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# Tracking human travel using stable oxygen and hydrogen isotope analyses of hair and urine

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**The stable oxygen and hydrogen isotope compositions of organic samples are increasingly being used to investigate patterns of animal migration. Relatively few studies have applied these techniques to modern humans, despite a variety of potential forensic applications. We analyzed drinking water and food at two geographic locations, East Greenbush, New York (USA) and Fairbanks, Alaska (USA), with different $\delta^{18}O$ and $\delta D$ values for precipitation and tap water. Foods varied widely in measured $\delta^{18}O$ and $\delta D$ values, but not systematically by purchase location. We measured $\delta^{18}O$ and $\delta D$ values of scalp hair from five residents at each location. We used a mixing model to establish the proportion of oxygen and hydrogen in head hair derived from drinking water (~27% and ~36%, respectively). Finally, we analyzed the $\delta^{18}O$ and $\delta D$ values of facial hair and urine from a subject who traveled from Fairbanks to East Greenbush, on to the UK and back to Fairbanks. Urine $\delta^{18}O$ and $\delta D$ values responded immediately and strongly to travel-related change in drinking water, and were well described by a single-pool turnover model. Beard hair $\delta^{18}O$ values tracked changes in urine $\delta^{18}O$ closely, and oscillated between the values for the resident populations in both locations. In contrast, beard hair $\delta D$ values did not track changes in urine $\delta D$ as well, and retained a signature of the traveler's permanent residence. Our findings show that the $\delta^{18}O$ and $\delta D$ values of urine and facial hair (specifically $\delta^{18}O$) can provide a record of the geographical movements of humans.** Copyright © **2007 John Wiley & Sons, Ltd.**

Stable isotope analyses have become a popular way to gain insight into patterns of animal movement.[1–3] Many of these studies take advantage of the large-scale continental gradients in the stable hydrogen and oxygen isotope composition of meteoric water. Generally, precipitation in locations at higher latitudes is depleted in both $^{18}O$ and $^{2}H(D)$ compared with lower latitudes,[4–6] although topography and other factors can also influence the $\delta^{18}O$ and $\delta D$ values of precipitation.[7] Numerous studies have attempted to delineate animal populations and their movements using $\delta D$ signatures preserved in organic tissue, often in combination with stable isotope analyses of additional elements (e.g. C or N).[8–14] A number of studies have also used variation in $^{18}O$ to track animal movements, typically using inorganic materials (e.g. teeth[15,16]). Few studies have investigated the combined values of $\delta D$ and $\delta^{18}O$ in organic tissues;[17,18] however, technical developments have streamlined this type of analysis for organic samples[19–21] such that these analyses are becoming increasingly routine.

Interpretation of $\delta^{18}O$ and $\delta D$ values in organic tissues is complicated by the fact that oxygen and hydrogen can derive both from drinking water and diet.[17,18,22] In animal populations it is generally assumed that animals and the plants or animals that they eat draw on similar sources of water; thus, the $\delta^{18}O$ and $\delta D$ values of an animal's diet and drinking water should vary in parallel.[1,2,18] However, when an animal's diet includes items expected to have dissimilar $\delta^{18}O$ and $\delta D$ values from drinking water (e.g. a high latitude terrestrial animal with a marine dietary component), this assumption may not be correct (as others have noted[18]). Subsequently, interpretation of $\delta^{18}O$ and $\delta D$ values in organic tissue samples becomes even more complicated when applied to modern human populations.[17]

Isotopic analyses have a rich history in the human anthropological literature;[23–27] including the use of inorganic $^{18}O$ from teeth and bone to track past human movements.[28,29] Studies of modern humans are confined to organic tissues that can be non-invasively sampled (e.g. hair and nail), and have largely focused on $\delta^{13}C$ or $\delta^{15}N$ values as dietary indicators.[30–35] However, there is a growing interest in tracking modern human movements via values of hair and nail $\delta^{18}O$ and $\delta D$.[17] Studies of this type could find a wide variety of forensic applications; for example, establishing the likelihood of reported travel or location of origin.[1,17]

Studies using organic $\delta^{18}O$ and $\delta D$ values to track the movements of modern humans are required to consider the multiple sources of oxygen and hydrogen to nail and hair

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tissue, and the kinetics by which oxygen and hydrogen isotope signatures change. In many human populations foods are non-locally grown and may derive from a huge range of geographical origins (e.g.[36]). The input of oxygen and hydrogen from these globally homogenized food sources into hair and nail will dampen the expected tissue variation based on local $\delta^{18}O$ and $\delta D$ values in drinking water. Furthermore, the isotopic composition of atmospheric oxygen will contribute to the isotopic signature of body water,[37–39] and, ultimately, hair. Understanding the proportional contributions of diet, drinking water, and atmospheric $O_2$ to hair and nail oxygen and hydrogen will be important to interpreting $\delta^{18}O$ and $\delta D$ values in humans.[38] Sharp and colleagues[18] tackled this question for $\delta D$ values in human hair, using a $\delta D$-labeling study and paired $\delta D$ measurements in urine and hair. Information on the magnitude and rates of $\delta^{18}O$ and $\delta D$ changes in human tissue during known travel will help to further calibrate this emerging tool for human forensics.

In this study we investigated the $\delta^{18}O$ and $\delta D$ values of hair from residents in two locations (East Greenbush, New York, USA and Fairbanks, Alaska, USA) that have distinctly different precipitation $\delta^{18}O$ and $\delta D$ values. We hypothesized that the $\delta^{18}O$ and $\delta D$ values of the drinking water in these two locations would also be distinctly different, with the water from our high latitude site (Fairbanks) having lower $\delta^{18}O$ and $\delta D$ values than our lower latitude site (East Greenbush). Because a portion of the organic hydrogen and oxygen in humans derives from diet, as well as from drinking water, we wished to examine the magnitude of $\delta^{18}O$ and $\delta D$ variation in a suite of food items. We collected a variety of food samples from both Fairbanks and East Greenbush for $\delta^{18}O$ and $\delta D$ analysis. As American diets typically include a majority of non-locally grown and nationally distributed foods, we hypothesized that the $\delta^{18}O$ and $\delta D$ values of food items from our two locations would show a large range of variation, but that their average signatures would not differ significantly. Using the tap water and resident hair data we constructed a mixing model to assess the proportion of oxygen and hydrogen in hair derived from drinking water. Finally, we analyzed the $\delta^{18}O$ and $\delta D$ values of urine and facial hair taken from a human subject during travel between Fairbanks and locations at lower latitudes (East Greenbush, NY, USA; and Littlehampton, UK), as a test case for a forensic interpretation of human variation in hair $\delta^{18}O$ and $\delta D$ values.

## EXPERIMENTAL

### Sample collection

All our human sampling was conducted with the approval of the University of Alaska Fairbanks Institutional Review Board (IRB Protocol 04-55). Head hair samples from five resident individuals (termed here residents) from East Greenbush, NY and Fairbanks, AK were taken between October 2006 and November 2006. Root material was removed with a scalpel under a dissecting microscope, and only the basal 1 cm of the hair samples was retained for $\delta^{18}O$ and $\delta D$ analysis. Human head hair grows at a rate of approximately 1 cm per month.[40] The basal 1 cm portions were taken from at least five hairs from an individual to yield a sufficient sample mass (0.1–0.3 mg). To be considered a resident and to be included in our study it was required that a subject had remained in the same area for at least 2 months prior to the samples being taken; thus, the basal 1 cm of hair from our participants should reflect local diet and water only. Tap water samples were collected into 15 mL centrifuge tubes and were frozen until analysis. Ninety food items were bought from supermarkets in Fairbanks (Table 1) and East Greenbush (Table 2). The food items from Fairbanks included a week's worth of diet items collected by a high-school lab intern. The food items from East Greenbush were selected to include a subset of those items collected in Fairbanks, and some additional opportunistic samples. These samples were freeze-dried, ground and weighed for $\delta^{18}O$ and $\delta D$ analysis (described below). We note that freeze-drying food samples removes the water component, estimated to contribute ~20% of the total water budget of adults in the USA (not including beverages).[41] The extent to which this water tracks the organic O and H isotope signatures of diet is not known, but its contribution (and that of bottled beverages) would be expected to reduce the correspondence between body water and local drinking water.

A human subject (termed here the migrant) was also selected to study during a trip from Fairbanks to locations at lower latitudes (East Greenbush, NY, USA and Littlehampton, UK). The individual was a 34-year-old male and was a permanent resident in Fairbanks, AK at the time of study. The subject traveled from Fairbanks to East Greenbush on 21 December 2003, and then from East Greenbush to Littlehampton, UK on 30 December 2003. The subject returned to Fairbanks from the UK on 8 January 2004. The subject dry-shaved a patch of facial hair stubble and collected a urine sample each morning during his trip, and for 24 days after returning to Fairbanks. Beard hair samples were collected into 3 mL Eppendorf tubes, and urine samples were collected into 15 mL Falcon tubes and stored frozen until analysis. Drinking water samples were taken at points during this travel schedule. We note that the subject also traveled from Fairbanks to Massachusetts and New York from 20 to 26 November, 2003; and we assess whether this earlier trip impacted on signatures at the beginning of the study trip.

### Stable isotope analyses

For the analysis of the $\delta^{18}O$ and $\delta D$ values of water and urine, each of the samples was transferred into a 3 mL glass gas chromatography (GC) vial that was capped shut with no headspace. The vials were loaded into an A200SE liquid autosampler (CTC Analytics, Zwingen, Switzerland) and 0.2 μL of each sample was injected into an on-line pyrolysis, thermochemical reactor elemental analyzer (TCEA; Thermo Scientific, Woburn, MA, USA) coupled via continuous flow (Conflo III) to a Delta V isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). The hydrogen reference gas was University of Alaska Fairbanks Ultra High Pure grade hydrogen ($\delta D = -223‰$) and the carbon monoxide reference gas was from GTS Inc. (Allentown, PA, USA) and was their isotope grade gas ($\delta^{18}O = -2.7‰$). The quality control scheme involved analyzing laboratory working standards after every seventh sample. Laboratory working standards were internally calibrated Duckering





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**Table 1.** $\delta$D and $\delta^{18}$O values of food items bought from supermarkets in Fairbanks, AK (USA), and the food type category to which they were assigned. Mean $\delta D = -101 \pm 47‰$, mean $\delta^{18}O = 24 \pm 7‰$, n = 59

| Food item | Food type | δD‰ | $\delta^{18}$O‰ | Food item | Food type | δD‰ | $\delta^{18}$O‰ |
|---|---|---|---|---|---|---|---|
| Butter | dairy | −228.2 | 5.3 | Raisins (Chocolate Covered) | mixed | −40.6 | 33 |
| Cheese (American) | dairy | −145 | 13.3 | Yogurt (Yoplait Fat Free Strawberry) | mixed | −56.7 | 22.6 |
| Cheese (Natural Vermont Cheddar) | dairy | −187.5 | 7.4 | Yogurt (Yoplait Orange Crème) | mixed | −75.2 | 28.9 |
| Cheese Fondue | dairy | −198.9 | 13.9 | Coffee (Mocha Frappachino) | mixed | −94.6 | 27.6 |
| Eggs (Scrambled) with Cheese | meat/eggs | −172.2 | 12.3 | Cookie (Rasberry Milano) | mixed | −98.6 | 30.8 |
| Meat (Pork Steak with spices) | meat/eggs | −156.2 | 7.6 | Cookie (Taffy) | mixed | −128.8 | 24.8 |
| Meat (Turkey Breast) | meat/eggs | −141.1 | 14.6 | Cookie (Milano Chocolate) | mixed | −78.9 | 29.5 |
| Peanuts (Butter Toffee) | mixed | −105.5 | 31.9 | Cookie (Chocolate Chip) | mixed | −68.3 | 33.3 |
| Bagel (Turkey/Cheese/Sprouts) | mixed | −120 | 20.5 | Apple | fruit/vegetable | −97.1 | 23.8 |
| Bagel (Cranberry) | mixed | −88.9 | 29.1 | Apple | fruit/vegetable | −128 | 23.5 |
| Bagel (Turkey/Cheese) | mixed | −89.3 | 28 | Apple | fruit/vegetable | −69.2 | 29.2 |
| Bagel (Ham) | mixed | −125.5 | 21.3 | Apple | fruit/vegetable | −80.1 | 25.7 |
| Bread (White) | mixed | −90.6 | 28.3 | Broccoli | fruit/vegetable | −127.8 | 21.7 |
| Bread (English Muffin) | mixed | −113.6 | 26.6 | Broccoli | fruit/vegetable | −74 | 28 |
| Bread (Apple Cinnamon) | mixed | −112.9 | 28.5 | Carrots | fruit/vegetable | −47.1 | 23.7 |
| Bread (Almond Poppyseed Muffin) | mixed | −133.5 | 24.7 | Carrots | fruit/vegetable | −122.3 | 15.9 |
| Bread with Butter | mixed | −121 | 21.2 | Celery | fruit/vegetable | −76 | 25.1 |
| Cereal (Cranberry Almond Crunch) (box 1) | mixed | −61.4 | 29.9 | Grapes | fruit/vegetable | −13.4 | 30.1 |
| Cereal (Cranberry Almond Crunch) (box 2) | mixed | −35.7 | 30 | Grapes | fruit/vegetable | −19.6 | 34.9 |
| Cereal (Banana Nut Crunch) | mixed | −99.7 | 24.2 | Honey | fruit/vegetable | −90.4 | 24.1 |
| Chips (Lay's Potato) | mixed | −147.3 | 22.2 | Raisins | fruit/vegetable | −26.4 | 33.7 |
| Chips (BBQ Potato) | mixed | −155.3 | 26.4 | Peanuts (Salted) | fruit/vegetable | −174.7 | 22.8 |
| Chips (Spicy Cheddar Doritos) | mixed | −93.2 | 26.8 | Candy (Hershey's Classic Caramels) | refined sugar | −60.3 | 31.9 |
| Lasagne | mixed | −131.9 | 19.9 | Candy (Hershey's Classic Caramels) | refined sugar | −69.7 | 26.9 |
| Milkshake (Rasberry) | mixed | −98.3 | 23.4 | Candy (Snickers Bar) | refined sugar | −73.9 | 28.2 |
| Peanut Butter | mixed | −158.5 | 23.4 | Candy (Raspberry/Vanilla Sherbert) | refined sugar | −64.6 | 29.7 |
| Pizza (Cheese) | mixed | −163.1 | 11.4 | Fruit juice (Cranberry/Raspberry) | refined sugar | −26.6 | 29.1 |
| Pizza (Pepperoni) | mixed | −126.3 | 17 | Fruit juice (Cranberry) | refined sugar | −19.1 | 32.2 |
| Soup (Chicken and Rice) | mixed | −97 | 22.4 | Lemonade (Minute Maid) | refined sugar | −40.8 | 29.7 |
| Whipped Crème | mixed | −118.2 | 21.2 | | | | |

Building Millipore water (DMW), NIST (REF 8535 V-SMOW), GISP (8536) and SLAP (8537), and measured vs. expected had an $R^2$ of >0.99. Multiple $\delta^{18}$O and $\delta$D analyses of DMW (n = 15) conducted during the sample sequence yielded standard deviation (std dev.) = 0.4‰ and 1.7‰, respectively. Each sample and standard was analyzed in triplicate. Triplicate $\delta^{18}$O and $\delta$D analyses of separate DMW and water samples yielded std dev. of ≤0.3‰ and 1.6‰, respectively. All the $\delta^{18}$O and $\delta$D values reported in this paper are expressed in standard delta ($\delta$) notation in parts per thousand (‰) relative to Vienna Standard Mean Ocean Water (V-SMOW).

Between 0.1 and 0.3 mg of each of the freeze-dried organic samples (beard hair and food items) were weighed using a microbalance. All samples were then freeze-dried for ≥10 days prior to stable isotope analysis, at which point they were taken straight from the freeze drier and loaded into a Costech Zero-Blank autosampler (Costech Analytical Technologies, Valencia, CA USA) that was purged with research grade helium. The helium was dried prior to use in the autosampler by passing it through an Alltech All-pure column (Grace, Deerfield, IL USA). By freeze drying our solid samples for ≥10 days our approach was consistent with that described by Bowen *et al.*,[42] to increase analytical

**Table 2.** $\delta$D and $\delta^{18}$O values of food items bought from a supermarket in East Greenbush, NY (USA), and the food type category to which they were assigned. Mean $\delta D = -107 \pm 41‰$, mean $\delta^{18}O = 26 \pm 6‰$, n = 30

| Food item | Food type | δD‰ | $\delta^{18}$O‰ | Food item | Food type | δD‰ | $\delta^{18}$O‰ |
|---|---|---|---|---|---|---|---|
| Butter | dairy | −185.5 | 3 | Celery | fruit/vegetable | −89.8 | 23.8 |
| Cheddar cheese | dairy | −177.8 | 17.5 | Cranberries | fruit/vegetable | −91.1 | 25.6 |
| Eggs (scrambled) with Cheese | meat/eggs | −167.2 | 22.2 | Green bean | fruit/vegetable | −98.8 | 26.8 |
| Ground beef | meat/eggs | −167.3 | 17.6 | Peach | fruit/vegetable | −83.1 | 27.8 |
| Ham | meat/eggs | −136.2 | 28.8 | Peach half, with skin | fruit/vegetable | −100.4 | 26.6 |
| Apple sauce (with sugar) | mixed | −71 | 28.1 | Raisins | fruit/vegetable | −71.6 | 31 |
| Banana nut muffin | mixed | −139.5 | 25.5 | Strawberry | fruit/vegetable | −119 | 27.2 |
| Bread | mixed | −97.3 | 30.2 | Tomato | fruit/vegetable | −110.7 | 26.7 |
| Cookie (chocolate chip) | mixed | −102.2 | 31.4 | Yellow squash | fruit/vegetable | −88.7 | 28.5 |
| Lasagna | mixed | −124.3 | 26.8 | Flour (unbleached) | fruit/vegetable | −79.9 | 30.9 |
| Peanut butter | mixed | −141.6 | 26.1 | Honey | fruit/vegetable | −80.4 | 24.3 |
| Potato chips | mixed | −136.3 | 28.4 | Peanuts (salted) | fruit/vegetable | −162 | 31.9 |
| Basil | fruit/vegetable | −118 | 22.4 | Cocoa (Nesquik) | refined sugar | −97.3 | 24.9 |
| Broccoli | fruit/vegetable | −87 | 32.4 | Sugar | refined sugar | −19.9 | 34.4 |
| Carrot | fruit/vegetable | −87.6 | 24.2 | Lemonade | refined sugar | −3.6 | 28 |





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precision and accuracy associated with the $\delta^{18}O$ and $\delta D$ values of organic samples. Measurements of the $\delta^{18}O$ and $\delta D$ values of organic samples were made using the TCEA (Thermo Scientific) coupled to the Conflo III and the Delta V isotope ratio mass spectrometer (Thermo Scientific). $\delta^{18}O$ and $\delta D$ values of sample gases (CO and H) from each sample were measured relative to calibrated reference gases (CO and H). The $\delta^{18}O$ values of samples were calibrated relative to international stable isotope standards (NBS N-1, NBS-18, NBS-19, and an internal calcite standard) and an internally calibrated keratin standard (Bowhead Whale Baleen Keratin – BWBII[43] (measured vs. expected $R^2 = >0.99$).

The exchange of keratin hydrogen with atmospheric water can influence $\delta D$ measurements of keratin samples.[18,42–44] The $\delta D$ values of hair samples were therefore calibrated relative to a suite of keratin standards of known $\delta D$ (BWBII, Hoof Keratin Standard – CHS and Feather Keratin Standard – CFS[43]), which were analyzed with each run of organic samples (measured vs. expected $R^2 = >0.99$) to account for exchangeable hydrogen. The $\delta^{18}O$ and $\delta D$ analyses of laboratory working organic standards (benzoic acid; Fisher Scientific, Pittsburgh, PA, USA; lot No 947459, and BWBII) also included throughout each run after at least every tenth sample yielded std dev. analytical precisions of $\pm \leq 0.7‰$ and $\pm \leq 1‰$, respectively. Blanks were analyzed after every twentieth sample.

### Data analysis

All statistical analyses were performed using JMP IN version 5.2.1 (SAS Institute, Cary, NC, USA). We compared the mean $\delta^{18}O$ and $\delta D$ values of tap waters and foods collected in East Greenbush and Fairbanks using *t*-tests, and tested the relationship between $\delta^{18}O$ and $\delta D$ values of food samples using linear regression. We tested the effect of collection location and food type on food $\delta^{18}O$ and $\delta D$ values using analysis of variance (ANOVA), and assessed differences among food types with Tukey's HSD contrasts. Isotopic differences in hair samples from Fairbanks and East Greenbush resident populations were assessed using t-tests. Urine samples from the 'migrant' subject after his return to Fairbanks were used to model body water turnover, using a single-pool turnover model of the form $y = a + b\,(e^{-r \cdot day})$, where r = the fractional turnover rate and the 'half-life' (or time for 50% of isotopic change to occur) is calculated as half life $= -\ln(0.5)/r$. The turnover model was fit to the data using the non-linear fitting platform in JMP IN. We assessed the relationship between $\delta^{18}O$ and $\delta D$ values in urine and hair using linear regression. We lagged urine samples to account for the time it takes for hair to be formed in the follicle and appear above the skin. For human scalp hair, the time required for newly synthesized hair to appear above the skin has been estimated at 9.7 days based on uptake of blood mercury;[40] and in a study involving isotopically labeled drinking water, Sharp *et al.*[18] report a lag of 8 days between urine and beard hair $\delta D$. However, each of these studies was conducted on a single individual, so the expected range of variation in lag times for human beard or head hair formation is not known. Here we observe a 5-day shift between the isotopic maxima of both the $\delta^{18}O$ and $\delta D$ values in hair, compared with those of urine. Thus, we lag the hair data by 5 days when we compare it directly with urine data.

## RESULTS

### Stable oxygen and hydrogen isotope composition of drinking water and food

As expected, Fairbanks drinking water (n = 23) was depleted in the heavier stable isotopes of oxygen and hydrogen relative to tap water from East Greenbush (n = 6) ($\delta^{18}O = -19.9 \pm 0.4‰$ vs. $-8.8 \pm 0.9‰$, $\delta D = -156.2 \pm 1.7‰$ vs. $-54.8 \pm 7.4‰$, Figs. 1 and 2).[4,5] The water data fell along the global meteoric water line (GMWL) (Fig. 1).

Although the $\delta^{18}O$ and $\delta D$ values of food samples varied widely ($\delta^{18}O = -3‰$ to 34.9‰; $\delta D = -228.2‰$ to $-40.8‰$) (Fig. 1), there was no significant difference in the mean $\delta D$ or $\delta^{18}O$ values of food items purchased in Fairbanks (Table 1) and of those purchased in East Greenbush (Table 2) ($\delta D$: $t = 0.697$, $P = 0.489$; $\delta^{18}O$: $t = -1.3$, $P = 0.192$; Fig. 2). A positive, linear relationship existed between $\delta D$ and $\delta^{18}O$ values for the entire sample of food items shown in Fig. 1 ($R^2 = 0.538$, $P < 0.0001$, Eqn: $\delta D = 5.09 \cdot \delta^{18}O - 230$). The relationship follows the GMWL but with a shallower slope (5.09, with 95% lower and upper confidence limits = 4.08 and 6.09, respectively)

We characterized food items according to five different categories: dairy, meat/eggs, fruit/vegetables, refined sugar (e.g. candy, sweetened beverages, sugar), and mixed (e.g. pizza or sweetened flavored yogurt). Dairy and meat/eggs were the most depleted in both $^2H$ and $^{18}O$, whereas foods based on refined sugars were the most enriched (Fig. 2). We tested the effects of sampling location and food category on $\delta^{18}O$ and $\delta D$ values using ANOVA (Table 3). Food category had a highly significant effect on both isotopes, while sampling location was non-significant for $\delta D$ but weakly

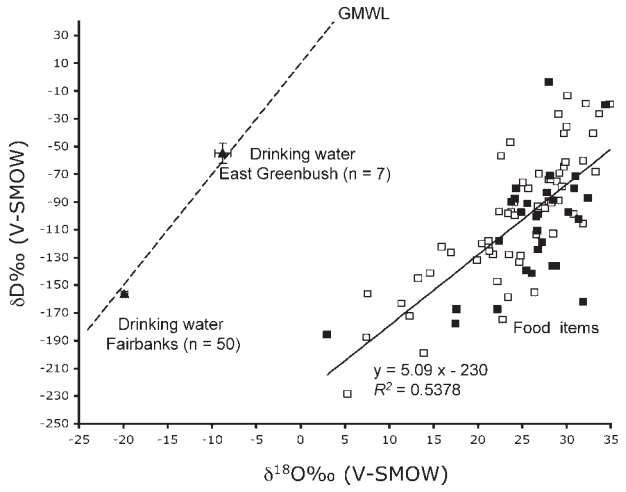


**Figure 1.** $\delta D$ and $\delta^{18}O$ values of drinking water from Fairbanks, AK (USA) and East Greenbush, NY (USA) (filled triangles), and foods from East Greenbush (filled squares) and Fairbanks (open squares). Bold solid line represents the regression line placed through all of the food samples. Dashed line = the Global Meteoric Water Line ($\delta D = 8\delta^{18}O + 10$).





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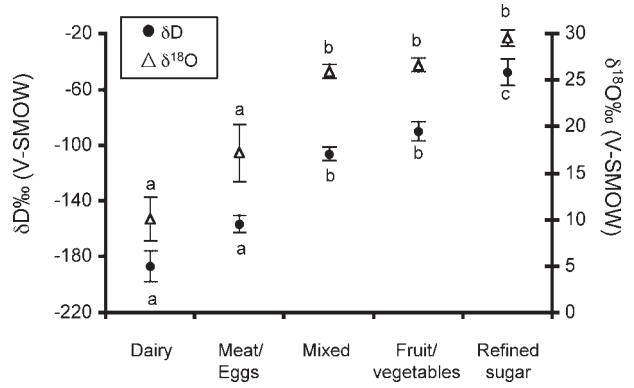


**Figure 2.** Variation in $\delta$D and $\delta^{18}$O values among categories of foods obtained in East Greenbush, NY and Fairbanks, AK. The effect of food type and purchase location on food $\delta$D and $\delta^{18}$O was assessed with ANOVA (Table 4). Means marked with different letters are significantly different in isotope ratio (Tukey's HSD, $P < 0.05$).

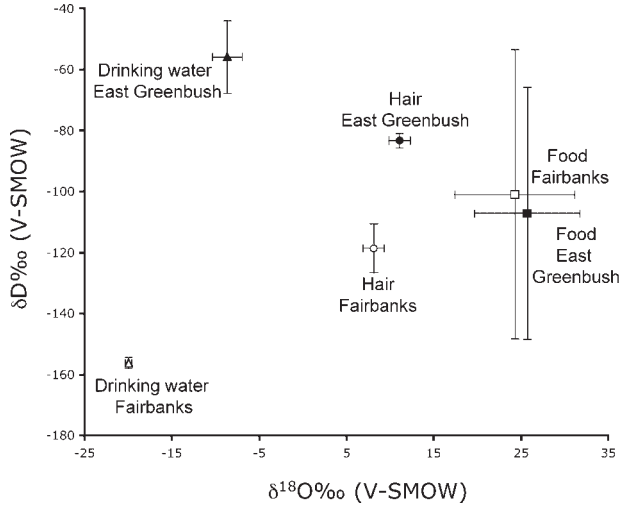


**Figure 3.** Hair $\delta$D and $\delta^{18}$O values from five residents from Fairbanks, AK and five residents from East Greenbush, NY, relative to the mean isotopic composition of drinking water and foods sampled in these locations.

significant for $\delta^{18}$O values. This significant difference was probably due to the meat/egg samples, which were $^{18}$O-enriched in East Greenbush relative to Fairbanks (Tables 1 and 2).

## Stable oxygen and hydrogen isotope composition of hair from residents

Hair from five residents of East Greenbush was enriched in both $^{18}$O ($\delta^{18}O = 11.1 \pm 1.2$‰) and $^{2}$H ($\delta D = -83.3 \pm 2.4$‰) compared with hair from five residents of Fairbanks ($\delta^{18}O = 8.1 \pm 1.2$, $\delta D = -118.6 \pm 8.0$‰) (Fig. 3, Table 4). Both differences were statistically significant ($t$-tests: $t = 3.94$, $P = 0.0043$ for $\delta^{18}$O and $t = 9.43$, $P < 0.0001$ for $\delta$D, both with $df = 8$). We calculate the contribution of oxygen and hydrogen from drinking water to hair in residents using the following mixing expression:[18,45]

$$\%\ \text{hair oxygen from drinking water} = (\delta^{18}O_{\text{NY hair}} - \delta^{18}O_{\text{AK hair}})/(\delta^{18}O_{\text{NY water}} - \delta^{18}O_{\text{AK water}})$$

using tap water values for Fairbanks and East Greenbush in the denominator and the average values of hair $\delta^{18}$O for residents of each location (reported above) in the numerator. We note that the use of this expression assumes that other sources of hair oxygen in these populations (e.g. diet, atmospheric $O_2$) do not differ isotopically between locations, nor do their fractional contributions of oxygen to hair. We use the same expression to calculate the % hair hydrogen from drinking water. Based on these assumptions, we calculate that 27% of human hair oxygen, and 36% of human hair hydrogen, derives from drinking water. Our estimate of the contribution of drinking water to human hair hydrogen is very similar to the 31% reported by Sharp et al.[18]

**Table 3.** The effect of sampling location and food category on food $\delta^{18}$O and $\delta$D values tested with ANOVA. Results demonstrate that food type has a much greater influence on food $\delta^{18}$O and $\delta$D values than purchase location

| | $\delta^{18}$O‰ | | | | $\delta$D | | | |
|---|---|---|---|---|---|---|---|---|
| Effect: | SS | df | F | $P$ | SS | df | F | $P$ |
| Location | 98 | 1 | 4.86 | 0.0302 | 1283 | 1 | 1.25 | 0.2665 |
| Food type | 2049 | 4 | 25.43 | <0.0001 | 96061 | 4 | 23.42 | <0.0001 |
| Error | 1672 | 83 | | | 85092 | 83 | | |

## Stable oxygen and hydrogen isotope composition of hair and urine during travel between regions with distinct water isotope composition

Both $\delta^{18}$O and $\delta$D values in urine from our migrant research subject changed rapidly, smoothly, and dramatically in response to travel (Fig. 4, Table 5). Changes in hair $\delta^{18}$O and $\delta$D values basically followed those of urine, but with more scatter (Fig. 4). Hair samples lagged behind the values of urine $\delta^{18}$O and $\delta$D by ~5 days. Once the 5-day lag was accounted for, changes in hair $\delta^{18}$O values tracked urine fairly closely (Fig. 5(a)). The relationship between hair and urine $\delta^{18}$O values was well described by a linear relationship:

**Table 4.** $\delta$D and $\delta^{18}$O values of scalp hair from resident humans in East Greenbush, NY (USA) and Fairbanks, AK (USA)

| Individual number | $\delta^{18}$O‰ | $\delta$D |
|---|---|---|
| AK1 | 9.55 | −109.60 |
| AK 2 | 9.14 | −121.05 |
| AK 3 | 6.71 | −126.75 |
| AK 4 | 7.24 | −124.97 |
| AK 5 | 8.04 | −110.59 |
| **Mean** | **8.14** | **−118.59** |
| **Standard deviation** | **1.21** | **8.03** |
| NY1 | 11.71 | −85.31 |
| NY2 | 12.02 | −83.97 |
| NY3 | 11.27 | −79.94 |
| NY4 | 8.97 | −81.75 |
| NY5 | 11.44 | −85.38 |
| **Mean** | **11.08** | **−83.27** |
| **Standard deviation** | **1.21** | **2.37** |





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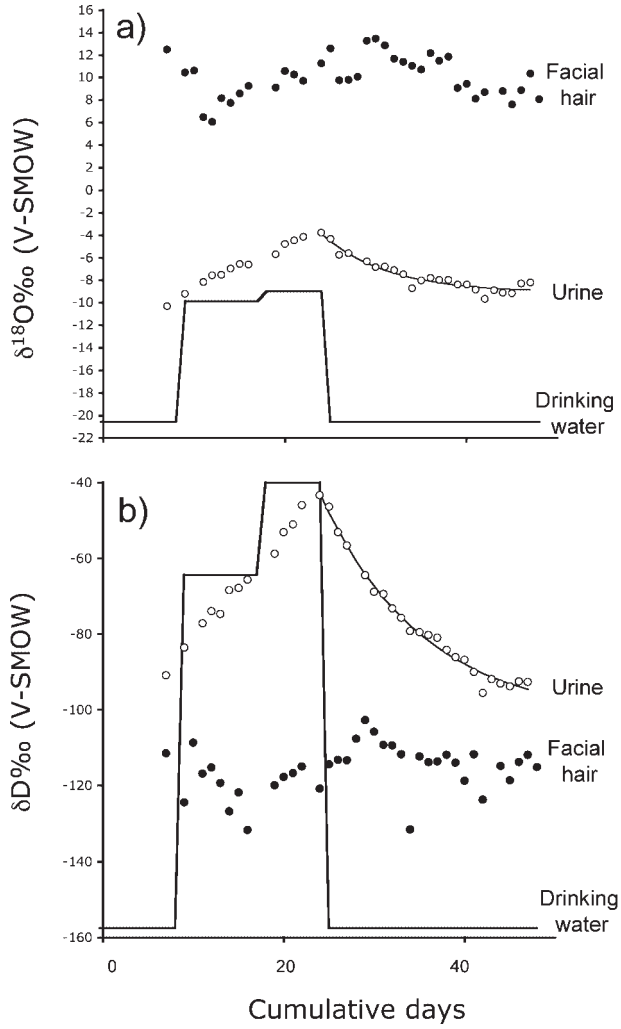


**Figure 4.** $\delta^{18}O$ (a) and $\delta D$ (b) values of beard hair and urine of a human subject traveling from Fairbanks, AK to East Greenbush, NY and Littlehampton (UK). The duration of the trip is indicated by the changes in drinking water $\delta D$ and $\delta^{18}O$ values, to values characteristic of tap water at the destinations. Lines through the urine data represent the best fit of a single-pool turnover model ($a + be^{-r \cdot day}$).

hair $\delta^{18}O = 0.79$ (urine $\delta^{18}O) + 15.709$ ($R^2 = 0.554$, $P < 0.0001$; Fig. 5(a)). Data from two collection days (Fig. 5(a)) fit the linear model poorly; removing those data points changed the slope of the relationship between urine and hair $\delta^{18}O$ values to 1.01. In contrast, the relationship between lagged hair and urine $\delta D$ was only weakly positive and not well described by a linear relationship ($R^2 = 0.111$, $P = 0.083$; Fig. 5(b)). The magnitude of isotopic change in hair $\delta D$ values was much less than that revealed in the urine samples and never approached that of East Greenbush residents.

The decline in urine $\delta^{18}O$ and $\delta D$ values after the subject's return to Fairbanks was well described by a single-pool turnover model (see lines of fit in Fig. 4). The fractional turnover rate of urine $\delta^{18}O$ was estimated to be 0.14 days$^{-1}$ for $\delta^{18}O$ (lower and upper 95% confidence interval (CI) = 0.09 and 0.20, respectively), corresponding to a half life of 5.0 days. For urine $\delta D$ values, the fractional turnover rate was 0.09 (lower and upper 95% CI = 0.07 and 0.11), corresponding to a half-life of 7.9 days. If we use the less variable half-life estimate of 7.9 days from the $\delta D$ data, we calculate that turning over 90% of total body water (as reflected by urine) requires ~24 days.

We note that beard hair sample values declined over the first week of travel, despite travel to regions with relatively enriched drinking water (Fig. 4). We interpret this decline in light of a previous trip made by the research subject to NY (USA) and MA (USA) 25 days prior to the experimental trip presented in this study. According to our calculations above, urine would be only just returning to Fairbanks values at the onset of the experimental trip, with beard hair $\delta^{18}O$ and $\delta D$ values lagging behind urine values by at least 5 days.

## DISCUSSION

Food items from East Greenbush and Fairbanks were highly variable in values of both $\delta^{18}O$ and $\delta D$, but generally did not differ isotopically by region of purchase. This result is consistent with the fact that modern American diets are composed of foods that are nationally distributed, and from a wide range of geographic origins. The similar slope of the regression line through the food data compared with the GMWL supports the assertion that geographic location and the $\delta^{18}O$ and $\delta D$ values of water sources used to produce the food items are key influences on the isotopic composition of foods. Food $\delta^{18}O$ and $\delta D$ values varied strongly and consistently according to the type of food sampled; for example, dairy, meat and eggs were significantly depleted in $^{18}O$ and $^{2}H$, and foods sweetened with refined sugar (sweetened beverages, candy) were significantly enriched. This consistent difference may reflect a lower latitude of ingredient origins; for example, US cane sugar cultivation is more predominant in the south, whereas dairy production is more common in the north. However, it may also reflect differences in the biochemical composition of those foods, if different organic compounds (e.g. proteins, lipids) differ in biosynthetic fractionation. Because $\delta^{18}O$ and $\delta D$ values varied with food type, dietary differences may confound geographical interpretation of human $\delta^{18}O$ and $\delta D$ values (e.g. vegetarians vs. non-vegetarians). Variability in the $\delta^{18}O$ and $\delta D$ values of the complex suite of items that composes modern human diets warrants further investigation.[42]

Despite the isotopic overlap and variability in diets, hair $\delta^{18}O$ and $\delta D$ values differed significantly between residents of Fairbanks and East Greenbush and showed little variation within each population. These differences were consistent with differences in tap water $\delta^{18}O$ and $\delta D$ in the two regions. This result demonstrates that residents of different geographic locations can have distinct isotopic signatures even when the diet is isotopically homogeneous. We estimated the proportional contribution of oxygen and hydrogen from drinking water to human hair as 27% of oxygen and 36% of hydrogen. Our estimate of the %H in human hair derived from drinking water agrees well with that of 31% reported by Sharp *et al.*[18] To our knowledge, we have provided one of the first estimates of the %O in human hair to derive from drinking water, based on stable isotope analyses. The tendency for human hair to reflect local water $\delta^{18}O$ as well as $\delta D$ provides another tool for tracking human movement or origin in forensic applications.





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**Table 5.** $\delta$D and $\delta^{18}$O values of beard hair and urine from a 'migrant' research subject during travel from Fairbanks, AK (USA), to East Greenbush, NY (USA) and Littlehampton, Sussex (UK). We also present $\delta$D and $\delta^{18}$O values of local tap water at each location (n.d. = no data). Data are presented graphically in Figure 4

| Cumulative days | Location | $\delta^{18}$O hair | $\delta$D hair | $\delta^{18}$O urine | $\delta$D urine | $\delta^{18}$O water | $\delta$D water |
|---|---|---|---|---|---|---|---|
| 0 | Fairbanks | n.d. | n.d. | n.d. | n.d. | −20.6 | −157.5 |
| 1 | Fairbanks | n.d. | n.d. | n.d. | n.d. | −20.6 | −157.5 |
| 2 | Fairbanks | n.d. | n.d. | n.d. | n.d. | −20.6 | −157.5 |
| 3 | Fairbanks | n.d. | n.d. | n.d. | n.d. | −20.6 | −157.5 |
| 4 | Fairbanks | n.d. | n.d. | n.d. | n.d. | −20.6 | −157.5 |
| 5 | Fairbanks | n.d. | n.d. | n.d. | n.d. | −20.6 | −157.5 |
| 6 | Fairbanks | n.d. | n.d. | n.d. | n.d. | −20.6 | −157.5 |
| 7 | Fairbanks | 12.5 | −111.4 | −10.3 | −90.8 | −20.6 | −157.5 |
| 8 | Fairbanks | n.d. | n.d. | n.d. | n.d. | −20.6 | −157.5 |
| 9 | E. Greenbush | 10.5 | −124.3 | −9.2 | −83.6 | −9.8 | −64.4 |
| 10 | E. Greenbush | 10.6 | −108.6 | n.d. | n.d. | −9.8 | −64.4 |
| 11 | E. Greenbush | 6.5 | −116.8 | −8.2 | −77.1 | −9.8 | −64.4 |
| 12 | E. Greenbush | 6.1 | −115.2 | −7.5 | −73.9 | −9.8 | −64.4 |
| 13 | E. Greenbush | 8.2 | −119.2 | −7.5 | −74.6 | −9.8 | −64.4 |
| 14 | E. Greenbush | 7.8 | −126.8 | −7.0 | −68.4 | −9.8 | −64.4 |
| 15 | E. Greenbush | 8.6 | −121.7 | −6.6 | −67.8 | −9.8 | −64.4 |
| 16 | E. Greenbush | 9.3 | −131.6 | −6.6 | −65.6 | −9.8 | −64.4 |
| 17 | E. Greenbush | n.d. | n.d. | n.d. | n.d. | −9.8 | −64.4 |
| 18 | Littlehampton | n.d. | n.d. | n.d. | n.d. | −9.0 | −40.0 |
| 19 | Littlehampton | 9.1 | −119.9 | −5.7 | −58.8 | −9.0 | −40.0 |
| 20 | Littlehampton | 10.6 | −117.7 | −4.8 | −53.1 | −9.0 | −40.0 |
| 21 | Littlehampton | 10.3 | −116.7 | −4.4 | −51.0 | −9.0 | −40.0 |
| 22 | Littlehampton | 9.7 | −114.8 | −4.1 | −45.9 | −9.0 | −40.0 |
| 23 | Littlehampton | n.d. | n.d. | n.d. | n.d. | −9.0 | −40.0 |
| 24 | Littlehampton | 11.3 | −120.7 | −3.8 | −43.4 | −9.0 | −40.0 |
| 25 | Littlehampton | 12.6 | −114.3 | −4.3 | −46.4 | −9.0 | −40.0 |
| 26 | Fairbanks | 9.8 | −113.2 | −5.7 | −53.2 | −20.6 | −157.5 |
| 27 | Fairbanks | 9.8 | −113.2 | −5.6 | −56.6 | −20.6 | −157.5 |
| 28 | Fairbanks | 10.1 | −107.6 | n.d. | n.d. | −20.6 | −157.5 |
| 29 | Fairbanks | 13.3 | −102.7 | −6.3 | −64.5 | −20.6 | −157.5 |
| 30 | Fairbanks | 13.5 | −105.8 | −6.8 | −68.8 | −20.6 | −157.5 |
| 31 | Fairbanks | 12.9 | −109.2 | −6.8 | −69.5 | −20.6 | −157.5 |
| 32 | Fairbanks | 11.7 | −109.4 | −7.1 | −73.2 | −20.6 | −157.5 |
| 33 | Fairbanks | 11.4 | −111.7 | −7.5 | −75.6 | −20.6 | −157.5 |
| 34 | Fairbanks | 11.1 | −131.5 | −8.7 | −79.2 | −20.6 | −157.5 |
| 35 | Fairbanks | 10.7 | −112.3 | −8.0 | −79.4 | −20.6 | −157.5 |
| 36 | Fairbanks | 12.2 | −113.8 | −7.8 | −80.3 | −20.6 | −157.5 |
| 37 | Fairbanks | 11.5 | −113.6 | −8.0 | −81.0 | −20.6 | −157.5 |
| 38 | Fairbanks | 11.8 | −111.8 | −8.0 | −84.2 | −20.6 | −157.5 |
| 39 | Fairbanks | 9.1 | −113.9 | −8.4 | −86.1 | −20.6 | −157.5 |
| 40 | Fairbanks | 9.4 | −118.7 | −8.4 | −86.8 | −20.6 | −157.5 |
| 41 | Fairbanks | 8.2 | −111.7 | −8.8 | −90.0 | −20.6 | −157.5 |
| 42 | Fairbanks | 8.7 | −123.7 | −9.6 | −95.5 | −20.6 | −157.5 |
| 43 | Fairbanks | n.d. | n.d. | −8.9 | −91.9 | −20.6 | −157.5 |
| 44 | Fairbanks | 8.8 | −114.8 | −9.1 | −93.0 | −20.6 | −157.5 |
| 45 | Fairbanks | 7.6 | −118.5 | −9.1 | −93.8 | −20.6 | −157.5 |
| 46 | Fairbanks | 8.9 | −113.7 | −8.3 | −92.5 | −20.6 | −157.5 |
| 47 | Fairbanks | 10.4 | −111.8 | −8.2 | −92.6 | −20.6 | −157.5 |
| 48 | Fairbanks | 8.1 | −115.1 | n.d. | n.d. | −20.6 | −157.5 |

A trip between regions with very dissimilar drinking water $\delta^{18}$O and $\delta$D by a 'migrant' human study subject was reflected in changing $\delta^{18}$O and $\delta$D values of urine. These changes were followed fairly closely by beard hair $\delta^{18}$O values and less closely (if at all) by beard hair $\delta$D values. Urine $\delta^{18}$O and $\delta$D values were very responsive markers of travel, both changing dramatically within a day of initiating travel and exhibiting very little scatter around increasing and decreasing trends. Although urine contains solutes containing oxygen and hydrogen (primarily urea), the vast majority of urine oxygen and hydrogen derives from water. If we assume a relatively high human urine urea concentration of 1000 mmol/L, only 1.8% of total urinary oxygen and 3.6% of total urinary hydrogen derive from urea, compared with 98.2% oxygen from water and 96.4% hydrogen from water. These calculations are based on a wt % of oxygen and hydrogen in urea (26.7% and 6.7%) and water (88.9% and 11.1%), respectively. Sharp *et al.*[18] found that distilling urine had little influence on its $\delta$D value. Thus, the signature of urine should be dominated by that of body water. If it were feasible to collect urine, it would provide a very high-fidelity marker of recent travel between regions with dissimilar water $\delta^{18}$O and $\delta$D values.





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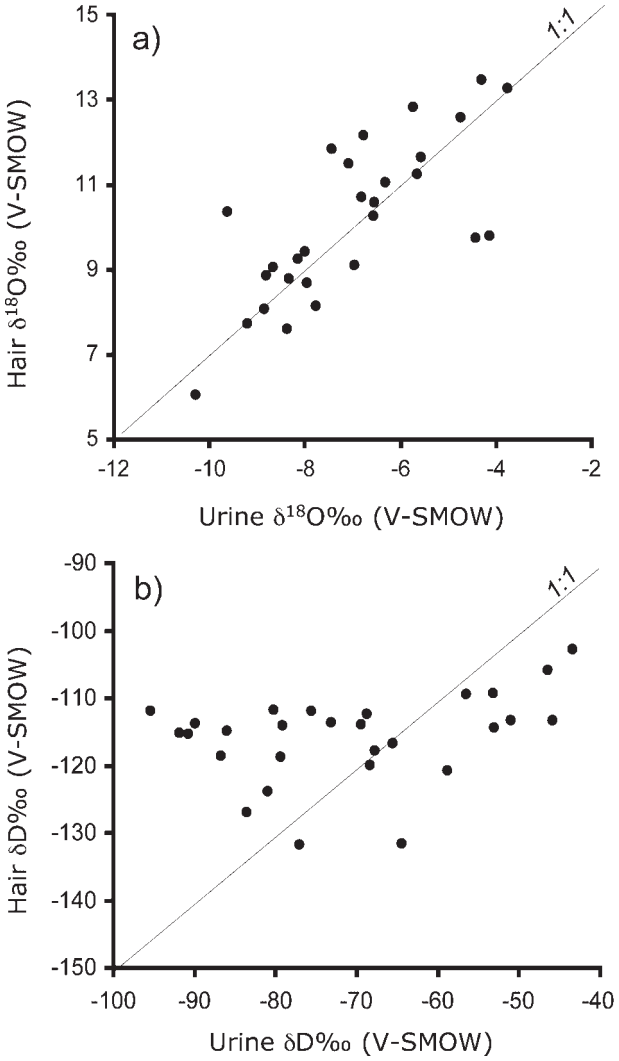


**Figure 5.** The relationship between (a) $\delta^{18}O$ and (b) $\delta D$ values in urine and beard hair. Urine samples were lagged by 5 days as described in the Experimental section. Lines indicate a slope of unity.

Beard hair $\delta^{18}O$ and $\delta D$ values varied in how well they were predicted by travel-related change in body water (represented by urine $\delta^{18}O$ and $\delta D$ values). Beard hair $\delta^{18}O$ values tracked urine $\delta^{18}O$ values fairly well, with an $R^2$ of 0.55 across all days of travel (once a growth lag of 5 days had been applied). In contrast, values of beard hair $\delta D$ tracked urine $\delta D$ poorly, and tended to resemble the Fairbanks resident hair $\delta D$ values despite travel out of the region. This result is puzzling given that we calculate that 36% of hair hydrogen derives from drinking water, based on head hair samples from resident populations. It is possible that the poor correspondence between beard hair and urine could result from skin flake or follicle contamination from shaving. However, this type of contamination does not explain why beard hair reflected changes in body water for one isotope ($^{18}O$) but not the other ($^{2}H$). We also note that hair samples were not lipid extracted. It is possible that contamination from skin or hair oil might explain the poorer correspondence between urine and beard hair $\delta D$ values compared with urine and beard hair $\delta^{18}O$ values. Head hair may be the more commonly used tissue for many forensic applications; thus, it would be useful to contrast temporal changes in beard and head hair $\delta D$ and $\delta^{18}O$ values under common cleaning conditions. It is also possible that hair $\delta D$ values reflect input from an internal H reservoir with a slow turnover time; for example, amino acids from body proteins (Z. D. Sharp, personal communication). If this is this case, hair $\delta D$ values will not provide a good marker for short-term travel events.

Hair $\delta^{18}O$ values in the migrant varied between resident values for both locations, despite relatively short trips (~1–2 weeks each). Both the expected geographical variation in a marker, as well as the fidelity with which it is recorded in tissue, contribute to the utility of a given isotope for reconstructing patterns of movement. The close relationship between hair $\delta^{18}O$ values and changing urine (body water) $\delta^{18}O$ values in our study suggests that $^{18}O$, while less commonly used in studies of migration, may have promise as a marker in human and wildlife forensics.

## CONCLUSIONS

Our findings show that $\delta^{18}O$ and $\delta D$ values of human hair both reflect regional differences in drinking water. Travel by a human subject between regions with isotopically distinct drinking water caused dramatic changes in urine $\delta D$ and $\delta^{18}O$ values. These changes were tracked fairly well by beard hair $\delta^{18}O$ values, and fairly poorly by beard hair $\delta D$ values. As with studies of animal migration, isotopic changes in humans resulting from travel will be most apparent when individuals move across isotope gradients (i.e. changes in latitude and altitude) rather than along isotope gradients (changes in longitude). We calculate that body water isotope composition continued to change for ~3 weeks or more following travel, with hair isotope composition lagging further behind. Thus, hair $\delta D$ values and $\delta^{18}O$ values will become considerably more complicated to interpret if an individual takes numerous short trips to locations with very different isotopic compositions of drinking water. There is a need to better understand the roles of food vs. drinking water in determining stable oxygen and hydrogen isotopic variation in organic samples (e.g. hair), and we look forward to the further development of these tools.

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## REFERENCES


1. Bowen GJ, Wassenaar LI, Hobson KA. *Oecologia* 2005; **143**: 337.
2. Hobson KA. *Oecologia* 1999; **120**: 314.
3. West JB, Bowen GJ, Cerling TE, Ehleringer JR. *Trends Ecol. Evolution* 2006; **21**: 408.
4. Bowen GJ, Revenaugh J. *Water Resour. Res.* 2003; **39**: Art. No. 1299.
5. Bowen GJ, Wilkinson B. *Geology* 2002; **30**: 315.
6. Dutton A, Wilkinson BH, Welker JM, Bowen GJ, Lohmann KC. *Hydrological Processes* 2005; **19**: 4121.
7. Ingraham NL. In *Isotope Tracers in Catchment Hydrology*, Kendall CK, McDonnell JJ (eds). Elsevier: Boston, 2003; 87.
8. Chamberlain CP, Blum JD, Holmes RT, Feng XH, Sherry TW, Graves GR. *Oecologia* 1997; **109**: 132.
9. Greenberg R, Mara P, Wooller MJ. *Auk* 2007; in press.
10. Hobson KA, Wassenaar LI. *Oecologia* 1997; **109**: 142.
11. Lott CA, Meehan TD, Heath JA. *Oecologia* 2003; **134**: 505.
12. Rubenstein DR, Chamberlain CP, Holmes RT, Ayres MP, Waldbauer JR, Graves GR, Tuross NC. *Science* 2002; **295**: 1062.
13. Wassenaar LI, Hobson KA. *Ecological Applications* 2000; **10**: 911.
14. Whitledge GW, Johnson BM, Martinez PJ. *Can. J. Fisheries Aquatic Sci.* 2006; **63**: 1746.
15. Hoppe KA. *Paleobiology* 2004; **30**: 129.
16. Hoppe KA. *Earth Planet. Sci. Lett.* 2006; **244**: 408.
17. Fraser I, Meier-Augenstein W, Kalin RM. *Rapid Commun. Mass Spectrom.* 2006; **20**: 1109.
18. Sharp ZD, Atudorei V, Panarello HO, Fernandez J, Douthitt C. *J. Archaeol. Sci.* 2003; **30**: 1709.
19. Kornexl BE, Gehre M, Höfling R, Werner RA. *Rapid Commun. Mass Spectrom.* 1999; **13**: 1685.
20. Koziet J. *J. Mass Spectrom.* 1997; **32**: 103.
21. Werner RA. *Isot. Environ. Health Stud.* 2003; **39**: 85.
22. Birchall J, O'Connell TC, Heaton THE, Hedges REM. *J. Anim. Ecol.* 2005; **74**: 877.
23. Schwarcz HP, Schoeninger MJ. *Yearbook Phys. Anthropol.* 1991; **34**: 283.
24. Ambler RP, Macko SA, Sykes B, Griffiths JB, Bada J, Eglinton G. *Philos. Trans. R. Soc. London, Series B* 1999; **354**: 75.
25. Fogel ML, Tuross N, Johnson BJ, Miller GH. *Org. Geochem.* 1997; **27**: 275.
26. Macko SA, Engel MH, Andrusevich V, Lubec G, O'Connell TC, Hedges REM. *Philos. Trans. R. Soc. London, Series B* 1999; **354**: 65.
27. Richards MP. *Eur. J. Clin. Nutr.* 2002; **56**: 1270.
28. Evans JA, Chenery CA, Fitzpatrick AP. *Archaeometry* 2006; **48**: 309.
29. Muller W, Fricke H, Halliday AN, McCulloch MT, Wartho JA. *Science* 2003; **302**: 862.
30. Bol R, Pflieger C. *Rapid Commun. Mass Spectrom.* 2002; **16**: 2195.
31. Minagawa M. *Appl. Geochem.* 1992; **7**: 145.
32. O'Connell TC, Hedges REM. *Am. J. Phys. Anthropol.* 1999; **108**: 409.
33. Petzke KJ, Boeing H, Klaus S, Metges CC. *J. Nutr.* 2005; **135**: 1515.
34. Schoeller DA, Minagawa M, Slater R, Kaplan IR. *Ecol. Food Nutr.* 1986; **18**: 159.
35. Williams JH, O'Connell TC. *J. Gerontol. Series A – Biol. Sci. Med. Sci.* 2002; **57**: M797.
36. Kelly S, Heaton K, Hoogewerff J. *Trends Food Sci. Technol.* 2005; **16**: 555.
37. Longinelli A. *Geochim. Cosmochim. Acta* 1984; **48**: 385.
38. Luz B, Kolodny T, Horowitz M. *Geochim. Cosmochim. Acta* 1984; **48**: 255.
39. Sharp Z. *Stable Isotope Geochemistry*. Pearson/Prentice Hall: Upper Saddle River, New Jersey, USA, 2007.
40. Cernichiari E, Toribara TY, Liang L, Marsh DO, Berlin MW, Myers GJ, Cox C, Shamlaye CF, Choisy O, Davidson P, Clarkson TW. *Neurotoxicology* 1995; **16**: 613.
41. Food and Nutrition Board, *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate*. Institute of Medicine of the National Academies. The National Academies Press: Washington, DC, 2004.
42. Bowen GJ, Chesson L, Nielson K, Cerling TE, Ehleringer JR. *Rapid Commun. Mass Spectrom.* 2005; **19**: 2371.
43. Wassenaar L, Hobson K. *Rapid Commun. Mass Spectrom.* 2006; **16**: 2505.
44. Wassenaar LI, Hobson KA. *Isot. Environ. Health Stud.* 2003; **39**: 211.
45. O'Brien DM, Fogel ML, Boggs CL. *Proc. Natl. Acad. Sci. USA* 2002; **99**: 4413.






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