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Spatial distributions of carbon, nitrogen and sulfur isotope ratios in human hair across the central United States

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We present data on the carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulfur ($\delta^{34}S$) isotope ratios of human hair collected in the central portions of the USA. These elements are incorporated into hair from the diet and thus provide a record of dietary inputs that may also document geospatial patterns. We detected regional differences in hair $\delta^{34}S$ values across the USA, with the lowest values in the northern Great Plains and increasing values towards the east, west and south. In contrast, no statistically significant patterns were detected in the spatial variation of human hair $\delta^{13}C$ and $\delta^{15}N$ values. Using $\delta^{34}S$ values and a Geographic Information System approach, we created a map ('sulfur isoscape'). The accuracy of the map was tested using hair samples not included in its generation. We conclude that sulfur isotope analysis may represent a new tool to investigate the movements and/or region-of-origin of humans.

The ability to use stable isotope analysis to assess the regions-of-origin of humans and food products strongly depends on an understanding of the spatial distribution of isotope ratios.[1] Because the isotope ratios in drinking water vary predictably across landscapes,[2,3] much emphasis has been placed on understanding how the spatial patterns within the hydrogen and oxygen isotopes of water are translated into human tissues.[4–6] In this context, a semi-mechanistic model describing the incorporation of H and O atoms into human hair has been developed.[4] This model, and its subsequent modification by Bowen *et al.*,[5] allow the prediction of region-of-origin and reconstruction of human movements across isotopic gradients by measuring the isotope ratios of hydrogen (δ^2H) and oxygen ($\delta^{18}O$) in hair keratin and back-calculating the isotopic composition of drinking water. The δ^2H and $\delta^{18}O$ values in human hair tend to be highly correlated, providing redundant geo-location information.[7] Additional stable isotope tracers, such as carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulfur ($\delta^{34}S$), might complement δ^2H and $\delta^{18}O$ analyses,[8,9] but the existence of human-related C, N and S isotopic gradients across landscapes has not been fully established, particularly when considering tissues from modern humans with a 'continental supermarket' diet.

The stable isotope ratios of carbon, nitrogen and sulfur have been primarily used for dietary reconstruction of both modern and ancient consumers. The use of these 'dietary isotopes' for region-of-origin assignment of humans is based on the principle that these isotopes reflect geographically distinct dietary patterns.[10–16] Previous research has shown that residents of different countries present distinctive keratin isotope ratios, primarily in $\delta^{13}C$ and $\delta^{34}S$ values, due to varied diets and food industry practices.[15] For example, $\delta^{13}C$ values obtained from hair and fingernails have been used to distinguish US residents from western Europeans.[10–14] This distinction is possible because carbon isotope ratios largely indicate the incorporation of ^{13}C-depleted values from C_3 plants (e.g., wheat, temperate grasses) or ^{13}C-enriched values from C_4 plants (e.g., maize, tropical grasses) consumed directly as a staple food or indirectly through animals raised on these plant types. In the USA, maize is the major component of livestock diet while C_3 grasses are still a major food for European livestock.[17–20]

In the case of nitrogen isotope ratios, several studies have detected significant differences among modern human tissue samples from different countries.[13,15] Nardoto *et al.*[13] found that fingernails from Brazilian subjects have higher $\delta^{15}N$ values than fingernails sampled from the USA and western Europe. In addition, Mutzel *et al.*[15] found that hair samples from Russia and Denmark tend to have $\delta^{15}N$ values in excess of 10‰, while hair samples from Pakistan tend to have values lower than 6‰, and samples from other European, Asian and Latin-American countries have $\delta^{15}N$ values between these two extremes. Although multiple and diverse factors could cause these differences, the authors cite the following primary mechanisms: the consumption of different amounts of animal protein (particularly marine protein), the use of different farming practices (use of synthetic versus organic fertilizers) or the use of plant material from arid areas for human consumption or animal feed.[13,15]

Considering sulfur isotope ratios, human hair $\delta^{34}S$ values have been found to be significantly lower in hair samples from inland regions (e.g., interior North America and Europe) than in samples from coastal locations (e.g., Chile,

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coastal Australia).[6,15,21] The two primary factors thought to be responsible for these isotopic differences are (1) a higher consumption of marine-derived protein with elevated $\delta^{34}S$ values by inhabitants of coastal regions and (2) the deposition of ^{34}S-enriched molecules from the ocean on coastal soils and vegetation.[6] However, it has been long recognized that bedrock geochemistry and atmospheric deposition (natural and anthropogenic) are also important factors in determining the sulfur isotopic composition of a particular area, which could lead to the generation of geographic patterns in $\delta^{34}S$ values.[21,22]

The differences in C, N and S isotope values between countries and cultures summarized above are undoubtedly useful in the provenancing of humans. To further extend the applicability of isotopes for human provenancing, we explored the spatial distribution of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values in human scalp hair across the United States using the same samples utilized by Ehleringer *et al.*[4] We show that the $\delta^{34}S$ values present a spatial pattern characterized by low values in the central northern region of the country and increasing values towards the eastern, western, and southern regions. Furthermore, we present a spatially explicit prediction map (isoscape) of $\delta^{34}S$ values in human hair across the central U.S.

EXPERIMENTAL

Sample acquisition

We collected discarded human scalp hair from barbershops and hair salons, as well as hair donated by anonymous volunteers from 73 cities or towns across 25 states of the contiguous USA between 2003 and 2007 (Fig. 1, Table 1 SI, Supporting Information). To increase the likelihood of collecting hair only from locals we primarily sampled cities or towns with populations <100 000, although seven cities included in our analyses had populations larger than 100 000 individuals (Table 1 SI, Supporting Information). No information was recorded regarding the age, gender, diet, and health or travel history of the individuals from whom the discarded hair samples were collected. At the time of collection, discarded hair was examined for color and texture to determine that it belonged to one person; hair samples were placed in separate paper envelopes.

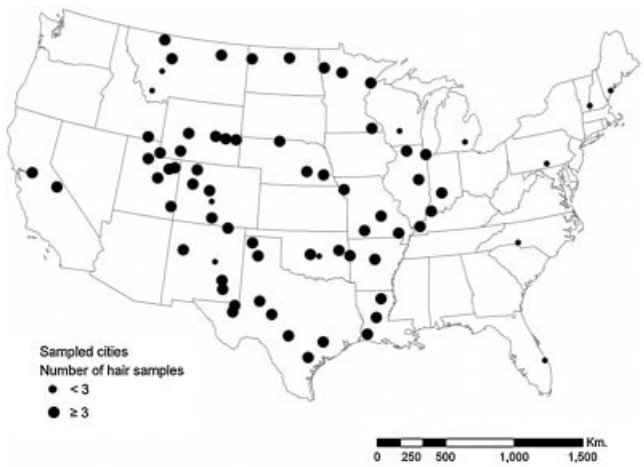

Figure 1. Map showing the location of the 73 sampled cities. Size of marker represents number of hair samples collected per city.

Table 1. Descriptive statistics for carbon, nitrogen and sulfur isotope ratios in human hair collected across the contiguous USA

	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Average	−16.8‰	8.8‰	3.4‰
SD	0.8‰	0.4‰	1.1‰
Minimum	−21.6‰	6.5‰	−1.2‰
Maximum	−14.7‰	9.7‰	9.9‰
Sample size	206	206	228

Sample preparation

Prior to analysis, all hair samples were washed twice in a 2:1 chloroform/methanol mixture to remove lipids and other surface contaminants. Washed samples were air-dried and ground to a fine powder using a ball mill (Retsch, Haan, Germany). For $\delta^{13}C$ and $\delta^{15}N$ analyses, 500 μg (±10%) of material was loaded into tin capsules; for $\delta^{34}S$, 900 μg (±10%) was loaded into tin capsules.

Sample analyses

Hair samples were analyzed using an isotope ratio mass spectrometer operated in continuous flow mode. Analyses of hair samples for combined carbon and nitrogen as well as sulfur isotopes were conducted using separate instruments, but the analytical procedures were similar. Tin capsules were loaded into a zero-blank autosampler interfaced with an elemental analyzer (Carlo Erba, Milan, Italy) where they were flash combusted to produce N_2 and CO_2 or SO_2. For sulfur isotope analysis we followed the buffering system developed by Fry *et al.*[23] to minimize the need for oxygen isotope corrections of the SO_2 gas. In this system, a second column packed with quartz chips is placed downflow of the combustion column and the water scrubber to provide a large uniform oxygen reservoir where SO_2-SiO_2 equilibration occurs and where the oxygen isotope composition of the SO_2 gas is thus buffered.[23] The resulting gases were chromatographically separated and carried to the mass spectrometer (Finnigan-MAT Delta S: Thermo Scientific, Bremen, Germany). Hair samples were analyzed alongside a set of internal laboratory reference materials [powdered keratin for C and N ($\delta^{13}C = -24.0$‰, $\delta^{15}N = 5.9$‰); and silver sulfide ($\delta^{34}S = 17.9$‰), zinc sulfide ($\delta^{34}S = -31.9$‰), and ground feathers ($\delta^{34}S = 16.7$‰) for S] that had been previously calibrated against international standards. Results for $\delta^{13}C$ values are presented on the Vienna Pee Dee Belemnite (VPDB) scale, those for $\delta^{15}N$ values on the AIR scale, and for $\delta^{34}S$ values on the Vienna Canyon Diablo Troilite (VCDT) scale. The analytical precision (1σ), based on long-term measurements of internal laboratory reference materials for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$, was 0.1‰, 0.2‰ and 0.4‰, respectively. Stable isotope ratios are reported using the standard δ-notation relative to an international standard in

units 'per mil' (‰) as follows:

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

where R_{sample} and $R_{standard}$ are the molar ratios of the heavy to light isotopes of the sample and standard, respectively.

Statistical and geostatistical analyses

Data analysis and statistical calculations were conducted using R.[24] Geostatistical analyses were performed using the geographic information system software ArcMAP™ 9.3.[25] Visual inspection of the semivariogram plots created using the Geostatistical Analyst tool in ArcMAP™ was used to evaluate the presence of autocorrelations in the dataset.[25] Interpolations were conducted by ordinary kriging with two different semivariogram models, spherical and exponential.[26] Prediction maps of isotope values were constructed by interpolating the calculated average isotope value for each city with three or more hair samples and standard deviations equal to or smaller than twice the analytical precision of the measurement (e.g., cities with standard deviation (SD) $\leq$0.8‰ for $\delta^{34}S$ were used). This arbitrary cutoff was chosen as an attempt to exclude cities with high isotopic variation, potentially due to non-local samples (e.g., visitors, tourists), and represents the 95% confidence interval under a normal distribution.

Nonparametric Kruskal-Wallis analysis of variance by ranks was used to compare isotope data among cities, while parametric correlations were used to test for covariation between isotope ratios and geographic or population parameters (e.g., latitude, longitude, population size).

RESULTS

Sample sizes varied from one to ten hair samples per city (Fig. 1). Descriptive statistics for the measured $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values are summarized in Table 1. A weak but statistically significant correlation was detected between the $\delta^{13}C$ and $\delta^{15}N$ values of human hair ($r = 0.40$, $p < 0.001$, $n = 206$). The $\delta^{34}S$ values were not significantly correlated with the $\delta^{13}C$ or $\delta^{15}N$ values ($r^2 < 0.01$, $p > 0.8$ and $n = 206$ for both tests).

The carbon, nitrogen and sulfur isotope ratios of human hair differed significantly among cities (for cities with three or more hair samples, Kruskal-Wallis analysis of variance by ranks: $p < 0.001$ for all tests). The calculated average carbon and sulfur isotope values per city were both significantly correlated with latitude (for $\delta^{13}C$, $r = -0.40$, $p < 0.002$, $n = 58$; for $\delta^{34}S$, $r = -0.47$, $p = < 0.001$, $n = 60$). There were no significant correlations between other geographic variables examined (latitude, longitude, or elevation) and average isotope ratios of hair by city ($r^2 < 0.05$ and $p > 0.05$ for all comparisons, Fig. 2). The $\delta^{34}S$ values were lowest in the northern Great Plains and higher towards the east, west and south (Fig. 2(c)). Low $\delta^{34}S$ values were also detected in central California (Fig. 2(c)). The mean $\delta^{13}C$ values were lower in the northwest and increased towards the southeast (Fig. 2(a)).

We detected appreciable variation in the standard deviations (SD) of isotope ratios of human hair among cities (Fig. 2, Table 2). In particular for $\delta^{13}C$ values, the average SD was 5 times larger than our estimated analytical precision ($1\sigma = 0.1$‰) and the maximum SD was 18 times larger than the analytical precision. On the other hand, the standard deviations for $\delta^{15}N$ and $\delta^{34}S$ values did not show as much variation (Table 2); the average SD and maximum SD were 1.5 and 4.5 times larger than our estimated analytical precisions

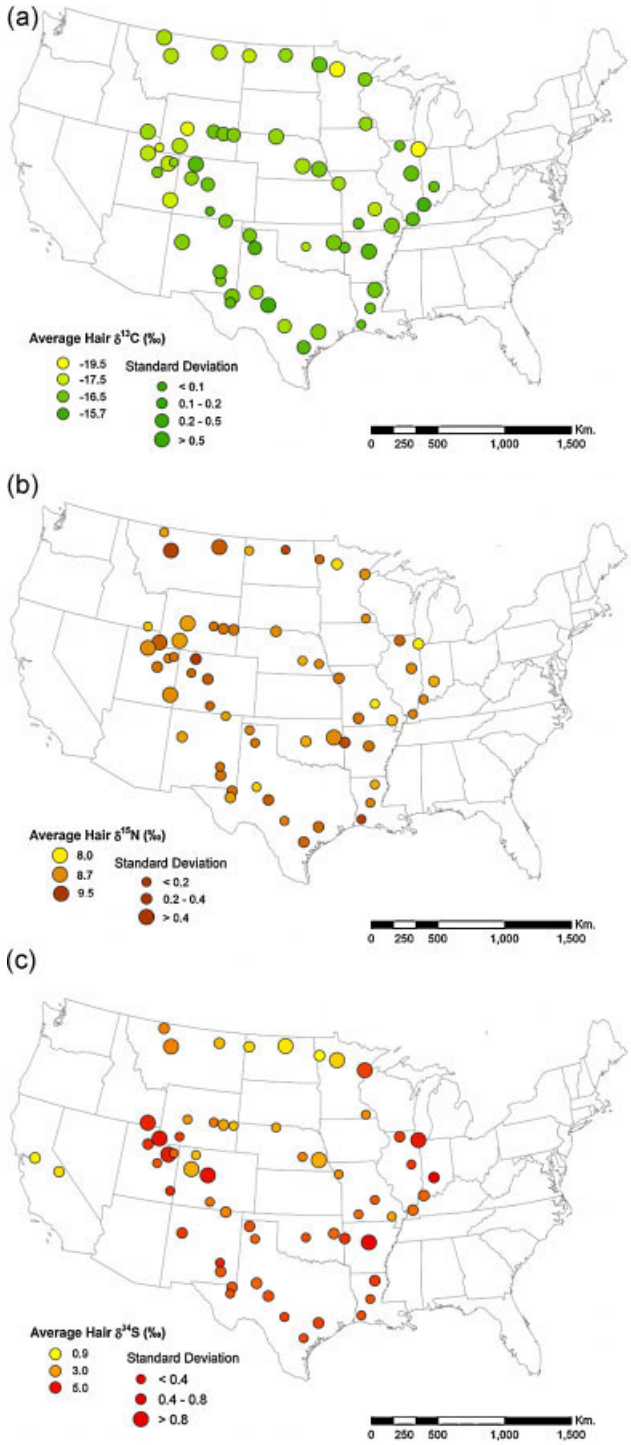

Figure 2. Maps showing the average $\delta^{13}C$ (a), $\delta^{15}N$ (b), and $\delta^{34}S$ (c) values, as well as the per-city standard deviations for cities with three or more hair samples. Color ranges correspond to isotope values (lighter colors describe lower values and darker colors describe higher values) and marker sizes symbolize standard deviations (smaller symbols represent low variability and larger symbols represent higher variability).

Table 2. Stable isotope variability by city, for cities with three or more hair samples

SD by City	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Average SD	0.5‰	0.3‰	0.6‰
Minimum SD	0.1‰	0.1‰	0.1‰
Maximum SD	1.8‰	0.9‰	1.8‰
Number of cities	58	58	60

for both elements ($1\sigma = 0.2$‰ for $\delta^{15}N$ and 0.4‰ for $\delta^{34}S$). The standard deviations of the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values by city were not correlated with latitude, longitude, population size or sample size ($r^2 < 0.25$; $p > 0.01$ for all comparisons).

Construction of a $\delta^{34}S$ isoscape of human hair

For geostatistical analysis and interpolations we used only samples from cities characterized by a SD equal to or smaller than twice the analytical precision. As described in the Experimental section, this cutoff was selected to reduce the chance of including non-locals (e.g., visitors, tourists). In the resulting dataset, only sulfur isotopes presented a significant geographic variation, correlated with latitude ($r = -0.52$, $p = 0.0001$, n = 46, Fig. 2(a)). The visual inspection of the semivariogram obtained using the Geostatistical Analyst tool in ArcMAP™ software showed the presence of a spatial autocorrelation between the cities. Furthermore, the visual examination of the mean $\delta^{34}S$ value per city showed that the spatial relationship was maintained, with lower values in the north-central sector of the geographic range and higher values towards the eastern, western, and southern regions (Fig. 2(c)). In contrast, using only the smaller dataset containing cities with low isotope variability, no significant correlation with any spatial variable (latitude, longitude, elevation) was found for the $\delta^{13}C$ and $\delta^{15}N$ values even when the threshold of inclusion was increased up to three times the analytical precision ($r^2 < 0.01$ and $p > 0.05$ for all correlations, Figs. 2(a) and 2(b)). Furthermore, a visual inspection of the semivariograms showed no discernable spatial autocorrelation between the cities.

We created isotope surfaces depicting prediction maps by interpolating the mean $\delta^{34}S$ values for 44 cities across the central USA (cities with SD <0.8‰); we excluded the two cities from California because the spatial distance to the closest city was too large and there were insufficient data for interpolation. The prediction map created by ordinary kriging with a spherical semivariogram model had a slightly lower root-mean-square error (RMSE = 0.46) than the isoscape produced with an exponential model (RMSE = 0.49). Figure 3 represents the isoscape produced with a spherical semivariogram model. We tested this prediction map by comparing the measured average $\delta^{34}S$ values with the predicted $\delta^{34}S$ values for 21 cities contained within the isoscape but not included in the interpolation procedure due to their larger standard deviations (n = 14 with SD >0.8‰) or their small sample size (n = 7 with one or two hair samples). The average $\delta^{34}S$ value of 14 of the 21 cities was predicted correctly within the analytical precision (Fig. 4), with a mean difference between measured and predicted values of 0.2‰ (±0.6‰).

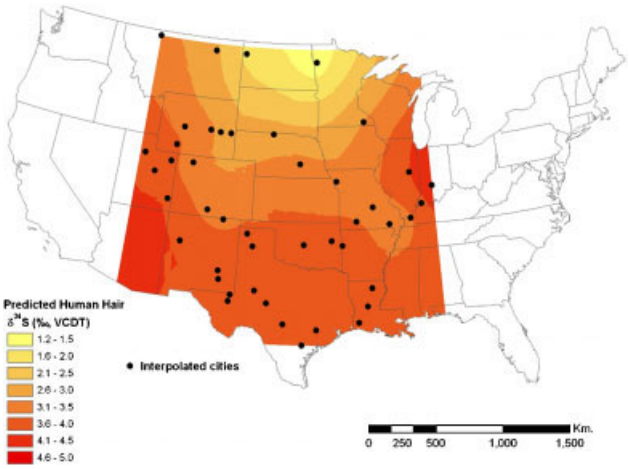

Figure 3. A $\delta^{34}S$ isoscape representing a prediction map produced by ordinary kriging interpolation of values for 44 cities with standard deviations less than or equal to 0.8‰. See text for a description of the interpolation method.

DISCUSSION

The carbon and nitrogen isotope ratios measured in our study are in agreement with values previously published for the USA.[10,14] Our findings reinforce the idea that the average diet of U.S. residents contains a significant C_4-plant component[18,19] and, although the data suggest a higher C_3-plant component in diets in the northeast region, more research will be necessary before any firm conclusions can be

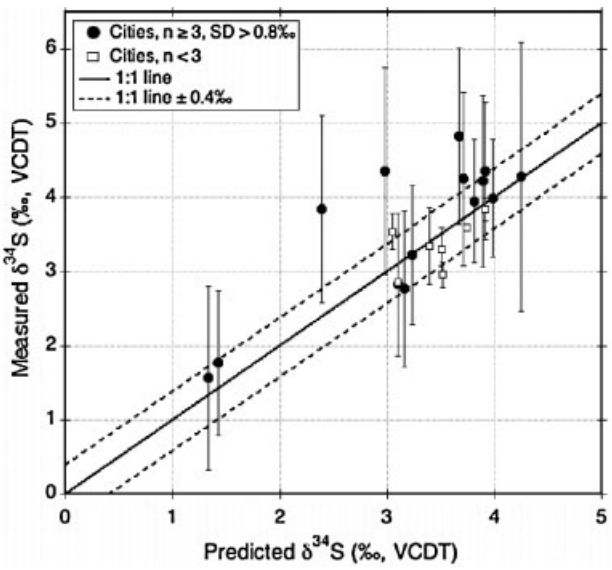

Figure 4. Comparison of predicted and measured $\delta^{34}S$ values for cities not included in the isoscape interpolation. Circles (n = 14) represent the $\delta^{34}S$ mean values (± standard deviation) for cities with three or more hair samples but with standard deviations larger than 0.8‰ (see text for a description of this cutoff value). Squares (n = 7) represent the $\delta^{34}S$ values for cities where only one or two hair samples were collected. The solid line is the 1:1 line, while the dashed lines represent the 1:1 line ± 0.4‰ (our estimated analytical precision).

reached. In addition, our research has considerably expanded the documented range of the sulfur isotopic composition of hair for the U.S. population; the only previously published value corresponded to a single hair sample from Omaha, Nebraska presented by Bol *et al.*[14]

Our objective was to explore the spatial distribution of carbon, nitrogen and sulfur isotope ratios in human hair across the U.S. As a first iteration and to prevent the inclusion of cities with excessively high isotopic variation we established a limit of acceptable variance within a city. In all cases the limit was set at standard deviations less than or equal to twice the analytical precision. Although this limit is probably too strict, it allows all included samples within a city to be considered as having the same isotopic ratio. However, we recognized that a better estimation of the natural variability within cities is needed. In the case of $\delta^{13}C$ values, the geographic pattern was only apparent when the full dataset (all cities) was used. Therefore, at this time, we cannot assert that this is indeed a real spatial pattern. In the case of $\delta^{15}N$ and $\delta^{34}S$ values, the removal of those cities with standard deviations greater than the limit did not affect the presence or absence of a spatial pattern. The inclusion of other cities may have affected the interpolated surface to some degree, but what would not have changed is the general pattern of low $\delta^{34}S$ values in the northern Great Plains (eastern Montana, North and South Dakota, western Minnesota) and higher values projecting outwards towards the east, south and west.

No independent dataset exists that allowed us to test the accuracy of the sulfur hair isoscape predictions. The accuracy of the predicted values was instead tested using those cities not used to develop the isoscape (either because the city had a standard deviation that exceeded the limit or did not contain enough samples). In this comparison only three cities (Conway, AR; Duluth, MN; Leadville, CO) reported averages much different from the predicted values (all showing positive offsets of ~1.3‰, Fig. 4). Additional and independent support for our prediction map comes from the sample from Omaha, Nebraska reported by Bol *et al.*;[14] this sample had a $\delta^{34}S$ value of 2.4‰, only 0.5‰ lower than our predicted value for that city ($\delta^{34}S$ value predicted for Omaha = 2.9‰). Furthermore, we estimated that just 112 km (70 miles) separates Omaha, NE, from the closest area with a predicted value of 2.4 ± 0.4‰.

Determinants of isotope ratio spatial distribution

Consumers obtain carbon, nitrogen and sulfur atoms from organic compounds in their diet. Thus, the spatial pattern of the isotopic composition detected in hair keratin (in the case of $\delta^{34}S$ values) or lack of a pattern ($\delta^{13}C$ and $\delta^{15}N$ values) is a reflection of the isotopic characteristics of diet across a landscape. Carbon atoms in human hair are derived from dietary amino acids (both essential and, to some extent, non-essential amino acids), as well as from other carbon-containing molecules present in the diet (e.g., carbohydrates, lipids), while nitrogen- and sulfur-containing molecules are derived solely from dietary amino acids. Based on the reported larger ranges in the $\delta^{13}C$ values than in the $\delta^{15}N$ values of food,[18,19] it could be expected that the $\delta^{13}C$ values of hair would present a greater isotopic variability than the $\delta^{15}N$ values; this hypothesis is consistent with larger values of $\delta^{13}C$ standard deviations relative to the estimated analytical precision. For sulfur, the hair keratin isotopic composition is directly incorporated from a few dietary S-bearing amino acids (mostly cysteine and methionine) and little to no fractionation exists between diet and consumer tissues.[27,28] Due to this direct link, the spatial distribution of the $\delta^{34}S$ values in human hair would be influenced by two main components: variation in the relative contributions of different protein sources and variation in the isotopic composition of the protein sources.

Differences in the consumption of protein sources across the U.S. have been reported previously.[29–31] Smit *et al.*[29] showed that the primary sources of protein differ among ethnic groups, and to some extent between men and women and among age classes. According to Smit *et al.*,[29] African-Americans consumed a higher proportion of pork, fish and, poultry in their diet than Caucasians and Mexican-Americans; Caucasians consumed a higher percentage of dairy and grain protein than the other two groups; and Mexican-Americans reported the highest percent consumption of eggs, legumes, and beef for some age classes. Although Smit *et al.* [29] did not report differences across states, it could be assumed that as the ethnic composition of states vary,[32] so too would the sources of proteins being consumed. Of special importance for the interpretation of $\delta^{34}S$ values in human hair is the consumption of proteins from the ocean. Marine-derived proteins have on average higher $\delta^{34}S$ values than land-derived proteins.[8,33] Moya[30] and Moya *et al.*[31] analyzed fish consumption across several U.S. states and reported that coastal states (e.g., Florida) had higher consumption rates of marine fish and shellfish than inland states (e.g., North Dakota). These studies also report small differences among ethnic groups and an increase in fish consumption with household income and education.[30,31]

Although it is expected that the reported differences in dietary patterns might affect the $\delta^{34}S$ values of human hair across the sampled region, it is also expected that those differences would affect the $\delta^{13}C$ and $\delta^{15}N$ values. In particular, if marine fish are an important source of protein to certain regions, the $\delta^{15}N$ and $\delta^{13}C$ values should also reflect that source as marine animals tend to be more enriched in ^{13}C and ^{15}N than terrestrial sources.[7,8,32] However, the $\delta^{13}C$ and $\delta^{15}N$ values do not appear to be show the same geographic pattern. Furthermore, the lack of correlation between $\delta^{15}N$ and $\delta^{34}S$ values suggests a need for interpretation above and beyond a two end-member model involving terrestrial and marine foods as the primary determinant of the geographic sulfur pattern seen in our study.

The sulfur isotope ratios of hair may also vary across the landscape because the isotopic composition of the major protein sources (beef, dairy and poultry according to Smit *et al.* [29]) may vary across the landscape in addition to variations in dietary habits. To our knowledge there has not been any report on the $\delta^{34}S$ values of the dietary sources of protein across the USA, and only a handful of reports exist on the $\delta^{34}S$ values of wild animals across the same region.[34–36] In Europe, however, $\delta^{34}S$ analyses have been used to assess the region-of-origin of dairy, lamb meat and beef attributed to predictable differences across areas. The regional differences detected in the European studies agree with our findings, with higher $\delta^{34}S$ values in close proximity to the ocean and lower values towards more inland regions.[37–40]

The only datasets that report sulfur isotope values of a keratinaceous tissue in regions overlapping our study area are a study of raptor feathers [34] and two studies of waterfowl feathers.[35,36] Feathers from nine species of raptors collected across the USA and Canada showed lower $\delta^{34}S$ values in samples collected from the interior of the country than in those collected from coastal birds,[34] which is consistent with our data. The difference between inland and coastal birds was consistent across both dietary specialists (bird-eating raptors) and generalists.[34] The authors did not present a more detailed analysis of variation of the $\delta^{34}S$ values with location that would allow us to assess the presence of a feather isotope gradient within the continent in the same areas where we have collected hair samples.[34] An explanation for the higher values found in coastal birds could be (1) the consumption of marine-derived protein (especially the bird-eating raptors) and (2) the consumption of protein derived from coastal vegetation affected by the deposition of ^{34}S-enriched marine aerosols.[8,22,34]

In a separate study, Hebert and Wassenaar[35] analyzed $\delta^{34}S$ values from waterfowl feathers collected in four regions of North America: the northern Great Plains (including samples from North Dakota, South Dakota and Montana), California, northern Canada, and Alaska. Feathers from the Great Plains had the lowest $\delta^{34}S$ values (lower than 0.0‰), feathers from California had values between −10‰ and +10‰, and feathers from Alaska and northern Canada had positive values.[35] This general pattern of $\delta^{34}S$ distribution agrees with the spatial distribution of $\delta^{34}S$ values for human hair detected in our study. In a recent paper, Coulton *et al.* [36] sampled feathers from the same populations in the northern Great Plains and northern Canada, and found similar geographic differences to those found by Hebert and Wassenaar.[35] The low values detected in feathers sampled in the northern Great Plains were explained by a combination of two factors: first, the presence of ^{34}S-depleted bedrock and derived soils and second, plant uptake of ^{34}S-depleted sulfides generated in large quantities by anaerobic bacteria in marshes and wetlands.[35] The higher values measured in other areas were thought to have resulted, in part, from a combination of effects such as plant uptake of ^{34}S-enriched molecules from ocean spray, local bedrock geology and low $\delta^{34}S$ values produced by anaerobic bacteria. The authors also found a negative correlation between land use (as percentage of cropland) and $\delta^{34}S$ values for feathers collected in Canada, and argued that this may be due to differences in soil composition and geology or to the application of synthetic fertilizers at some sites.[35]

Whether the factors mentioned above are important in generating the spatial pattern of $\delta^{34}S$ values in human hair detected in our study is difficult to establish. However, any factor affecting the $\delta^{34}S$ values of plants is a likely contributor to the observed spatial variability in human hair. Other factors not discussed by the previous authors include anthropogenic sulfur deposition with varied $\delta^{34}S$ signatures depending on the source of S (petroleum, coal, natural gas and sulfide ores from smelting plants), as well as volcanic ash deposition.[22]

We have thus far assumed little to no fractionation between the sulfur isotopic composition of dietary proteins and hair.[27,28] If fractionation does occur, we have furthermore assumed that this effect is constant across the sampled regions. However, if our assumptions are incorrect, a differential fractionation across a region might also produce spatial differences in $\delta^{34}S$ values. Richards *et al.*[28] observed that a horse fed a nutritionally inadequate diet showed a much larger sulfur fractionation ($\Delta_{diet\text{-}hair} = +4‰$) between diet and its hair than did another horse under similar experimental settings but with a nutritionally adequate diet, potentially as a result of protein recycling. Under conditions of low cysteine intake, the essential amino acid methionine would function as a sulfur donor for the synthesis of new cysteine, which is the main S-bearing amino acid in hair, leading to a recycling of S-containing molecules and a potential isotopic fractionation.[41] In the USA, regions with diets deficient in protein seem unlikely given that modern U.S. residents consume, on average, amounts of protein that meet or exceed their nutritional requirements.[29,42]

CONCLUSIONS

Our analyses revealed a spatial pattern of $\delta^{34}S$ values across the U.S., a finding that is not unexpected given that the potential of sulfur isotope ratios as geographic tracers has previously been recognized.[8,21,22] It was unexpected, however, that such a clear pattern of spatial variability was found in scalp hair of modern Americans. Modern U.S. residents are thought to be a population with a 'continental supermarket' diet, where most if not all food types are available throughout the country.[4] Our findings suggest that this characterization might not be entirely accurate, and that regional differences in food resources or diet choice may still exist. Further research is needed to understand the primary determinants of the spatial pattern of $\delta^{34}S$ values in human hair, and to what extent it would be modified by changes in food distribution, dietary habits or farming practices.

Because the sulfur isotopic composition of hair does not respond directly to fundamental climatic or environmental characteristics of a particular location in the same way that $\delta^{2}H$ and $\delta^{18}O$ values do, its use is far from reaching a mechanistic modeling approach. However, through predictive mapping our study provides the basis for a new methodology in cases of human provenance that goes beyond a comparison of measured $\delta^{34}S$ data and reference samples in a database. We do recognize that the production of isoscapes would still rely on the acquisition of large datasets to accomplish adequate interpolations. However, the human hair $\delta^{34}S$ isoscape presented here represents a first step towards establishing a new tracer from which geographic information can be extracted. This prediction map could be used in a Geographic Information System alongside predictive layers of $\delta^{2}H$ and $\delta^{18}O$ values to obtain a more accurate approximation for region-of-origin assignments of human hair.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

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