird feathers and butterfly wings (both are largely keratin) and water in the region in which the tissue was produced are highly correlated (14, 15). Kreuzer-Martin *et al.* (1) showed that $\approx$70% of the oxygen and $\approx$30% of the hydrogen atoms in microbial spores ($\approx$50% proteinaceous) were derived from the water in the growth medium, whereas the remainder was derived from the organic compounds supplied as substrate (16).

The δ^2H and δ^{18}O values of keratin in human hair should be influenced by a number of factors during synthesis within the hair follicle, including all dietary and atmospheric sources of H and O. Even after synthesis, a fraction of the ^{2}H in hair is subject to continuous isotopic exchange with the environment and our discussion is therefore limited to only that proportion of the H atoms not subject to postsynthesis isotopic exchange (17). In advance of any synthesis reaction, the amino acids interact with their aqueous medium. There is an opportunity for complete O isotope exchange between the carboxyl component of amino acids and water during protein hydrolysis, but subsequent exchange only at much slower rates afterward (18, 19). Similarly, the H isotope exchange is extensive between amino acids and water except for C–H bonds (20, 21). As the basis of this study, we hypothesized that variations in the nonexchangeable δ^2H and δ^{18}O values in human keratin could provide insights into water and human diet across geographical regions if the hydrogen and/or oxygen isotopes from these sources were recorded in human hair. We first describe the observed patterns between the δ^2H and δ^{18}O values of randomly collected hair samples from across the United States and those of local tap water. Because drinking water and body water do not have the same isotope ratio values, we develop and test models to reflect how contributions of both dietary sources and local drinking waters to body water might be incorporated into the isotopes of keratin in human hair.

Results

On Relationships Between Tap Water and Hair Isotopes. Tap water and hair samples were collected from 18 states. Figs. 1 and 2 show that the tap-water samples spanned the range of δ^2H and δ^{18}O values reported from the contiguous states (11) (i.e., excluding the very lowest values anticipated for Alaska). The isotopically lightest tap waters were $-$140‰ and $-$18.0‰ for δ^2H and δ^{18}O, respectively, from northern Montana. At the other extreme, the isotopically heaviest waters were +4‰ and +1.3‰ for δ^2H and δ^{18}O, respectively, sampled in southern Oklahoma. The standard deviations among replicated water samples from a city were generally small, with 95% of all water samples having standard deviations of <5‰ and 1‰ for δ^2H and δ^{18}O, respectively. From this we concluded that most, if not all, of the cities or towns sampled were using an isotopically similar water source throughout the city.

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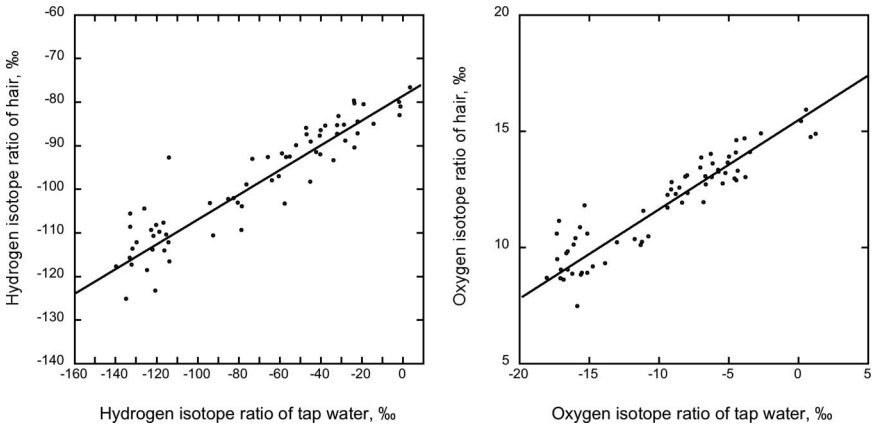

Fig. 1. Plots of the relationships between mean H isotope ratios (δ^2H) *(Upper)* and mean O isotope ratios (δ^{18}O) (*Lower*) of human scalp hair and tap water for samples randomly acquired in cities representing 18 states across the United States. The lines through the data in each plot represent model-predicted values based on local tap water and a continental supermarket diet.

Hair samples had isotopic compositions ranging from −125‰ to −77‰ for δ^2H and +7.4‰ to +15.9‰ for δ^{18}O (Figs. 1 and 2). The average standard deviation of triplicate samples from individual cities was 5.2‰ and 1.0‰ for δ^2H and δ^{18}O, respectively. We use average values of hair samples from cities in subsequent analyses.

We analyzed water and hair δ observations with use of least-squares regression analyses. When the mean H and O stable isotope values of human hair (δ^2H_h and $\delta^{18}O_h$, respectively) were regressed against the respective mean δ^2H and δ^{18}O values of tap water ($^2H_{we}$ and $\delta^{18}O_{we}$, respectively) there were highly significant linear relationships between these two parameters. For δ^2H values the regressed relationship was $\delta^2H_h = 0.271\delta^2H_{we} - 79‰$ ($r^2 = 0.86$, $P < 0.001$) and for δ^{18}O values the relationship was $\delta^{18}O_h = 0.353\delta^{18}O_{we} + 15.2‰$ ($r^2 = 0.86$, $P < 0.001$). A first-order interpretation of these results is that 27% and 35% of the H and O (respectively) in hair keratin were derived from local drinking water, with the remaining fraction coming from sources having isotope ratios that do not vary systematically with geography. These results are similar to feather (keratin) studies in birds where it is estimated that 26–32% of the H atoms are derived from water (13). To better understand how this local isotopic signature is incorporated in hair keratin and potential factors influencing these relationships we developed a semimechanistic model of isotopic transfer from diet to hair.

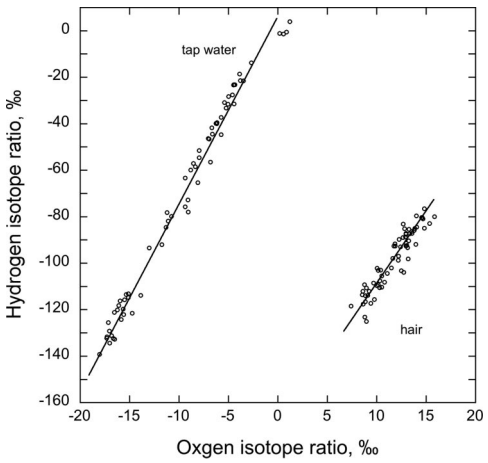

Fig. 2. A plot of the H (δ^2H) and O (δ^{18}O) isotope ratios for tap-water samples ($\delta^2H = 7.87\delta^{18}O + 4.4‰$, $r^2 = 0.984$, $P < 0.001$) and for human scalp hair ($\delta^2H = 5.73\delta^{18}O - 166‰$, $r^2 = 0.873$, $P < 0.001$) for samples randomly acquired in cities representing 18 states across the United States.

Model Derivation. Keratin is synthesized from amino acids in the hair follicle, and these keratin sheets form the basis of hair tissue. With the exception of a fraction of the H atoms that freely exchange with environmental water (17), H atoms in keratin are no longer subject to isotopic exchange and are recorders of isotopic inputs to and cycling within a person's body, as has been shown for carbon isotopes (2, 4, 6, 7). We develop a steady-state model that considers new inputs of amino acids to an individual's body, and assume that recycling of other body tissues is in equilibrium with dietary inputs. The H and O atoms in these amino acid sources are fixed at one or more points before keratin synthesis. Our model describes the sources and isotope effects determining the H and O isotopic composition of the amino acids ultimately incorporated into hair keratin.

The location at which the isotope ratios of amino acid H and O atoms are fixed will vary depending on the amino acid and pH. Essentially all of the O atoms in amino acids in proteins are associated with the C terminus and are subject to isotope exchange with water during hydration when peptides and amino acids are cleaved from proteins (18, 22). In the case of ingested proteins, this occurs in the stomach (low pH) and small intestine (neutral pH) with minimal subsequent exchange occurring after adsorption through the gut wall (19). Once the carbonyl O has been added during protein hydration, subsequent isotopic exchange with water under neutral pH values is slow (23). During subsequent protein synthesis to produce keratin, the O atoms either become carbonyl O in the protein or part of water by-product. The O atoms in the carbonyl O should largely record the isotopic composition of gut water during digestion, if amino acids are exposed to neutral pH values thereafter. Thus, the oxygen isotopic composition of keratin in human hair ($\delta^{18}O_h$) should relate to that of gut water ($\delta^{18}O_{wg}$) as

$$\delta^{18}O_h = \alpha_{ow} \cdot (1{,}000 + \delta^{18}O_{wg}) - 1{,}000 \quad [1]$$

where α_{ow} is the oxygen isotope fractionation associated with a carbonyl oxygen–water interaction.

In the case of hydrogen, not all H atoms of amino acids are subject to isotope exchange with cell or body water. Although the H atoms in carboxyl, amide, and sulfhydryl groups exchange with the H in water, this is not the case with H atoms in C–H bonds. Some fraction of the exchangeable H atoms (≈10% of total H) remain exchangeable after hair production (17). These H atoms

were effectively excluded from our measurements of keratin hair H through postanalysis correction (see *Materials and Methods*) and are not considered here. The remaining fraction of exchangeable H (f_e) is considered to have fully exchanged with water in the hair follicle up until the synthesis of keratin. The C-bound H ($1 - f_e$) can be subdivided into those on "essential" and those on "nonessential" amino acids. The C–H atoms of essential amino acids in keratin should reflect the δ^2H values of food sources. Nonexchangeable H atoms in nonessential amino acids in human hair may reflect either the δ^2H values of dietary amino acids or of amino acids synthesized in the body. The fraction of H atoms derived directly from the dietary food source (f_d) can be described as

$$f_d = (1 - f_e)\cdot(1 - f_s), \quad [2]$$

where f_e is the fraction of keratin H exchanged *in vivo* and f_s is the fraction of nonexchangeable keratin H fixed *in vivo*. On this basis, the δ^2H of the nonexchangeable hydrogen in keratin in human hair (δ^2H_h) should relate to water of the hair follicle (δ^2H_{wf}) as

$$\delta^2H_h = f_d\cdot\delta^2H_d + (1 - f_d)\cdot(\alpha_{hw}\cdot(1{,}000 + \delta^2H_{wf}) - 1{,}000), \quad [3]$$

where α_{hw} is the hydrogen isotope fractionation associated with synthesis of the amino acid and ultimately the polypeptide.

Hydrogen and oxygen in follicle and gut water are each derived from multiple sources. The δ^2H values of cells and their surrounding water environment differ because of the noninstantaneous exchange of intracellular and extracellular water (24). Therefore, we consider two water pools in a human, when describing the ways in which the H isotope ratio of tap water (δ^2H_{we}) in the geographic environment influences the H isotope ratio of water in the follicle (δ^2H_{wf}). We model the H isotopic composition of hair-follicle water (δ^2H_{wf}) as a mixture of extracellular (blood) water (δ^2H_{wb}) and H produced through the metabolism of dietary foods (δ^2H_d):

$$\delta^2H_{wf} = e\cdot\delta^2H_{wb} + (1 - e)\cdot\delta^2H_d, \quad [4]$$

where e is the fractional contribution of body water. O in gut water is derived primarily from gastric fluids, which we assume to be in isotopic equilibrium with body water ($\delta^{18}O_{wb}$), water from both ingested food ($\delta^{18}O_{wd}$), and water from drinking water ($\delta^{18}O_{we}$):

$$\delta^{18}O_{wg} = g_1\cdot\delta^{18}O_{wb} + g_2\cdot\delta^{18}O_{we} + (1 - g_1 - g_2)\cdot\delta^{18}O_{wd}, \quad [5]$$

where g_1 and g_2 are the fractional contributions of O from gastric juice and food water, respectively. Body water does not have the same isotope composition as drinking water (25); it is a mixture of both drinking water and of water derived from metabolism. Diatomic oxygen and dietary sources (including water in food and metabolized organic O) contribute O with contrasting isotope ratio values. We describe the $\delta^{18}O$ of water in humans ($\delta^{18}O_{wb}$) as [following Gretebeck *et al.* (25)]:

$$\delta^{18}O_{wb} = (a\cdot\delta^{18}O_{we} + b\cdot\delta^{18}O_d + c\cdot[\alpha_{O2}(1{,}000 + \delta^{18}O_{O2}) - 1{,}000])/(h\cdot\alpha_{fwlo} + j\cdot\alpha_{co2} + k), \quad [6]$$

where $\delta^{18}O_{we}$, $\delta^{18}O_d$, and $\delta^{18}O_{O2}$ are the oxygen isotope ratios of tap water, dietary sources, and atmospheric oxygen, respectively; a, b, and c are mixing fractions of drinking water, dietary, and atmospheric oxygen; h, j, and k are fractional losses of O as fractionated water, CO_2, and unfractionated water, respectively; and α_{O2}, α_{fwlo}, and α_{co2} are isotopic fractionation factors for the absorption of O_2 in body water, loss of O during breathing and transcutaneous evaporation, and loss of O as CO_2, respectively. The equivalent equation for the δ^2H of body water (δ^2H_{wb}), where there is no atmospheric source, is

$$\delta^2H_{wb} = (d\cdot\delta^2H_{we} + (1 - d)\cdot\delta^2H_d)/(m\cdot\alpha_{fwlh} + n), \quad [7]$$

where δ^2H_{we} and δ^2H_d are the H isotopic compositions of tap water and dietary sources; d is the fraction of body water derived from drinking water; m and n are the fractional losses of H as fractionated and unfractionated water; and α_{fwlh} is the isotopic fractionation factor for the loss of H during breathing and transcutaneous evaporation.

Previous work allows us to constrain many but not all of the parameters required by our model. Mixing ratios of H and O isotope sources (*a–d* and *h–n*) are based on the synthesis of Gretebeck *et al.* (25). The contribution of metabolic H to follicle water is unconstrained, but we adopt the assumption that these mixing ratios are similar to those for the whole body and set $e = d$. The mixing ratios of food water and body water sources to gut water are also poorly constrained, but the work of Malagelada *et al.* (26) suggests that the volumetric mixing ratio is similar to 25:75 food water to digestive juice in the average human. We consider drinking water as an additional component to gut water, but set this fractional contribution to zero in the current calculations for simplification; however, different combinations of food-derived and drinking water can be adjusted in the model to yield similar results. The fraction of H atoms in keratin derived directly from dietary protein was estimated based on an inventory of H atoms on individual amino acids. We calculate that 15% of all H atoms that remain fixed after keratin synthesis are exchangeable *in vivo* (f_e). Of the remaining H atoms, 54% are associated with essential amino acids and would be derived from diet, making the upper bound on $f_s = 46\%$. The total possible range for f_d is therefore from 46% to 85% (Eq. **2**).

Average values of local tap-water samples were used to estimate δ^2H_{we} and $\delta^{18}O_{we}$ for each city. Given the food-distribution systems available today and the general availability of all food types throughout the United States, we assume that the U.S. residents sampled here largely consume food from a "continental supermarket" diet that is isotopically homogeneous, and adopt average values for δ^2H_d and $\delta^{18}O_d$ of $-115‰$ and $+26.0‰$ based on our own unpublished data, which are similar to other recent observations (27, 28). The value of $\delta^{18}O_{wd}$ is determined from $\delta^{18}O_d$ based on the average isotopic enrichment of plant carbohydrates relative to water (29), and $\delta^{18}O_{O2}$ is assigned the global average value of $+23.5‰$. The value of α_{ow} is unknown, but could be as high as 1.027, the value for fully equilibrated carbonyl-oxygen isotope fractionation between acetone and water (29). Kreuzer *et al.* (1) calculated α_{ow} to be 1.0164 for microbial spore cell walls during non-log-phase growth.

Given these constraints and assumptions, four model parameters remain unconstrained. The values of two of these (f_s and g) influence the slope of model-predicted relationships between the isotopic composition of hair and local water, whereas the other two (α_{hw} and α_{ow}) affect the intercept of these relationships. These parameters were tuned to maximize the fit between model predictions and our observational data. For two of these parameters where some constraints were available (g and α_{ow}, see above), our tuned values were in reasonable agreement with these constraints. We note that there is significant interaction in the model between f_s and e, a parameter that is assumed but largely unconstrained. Because the current dataset does not allow us to deconvolve this interaction and fit the parameters independently, we take the approach of assuming one and tuning the other. It might be expected, however, that the degree to which nonessential amino acids are synthesized *in vivo* might vary among individuals and populations in response to changes in

dietary protein intake, whereas the relative rates of cellular metabolism and water exchange (which determine e) would be less likely to do so. This suggests that further isotopic data gathering might allow independent tuning of these parameters in the future.

The parameters of our body-water model are tabulated in supporting information (SI) Table 1 and their interrelationships are shown in SI Fig. 5.

Model Predictions and Implications. Model predictions were forecasted for both δ^2H_h and $\delta^{18}O_h$ by using the range of tap-water isotope ratios seen along the continental transect (Figs. 1 and 2). The tuned model maximizes the fit between predictions and data, achieving r^2 values equal to those of the linear regressions (85% and 86% for δ^2H_h and $\delta^{18}O_h$ values, respectively). One implication of the model is that prediction of the observed slopes between hair and water isotopic compositions requires a multicompartment model that takes into account the site of H and O fixation on amino acids within the body. This distinguishes the model developed here with a rapidly growing tissue from previous models describing, for example, the isotopic composition of mammalian tooth enamel apatite and carbonate (slower growing tissues), which appears to form in equilibrium with bulk body water (21). If H and O atoms in hair were derived directly from body water in an analogous manner, our model would predict that the slope of the relationship between the isotopic composition of hair and drinking water would be similar to 0.81 for δ^2H and 0.62 for $\delta^{18}O$, equal to the fractional contributions of drinking water to body water. That the observed slopes are much lower than these values provides strong evidence that amino acid H and O atoms are derived from secondary water pools within the body having a lower mixing ratio of drinking water to other H and O sources.

The scatter in the hair isotope ratio data about the model-prediction values in Figs. 1 and 2 exceeded measurement precision; we believe that these variations are biologically meaningful. If our model is correct and if the hair samples represented local individuals, then the deviations from model predictions could be indicative of dietary differences among the randomly sampled individuals from a single continental supermarket value. We used a continental supermarket average of $\delta^2H_d = -115‰$ and $\delta^{18}O_d = +26‰$, representing the average of food values in the literature and of unpublished observations we have compiled for different foods. Based on model calculations, the ranges of $-35‰$ to $-100‰$ for δ^2H_d values and of 20‰ to 35‰ for $\delta^{18}O_d$ values are sufficient to encompass all of the observed variations in δ^2H_h and $\delta^{18}O_h$ values shown in Figs. 1 and 2. This range of δ^2H_d and $\delta^{18}O_d$ values fits within the ranges of published food isotope ratio values (27, 31; L.A.C., D.W.P., T.E.C., and J.R.E., unpublished data).

We tested the robustness of the model predictions in Figs. 1 and 2 by analyzing a second set of individual hair samples, representing separate collections of randomly acquired hair samples from barbershops in the same cities originally sampled. We asked how well the model could be inverted to predict the isotope ratio of the tap water for the city in which the hair sample originated. For these 65 hair samples, we again achieved 85% and 86% agreement, respectively, between the predicted and observed "isotopic origins" of both the δ^2H_h and $\delta^{18}O_h$ values.

The relationships between δ^2H_h versus $\delta^{18}O_h$ and δ^2H_{we} versus $\delta^{18}O_{we}$ values (Fig. 2) were not similar in slope. The δ^2H_{we} versus $\delta^{18}O_{we}$ line calculated from these tap-water samples was $\delta^2H_{we} = 7.87\delta^{18}O_{we} + 4.4‰$ ($r^2 = 0.984$, $P < 0.001$, $n = 65$). If the four heaviest water samples, which were likely from enriched evaporated reservoir sources, were removed from the analysis, the δ^2H_{we} versus $\delta^{18}O_{we}$ water line calculated from these tap-water samples was $\delta^2H_{we} = 8.22\delta^{18}O_{we} + 8.9‰$ ($r^2 = 0.988$, $P < 0.001$, $n = 61$), which is indistinguishable from the global meteoric water line. However, the slope and intercept of the relationship between H and O isotope ratios in hair were different from any of the water lines. The δ^2H_h versus $\delta^{18}O_h$ line calculated for hair samples was $\delta^2H_h = 5.73\delta^{18}O_h - 166‰$ ($r^2 = 0.873$, $P < 0.001$, $n = 65$). If our model is correct, then we predict that other human societies (modern or ancestral) with dietary consumption patterns that differ from those of our current modern U.S. samples may exhibit slight differences in the slope of the δ^2H_h versus $\delta^{18}O_h$ relationship.

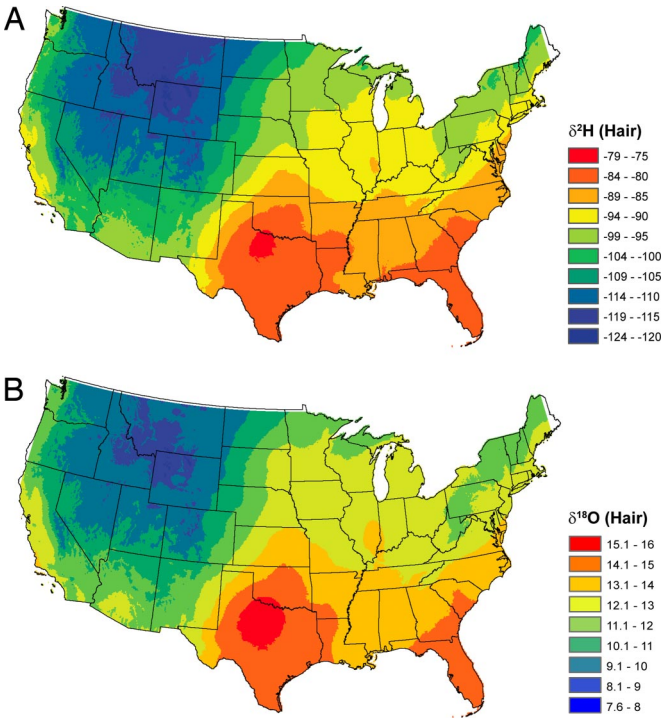

Fig. 3. Geographic Information System-generated maps of the predicted average H isotope ratios (δ^2H_h) *(A)* and average O isotope ratios ($\delta^{18}O_h$) *(B)* of human scalp hair across the coterminous United States.

Mapping the Distributions of δ^2H and $\delta^{18}O$ in Human Hair. Geographic maps of δ^2H_h and $\delta^{18}O_h$ values for hair across the coterminous United States were produced by taking maps of the δ^2H_{we} and $\delta^{18}O_{we}$ values based on 496 locations (at least one location per state) as spatial input (11) to predict δ^2H_h and $\delta^{18}O_h$ values by using regressions between the two parameters derived from Figs. 1 and 2. Fig. 3 *A* and *B* show the predicted average equilibrium δ^2H_h and $\delta^{18}O_h$ values, respectively, for different geographic locations across the coterminous United States.

Fig. 3 *A* and *B* both show that the isotopically heaviest hair is expected to occur in the southern parts of the United States. Specifically, the Gulf coast states and the central Texas through Oklahoma region are predicted to have the highest δ^2H_h and $\delta^{18}O_h$ values. In contrast, the most negative hair isotope values are predicted for the northern Rockies region.

Detecting Human Movements Across Regions with δ^2H_h and $\delta^{18}O_h$. Hair has been shown to be a sequential recorder of changes in diet (3, 12, 32–35). Similarly, geographic movements by an individual between locations with contrasting tap-water isotope ratios should be detectable. Fig. 4 illustrates this point, showing the hair's recording of an individual who had moved from Beijing, China to Salt Lake City, Utah. A single hair was used in this analysis, with each data point representing about a 4-week interval. Changes in the δ^2H_h and $\delta^{18}O_h$ values were detected in

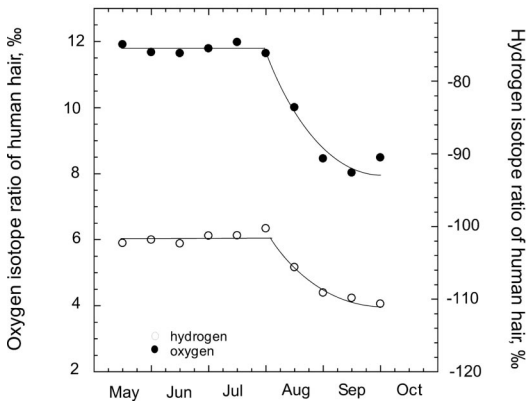

Fig. 4. Time sequence plots of the H (δ^2H_h) and O ($\delta^{18}O_h$) isotope ratios of human scalp hair along a basal-to-tip transect for an individual that moved from Beijing, China, to Salt Lake City, Utah.

the first hair interval produced after arriving in the new city. The exponential approach of the δ^2H_h and $\delta^{18}O_h$ values to the new location suggested a 95% turnover in δ^2H_h and $\delta^{18}O_h$ values of $<$3 months and transition rate is predictable by using a reaction progress model (33).

Discussion

A semimechanistic, multipool model of H and O isotopes in the human body is capable of reproducing the slope and intercept of observed relationships between isotopic compositions of hair and local drinking water by using reasonable parameter values and the assumption of continental supermarket dietary inputs. The isotopic signal from tap water was sufficient to account for the water inputs into the model, without the necessity of invoking other water sources such as the water in food. There may be two explanations for this observation. First, it is likely that the majority of water input to humans reflects a local source. Soft drinks, beer, coffees, teas, and reconstituted juices are likely to be derived from local waters. Milk, a common source of water in the United States, is likely to have been derived from animals growing in the region. The water used to cook vegetables, soups, and other dietary inputs is local water. Thus, it is perhaps not too surprising that these other water inputs would be isotopically related to local tap waters. Yet, given the extent to which individuals in the United States drink bottled waters and juices that might be imported into their region, a portion of the deviation in observations from expected values based on the regressions for data in Figs. 1 and 2 could have been associated with nonregional water sources. Second, it is also possible that a fraction of the barbershop hair samples may have been from visitors or individuals in transit (i.e., not native to the region where samples were collected). We cannot distinguish among these possibilities because of the random-sampling design, but do note that our finding that the two-pool model accounted for $>$85% of all variation in hair isotope ratios suggests that these confounding effects do little to compromise the isotopic signature in hair inherited from local water sources.

The scatter in the hair isotope ratio data about the regression line exceeded measurement precision and we believe that this scatter is likely associated with dietary differences among the randomly sampled individuals. Further studies are required to determine the extent to which dietary input differences in a region contribute to the H and O isotope variation in hair among individuals in a local population. The H and O isotopic composition of body water is influenced by both dietary inputs and drinking water, but the O isotopic composition of body water is also influenced by atmospheric O. That there were no overriding influences of dietary inputs on the δ^2H_h and $\delta^{18}O_h$ values allowed geographical water signals to be expressed and was perhaps expected in today's society. A near "constancy" of dietary input of δ^2H_d and $\delta^{18}O_d$ values would be expected with the continental supermarket diet enjoyed by most Americans today, where we tend to have foods derived from many common regions instead of only locally derived foods. In this regard, the analysis of historical or geographically isolated human populations may yield stronger relationships between local water and hair isotope ratios. The proposed model predicts changes in the slopes of δ^2H_h versus δ^2H_{we} and $\delta^{18}O_h$ versus $\delta^{18}O_{we}$, dependent on the degree to which food is part of a continental supermarket or locally derived. As the fraction of locally derived foods in the diet increases, the model predicts an increase in these slopes. Similarly, nitrogen balance in an individual's diet is expected to have an effect. As nitrogen balance decreases and protein deficiencies in the diet develop and/or increase, we expect that more of the amino acids involved in keratin synthesis will be synthesized *de novo* in the body rather than derived from dietary inputs. Such a decrease in protein availability in the diet is predicted by the model to result in a steeper relationship between δ^2H_h versus δ^2H_{we}, but to have no effect on $\delta^{18}O_h$ values. The examination of individuals with amino acid synthesis diseases or with dietary deficiencies (e.g., bulimia and anorexia nervosa) may be interesting tests of the model.

Implications of Spatial Isotope Ratio Variations in Human Hair. The prediction of spatially explicit patterns in the δ^2H_h and $\delta^{18}O_h$ values of human hair has many potential implications with respect to human movements and with respect to specifying regions of origin in anthropological, archeological, and forensic studies. One potential and practical application of the spatial variations predicted in Fig. 3 are estimating the region-of-origin of unidentified human remains in forensic investigations. Occasionally, medical examiners and police investigators are faced with the situation where they do not have identities of murder victims or discovered human remains. One of the potential applications of the model is to evaluate whether the hair associated with forensic material is isotopically consistent with hair expected from individuals of that region. If not, the geographical maps make explicit predictions of the regions in the United States that would be isotopically consistent with the observed hair isotope ratios for that individual. In addition, by using the range of known growth rates of human hair and models of tissue turnover (33), it may be possible to place constraints on the time when an individual moved from one locality to another. Although it is perhaps early to make explicit predictive use of Fig. 3 or forensic investigations, the quality and utility of such maps will become more useful to anthropological, archeological, and forensic studies as we better understand the tap-water variations across the United States and the mechanistic basis of dietary inputs to hair δ^2H_h and $\delta^{18}O_h$ values.

Materials and Methods

Human hair samples were collected as discarded, trash clippings from the floor and garbage containers in barbershops from 65 geographically distributed cities or towns across the United States. Three sets of discarded hair clippings were collected in cities or towns with populations of $<$100,000 to increase the probability that the hair sample was associated with a local citizen. No information is available regarding the identification and/or origins of the individuals from which discarded hair samples were obtained. We assume that these individuals were residents of the city in which the hair sample was collected and that they had no dietary differences. Hair samples were collected from 18 states: Arkansas, Colorado, Idaho, Illinois, Indiana, Kentucky, Louisiana, Minnesota, Missouri, Montana, Nebraska, New Mexico, North Dakota, Oklahoma, Texas, Utah, Wisconsin, and Wyoming. In each of these cities, we also collected three 25-ml vials of tap water from different locations within the city; the data presented are the means. These hair and tap-water samples were used to evaluate the relationships between hair and tap water. For later

inverse model evaluations of the regression models that would be produced, we collected an additional pair of hair and water samples that could be used to independently evaluate the models.

To evaluate the time trend associated with an individual moving from one geographic location to another, a single strand of hair was analyzed. This hair sample was cleaned in methanol and then sectioned into 1-cm increments for isotope ratio analyses. This research was conducted under University of Utah Institutional Review Board (IRB) permit 10249, although IRB permission was not required to sample trash, such as the discarded hair clippings from the floor of a barbershop.

Hair samples consisting of 20–40 strands of hair were treated as if they were homogeneous samples. The entire subsample was ground into a homogeneous, fine powder. Because there is partial isotopic exchange of H atoms in keratin with water (either in liquid or vapor form), all samples were analyzed together with hair reference materials for which the δ^2H of nonexchangeable H had been determined (17). Both reference material and unknown hairs were allowed to equilibrate with water vapor in the laboratory atmosphere, desiccated under vacuum for 7 days; 150-μg samples of each were weighed and analyzed. Measured values for the standard hairs were used to determine the nonexchangeable H isotope ratios of the samples from the measured values and isotope mass balance (17).

The δ^2H and δ^{18}O values of both hair and tap-water (40 μl) samples were measured by using an isotope ratio mass spectrometer operating in a continuous flow mode (ThermoFinnigan Delta Plus XL). The sample was pyrolyzed to form H_2 and CO in a thermochemical elemental analyzer furnace (TCEA; ThermoFinnigan) and analyzed for ^{2}H and ^{18}O content (30). Gases were transported in a continuous-flow fashion by using a He carrier gas from the point of the pyrolysis reaction through a GC separation column to the ionization source of the mass spectrometer. For hair, samples were stored on a zero-blank autosampler (Costech Analytical), thereby ensuring that water vapor could not interact with the sample during the analysis period. For tap-water analyses, 400-μl subsamples were placed in glass containers and then injected (10 μl) by using an autosampler that transferred the sample to the elemental analyzer for pyrolysis.

Stable isotope ratio abundances are expressed in ''delta'' notation (δ), relative to an international standard as

$$\delta(‰) = (R_{\text{sample}}/R_{\text{standard}} - 1)\cdot 1000‰,$$

where R_{sample} and R_{standard} are the molar ratios of the heavy to light isotopes (^{2}H/^{1}H, ^{18}O/^{16}O) of the sample and standard. All δ-values are expressed in per mil (‰) values relative to the international standard SMOW. Hair samples were analyzed by using two in-house laboratory reference materials in the laboratory: hair from Florida (isotopically heavy end member) and Utah (isotopically light end member). Water samples were analyzed by using University of Utah manufactured ''zero'' water (heavy end member) and deionized tap water (light end member). The 2σ precisions of δ^2H and δ^{18}O analyses for water samples were $\pm$0.9‰ and $\pm$0.2‰, respectively, and for hair samples were $\pm$4‰ and $\pm$0.4‰, respectively.

Isotope fractionation (α_{AB}) is defined as

$$\alpha_{AB} = (\delta_A + 1{,}000)/(\delta_B + 1{,}000)$$

and subsequently the isotope enrichment ε_{AB} is expressed as

$$\varepsilon_{AB} = (\alpha_{AB} - 1)\cdot 1{,}000,$$

with ε expressed in per mil (‰).

Geographic maps were produced by using the geographic information system program ArcGIS 9 (ESRI), which incorporated as a driver a spatially explicit map of the isotope ratios of tap water for the coterminous United States (11). Calculation capacities within ArcGIS were then used to relate hair and tap-water observations from this study to the predicted distributions of the hydrogen and oxygen isotope ratios of hair across the United States.

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