

# Stable isotopes in hair reveal dietary protein sources with links to socioeconomic status and health

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Carbon and nitrogen isotope ratios in hair sampled from 65 communities across the central and intermountain regions of the United States and more intensively throughout 29 ZIP codes in the Salt Lake Valley, Utah, revealed a dietary divergence related to socioeconomic status as measured by cost of living, household income, and adjusted gross income. Corn-fed, animal-derived proteins were more common in the diets of lower socioeconomic status populations than were plant-derived proteins, with individual estimates of animal-derived protein diets as high as 75%; United States towns and cities averaged 57%. Similar patterns were seen across the socioeconomic status spectrum in the Salt Lake Valley. It is likely that corn-fed animal proteins were associated with concentrated animal-feeding operations, a common practice for industrial animal production in the United States today. Given recent studies highlighting the negative impacts of animal-derived proteins in our diets, hair carbon isotope ratios could provide an approach for scaling assessments of animalsourced foods and health risks in communities across the United States.

carbon isotope | nitrogen isotope | CAFO | socioeconomic status | diet

Nearly 40% of the adults in the United States today are obese (1, 2). Carbohydrate-rich diets have garnered much attention within health discussions, as diets rich with simple carbohydrates are a factor in the rapid rise of obesity, diabetes, kidney disease, and other chronic diet-related diseases across the United States (3-8). In conjunction with increases in carbohydraterich diets, but less discussed, the intensification of animal protein production through large concentrated animal-feeding operations (CAFOs) has made animal protein more widely available. Over the past half-century, the diets in Western societies have shown trends toward greater abundances of both carbohydrates and proteins (3, 5, 9-11). Within the United States, mass production and availability of animal protein and greater accessibility of refined carbohydrates and sugars has facilitated this dietary homogenization (3-6, 8). Convenience, pricing, marketing, and improvements in crop yields, long-distance shipment infrastructure, and storage, are factors that have contributed to these dietary shifts. As evidence provided here will suggest, dietary divergence in the United States is related to socioeconomic status (SES). The consumption of corn-fed animal protein in the United States is more prevalent in the diets of lower SES populations. These results contrast with similarly designed studies in Brazil and Colombia, where meat is relatively more expensive and forms a smaller proportion of the diets of those individuals with lower SES (12-14). Here we estimate the proportions of corn-fed animal- vs. plant-based protein in human diets across the United States as assessed by measurements of carbon and nitrogen stable isotopes in human hair. We then relate hair stable isotopes and SES patterns with consumption of proteins traceable to corn sources. We build upon an extensive anthropological foundation that has used carbon and nitrogen isotopes to

investigate dietary differences associated with food chains (e.g., refs. 15–18) and with status, class, and gender (e.g., refs. 19, 20). We recognize that hair is composed of keratin, which contains both essential and nonessential amino acids, with nonessential amino acids derived from either dietary proteins or dietary carbohydrate sources (21–26). However, the bias toward synthesis of proteins from preformed amino acids in dietary proteins results in hair  $\delta^{13}$ C values underestimating the amounts of carbohydrates and fats in human diets (22).

American diets are increasingly derived from animals raised on corn-dominated diets in CAFO facilities (i.e., beef, poultry, pork, eggs, milk, and so forth) (27-30). While industrialized CAFO facilities can differ in their operations, they share common features. First, CAFOs benefited historically from federal incentives and subsidies on corn and soy beans, resulting in CAFOs as a widely adopted industrial practice. While CAFOs produce vast amounts of protein for human consumption, concentrating animals into confined spaces has negative impacts on both groundwater contamination and animal health (31, 32). Second, CAFOs typically utilize a mixture of 85 to 90% corn and 10 to 15% soybean as animal feed within the United States (29, 30, 33–36). Since corn utilizes the  $C_4$  photosynthetic pathway (33), it imparts a higher carbon isotope value into the animal protein that is subsequently transferred into human tissues as proteins are consumed (34, 35). Thus, a reliance on corn as a feed in animal feed today (36-38) affords the opportunity to use carbon isotopes as a tracer to quantify the importance of

## Significance

As Americans, our diets have among the highest protein consumption rates and we are experiencing increased rates of obesity across all age groups. Here we reveal that consumption of corn-fed animal proteins are more common among lower socioeconomic status populations, which places these populations at a potentially greater risk for increased health problems.

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The authors declare no competing interest.

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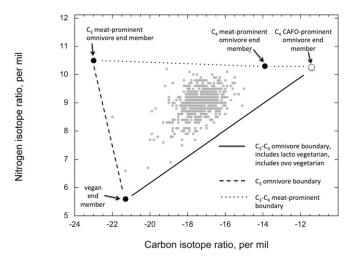
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**Fig. 1.**  $\delta^{13}$ C and  $\delta^{15}$ N values of scalp hair observed for individual modern consumers, with scalp hair samples collected randomly from barbershops and hair salons across the United States. The end members represent extremes in hair stable isotope values for modern consumers, based on the expected values for an individual based on extreme diet preferences. The correlation between  $\delta^{13}$ C and  $\delta^{15}$ N values among all individual hair values is significant (r = 0.475, n = 684, P < 0.0001); per mil.

corn-fed, animal-derived protein in the protein consumption of an individual's diet across an economic spectrum.

While there are many approaches to quantifying dietary choices, carbon ( $\delta^{13}$ C) and nitrogen ( $\delta^{15}$ N) stable isotope ratios of human scalp hair and blood have been shown to directly relate to an individual's dietary protein sources (24, 25, 39-43). We also recognize that carbon-derived carbohydrates can be incorporated into nonessential amino acids (23–26). The  $\delta^{13}$ C values of human hair reveal the proportion of protein originating from C<sub>3</sub> plants or C<sub>4</sub> plants in an individual's diet (16, 44-47). Since there is limited carbon isotope fractionation between plants and animal protein as food sources into the human diet, the  $\delta^{13}$ C values of animal protein reflect these same  $C_3/C_4$  consumption patterns (34, 35, 48), but not the specific foods consumed. Cool-season grasses, legumes, vegetables, shrubs, and trees are all  $C_3$  plants (46), and thus the  $\delta^{13}$ C value of animals fed these C<sub>3</sub> food sources will reflect C<sub>3</sub>like  $\delta^{13}$ C values. In contrast, corn (maize, Zea mays), warm season grasses, sorghum, millet, and sugar cane are important  $C_4$  plants (46), with corn serving as the primary feed in CAFO operations (29, 49, 50). We recognize that range-raised cattle in southern parts of the United States will also impart a  $C_4$  signal, similar to corn, as  $C_4$  grasses dominate those habitats (51).

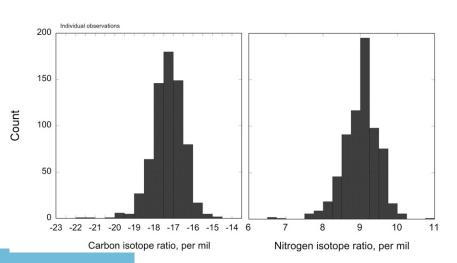
In contrast, hair  $\delta^{15}$ N values are generally used to estimate the proportions of plant vs. animal-derived foods to an individual's diet, where higher  $\delta^{15}$ N values represent greater consumption of animal-derived proteins (40, 47, 52–54). Nonmarine, animal-derived proteins include not only meat and poultry, but also milk, cheese, and eggs.

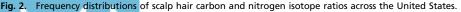
Our study used  $\delta^{13}$ C and  $\delta^{15}$ N values in scalp hair to evaluate three hypotheses related to variations in dietary choices and SES. Hypothesis 1 is that  $\delta^{13}$ C and  $\delta^{15}$ N values of human hair will be positively correlated, reflecting the proportions in two primary dietary sources: C<sub>4</sub>-derived animal protein and C<sub>3</sub>derived plant protein. Hypothesis 2 is that the proportions of C<sub>4</sub>-derived animal protein in a diet will be highest in areas of lower SES. Hypothesis 3 is that the proportions of C<sub>4</sub>-derived animal proteins in a diet and obesity will be positively correlated.

We also recognize that extensive consumption of  $C_4$  sugars,  $C_4$ -based beverages, and marine protein sources could complicate interpretation of hypotheses 2 and 3 (8, 16, 23–25, 55).

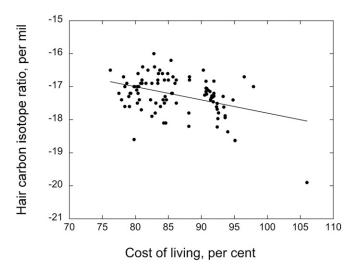
To explore hypothesis 1, we must first specify the dietary extremes that form the boundaries of hair  $\delta^{13}$ C and  $\delta^{15}$ N values. These consumer dietary end members of modern consumers are: 1) Strict vegetarians (i.e., vegan) consuming only plant protein sources; 2) meat-prominent omnivores, whose primary protein source is C<sub>4</sub>-fed animal protein with limited plant-based proteins; 3) meat-prominent omnivores, whose primary protein source is C<sub>3</sub>-fed animal protein or marine fishes with limited plant-based proteins; and 4) hypothetical CAFO-prominent omnivores with animal protein derived from corn-fed sources and limited plant-based proteins (Fig. 1).

Modern human diets broadly range from vegan to meatprominent omnivores, with most omnivores and both ovo- and lacto-vegetarians theoretically falling between these extremes. Data to define modern hair  $\delta^{13}$ C and  $\delta^{15}$ N values associated with dietary extremes were obtained from the literature (16, 40, 52, 56). Limited hair  $\delta^{13}$ C and  $\delta^{15}$ N values for vegans are available; we used a recent and perhaps the largest stable isotope study of European vegans to characterize the vegan  $\delta^{13}$ C and  $\delta^{15}$ N end member (56). It is entirely possible that future surveys of United States vegans may reveal higher  $\delta^{13}$ C values, reflecting corn as a more significant component of United States vegan diets. Data





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**Fig. 3.** A plot of the correlation between average carbon isotope ratio of human hair and the cost of living in cities and ZIP codes across the United States (r = 0.397, n = 94, P < 0.001).

to describe  $\delta^{13}$ C and  $\delta^{15}$ N values for C<sub>3</sub>- and C<sub>4</sub>-meat-prominent omnivores were derived from country-level datasets summarized by Hülsemann et al. (57), selecting Swede and Finnish end members as most representative of C3-meat-prominent omnivores and Mexicans as most representative of primarily C<sub>4</sub>meat-prominent omnivores. Within each national population, the meat-prominent omnivore end members are defined as individuals whose paired  $\delta^{13}$ C and  $\delta^{15}$ N values were 2 SDs from the mean national value. These isotopic end members form a triangular realm of possible hair  $\delta^{13}$ C and  $\delta^{15}$ N values and are consistent with observations of several other large-scale hair isotope surveys (12, 52, 58-60). We note that CAFO foods in the United States are 80 to 85% C4-based (e.g., refs. 61, 62) so that the true end member of CAFO-prominent omnivores diets would be several per mil higher than the diets of a C<sub>4</sub>-meatprominent omnivore end member shown in Fig. 1 [see also data in Bowen et al. (63)].

Differing dietary preferences will be reflected in hair  $\delta^{13}C$ and  $\delta^{15}N$  values and fall within a space bounded by the extreme-diet end members (Fig. 1). We expect that omnivores will fit within this triangular realm, reflecting differing proportions of the C<sub>3</sub>-animal, CAFO-animal, and plant protein sources in their diets. Lacto-vegetarian and ovo-vegetarian consumers are predicted to exhibit a  $\delta^{13}$ C and  $\delta^{15}$ N relationship indistinguishable from omnivores, since eggs and milk are likely to have been derived from animals raised on C<sub>4</sub> feed (64-66). Most individuals in the United States are omnivores (41, 60), and if corn is a significant part of the "American" diet (29), we expect that individual hair  $\delta^{13}$ C and  $\delta^{15}$ N values will fall along the slope specified by CAFO-meat prominent omnivore and vegan end members in Fig. 1. The slope of a United States hair  $\delta^{13}$ C and  $\delta^{15}$ N relationship is predicted to be positive with a slope of  $\sim 0.8$ , based on constraints of the two endmember paired isotope values. Nonetheless, it is possible that individual hair  $\delta^{13}$ C and  $\delta^{15}$ N values could deviate from this relationship if marine-derived protein constituted a significant fraction of that individual's dietary protein intake or if C<sub>4</sub>-derived sugars in the diet then contributed significantly to the formation on nonessential amino acids.

# What Is the "American" Protein?

For the hair samples of 684 individuals in this study, virtually all of the variations in paired  $\delta^{13}$ C and  $\delta^{15}$ N values were captured within the framework boundaries defined by the principal dietary

extremes (Fig. 1). Our sampling does not include coastal portions of the United States and, therefore, is constrained in scope. Since most observations do not lie at one of the three extremes, our conclusion is that American omnivores eat varying proportions of the three primary protein sources. Moreover, the  $\delta^{13}$ C and  $\delta^{15}$ N observations span a relatively modest range of  $\delta^{13}$ C and  $\delta^{15}$ N values. We found 96.0% of the hair  $\delta^{13}$ C values fell between a low of -20.0% and a high of -16.0%, with the mean value being -17.3% (Fig. 2). And, 97.3% of the hair  $\delta^{15}$ N values fell between a low of 7.5‰ and a high of 10.0%, with the mean value being 9.0‰ (Fig. 2). Similar values were observed in young children (42). Thus, while there were indeed variations in hair isotope values, these United States hair samples spanned a relatively narrow region of the total possible  $\delta^{13}$ C and  $\delta^{15}$ N values realm (Fig. 1).

Hypothesis 1 predicts a positive relationship among hair  $\delta^{13}C$ and  $\delta^{15}$ N values. Indeed, there was a highly significant correlation among hair  $\delta^{13}$ C and  $\delta^{15}$ N values of all individual hair observations (r = 0.475, n = 684, P < 0.0001), consistent with the concept that proteins contributing to an individual's diet were largely a mixture of just two primary sources: C<sub>3</sub>-plant protein and C<sub>4</sub>-derived animal protein. It is not possible to explain this correlation on the basis of a mixing of C3-derived and C4-derived animal proteins into an individual's diet, because of the observed covariance between the increased contributions of animalsourced food with increased  $\delta^{15}N$  values (Fig. 1). While it is clear that different individuals do consume different proportions of both C<sub>3</sub>- and C<sub>4</sub>-derived animal protein, the predominant factor driving variations in hair  $\delta^{13}$ C and  $\delta^{15}$ N values is variations in the proportions of plant- vs. C4/corn-fed, animal-derived protein sources.

# **Relationships between Hair Isotopes and SES**

At the national scale, hair  $\delta^{13}$ C values were negatively related to the cost-of-living index (Fig. 3). In support of hypothesis 2, the pattern of decreasing hair  $\delta^{13}$ C values with increasing cost of living is most easily explained as increased proportions of plantbased proteins contributing to diets of individuals in regions with higher costs of living. Mean household income, a second SES metric, was also negatively correlated with hair  $\delta^{13}$ C values (Table 1) at a level of significance (r = -0.338, n = 94, P =0.0009) similar to that of the more general cost-of-living index (r = -0.395, n = 94, P = 0.0001). It is notable that the locations sampled in this survey were generally regions where the cost of living was below the United States average.

It is noteworthy that hair carbon isotopes were also influenced by geographic location. Spanning a latitudinal range of 29° to 48°, the average hair  $\delta^{13}$ C values within a town or ZIP code were negatively correlated with latitude (r = -0.334, n = 94, P =0.001). The direction of variations in hair  $\delta^{13}$ C values with latitude was identical to documented patterns of C<sub>3</sub> ecosystems predominant in northern cooler climates and a C<sub>4</sub> ecosystem predominant in southern warmer climates (51, 67). However, a more likely explanation for the apparent latitude effect is not ecological, but more likely the north-to-south geographic

 Table 1. Correlations among carbon isotope ratios in human

 hair sampled across the United States and SES measures

	n	r	Р
$\delta^{13}$ C vs. cost of living	94	-0.395	<0.001
$\delta^{13}$ C vs. household income	94	-0.338	0.001
$\delta^{13}$ C vs. latitude	94	-0.335	0.001
$\delta^{15}$ N vs. cost of living	94	-0.174	0.0938
$\delta^{15}$ N vs. household income	94	-0.1284	0.218
$\delta^{15}$ N vs. latitude	94	-0.0467	0.655

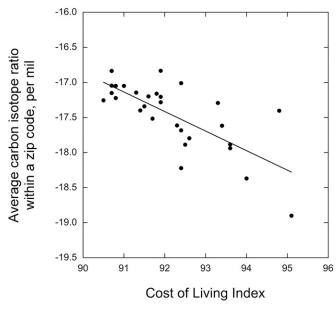
Table 2. Multiple linear relationships among carbon isotope ratios in human hair sampled across towns and cities of the United States (n = 94), SES measures, and latitude as a measure of geographic location

	Coefficient	SE	Р
Cost of living	-0.02612	0.01131	0.023
Household income	-0.00000317	0.00000831	0.222
Latitude	-0.03008	0.1239	0.018
R <sup>2</sup>		0.227	<0.001

gradient of SES across the central and southern states of the United States, where a majority of the sampling was conducted (*SI Appendix*). Southern portions of the United States had lower SES values than more northern states (68–71). To examine the interactions between SES parameters and hair  $\delta^{13}$ C values, we performed a multiple linear regression (Table 2). Here we observed that both cost of living (P = 0.0232) and regional location in the United States (latitude, P = 0.0175) were significant factors influencing SES interpretations of hair  $\delta^{13}$ C values.

We considered geographic location as a general factor influencing the  $\delta^{13}$ C values of animal proteins nationally and that SES factors were actually driving regional variation in hair  $\delta^{13}$ C values. In order to exclude latitude as a factor, we explored variations in hair  $\delta^{13}$ C values at the ZIP code level across a single metropolitan area: The Salt Lake Valley, Utah (SLV), with a population of ~1.1 million.

We found the individual hair  $\delta^{13}$ C values across the SLV varied extensively, ranging from -21.3% to -14.7%. This range was indistinguishable from the range of hair  $\delta^{13}$ C values previously observed in the United States (72) and among children within the SLV (42). Thus, hair  $\delta^{13}$ C values at this metropolitan scale were indistinguishable from the stable isotope ranges at the national level. However, among individual towns and cities sampled across the United States, we could not observe such large variations primarily because of limited sample sizes (n = 4to 6 individual hair samples per town/city). For subsequent SES evaluations across 29 ZIP codes in the SLV, individual hair  $\delta^{13}$ C



**Fig. 4.** A plot of the correlation between average carbon isotope ratio of human scalp hair and the cost of living in ZIP codes within the SLV (r = 0.725, n = 29, P < 0.001).

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values were averaged since no personal information was available for these samples.

Across the SLV metropolitan regional area, hair  $\delta^{13}C$ values were negatively related to cost of living, similar to the national trend but with higher explanatory capacity (Fig. 4) (r = -0.725, n = 29; P < 0.001). Across a mere 5% change in the cost of living, spanning a distance of less than 30 km, the average hair carbon isotope ratio across ZIP codes changed 1.8%. Both household income (P = 0.018)and adjusted gross income (P < 0.001) were also significantly correlated with hair  $\delta^{13}C$  values (Table 3). To evaluate the relative contributions of these three SES parameters, we performed a multiple linear regression (Table 4). Cost of living remained a significant factor (P = 0.026), as was adjusted gross income (P = 0.019). Household income was not significant (P = 0.068). While all three of these SES parameters are correlated with each other, they measure distinct factors and thus when considered in combination provide a higher  $R^2$  value  $(R^2 = 0.648)$  than was obtained through single-factor analyses (Table 3).

Of anecdotal interest and directly relatable to SES, the price for a haircut was also correlated with both hair  $\delta^{13}$ C values across the SLV. It is perhaps not surprising that the average price of a salon haircut within a ZIP code correlated well with the cost of living in that ZIP code area (r = -0.649, n = 29, P = 0.001), with both private and national chain barbershops and salons included in the analysis. Thus, at the ZIP code level, hair  $\delta^{13}$ C values correlated significantly with salon haircut price (Fig. 5), but the pattern with hair  $\delta^{15}$ N values was not statistically significant (r = -0.314, n = 29, P = 0.097). We had not imagined that it might be possible to estimate the average cost an individual had paid for their haircut knowing  $\delta^{13}$ C values. While salons sampled included both private (variable pricing) as well as national chain (fixed pricing) barbershops and salons with different pricing schemes, the statistical trend consistently held its significance even when all types of shops were melded into this single analysis.

In contrast to hair  $\delta^{13}$ C values,  $\delta^{15}$ N values were not significantly correlated with SES parameters at the national scale, but were at the metropolitan scale, where more data were available (Tables 1 and 3). Of note at the national level, hair  $\delta^{15}N$  values versus cost of living were not significant (Table 1) (r = -0.174, n = 94, P = 0.093). At the metropolitan scale of the SLV, correlation of hair  $\delta^{15}N$  values with cost of living was significant (r = -0.409, n = 29, P = 0.028) (Table 3). We had anticipated significant correlations between hair  $\delta^{15}N$  values and SES parameters, given the strong support of hypothesis 1 from hair  $\delta^{13}$ C values and expected food-chain nitrogen isotope enrichment effects. The lack of equivalently significant trends with SES parameters for both hair  $\delta^{13}C$  and  $\delta^{15}N$  values is surprising. Given that variations in  $\delta^{15}N$  values are generally interpreted as indicators of the proportions of animal vs. plant protein diets (25), we had anticipated significant correlations between hair  $\delta^{15}$ N values with each of the SES parameters. However, we note that carbon from C<sub>4</sub> carbohydrates (including

 
 Table 3. Correlations among carbon isotope ratios in human hair sampled across the SLV and measures of SES

	n	r	Р
$\delta^{13}$ C vs. cost of living	29	-0.740	<0.001
$\delta^{13}$ C vs. household income	29	-0.438	0.018
$\delta^{13}$ C vs. adjusted gross income	29	-0.653	<0.001
$\delta^{15}$ N vs. cost of living	29	-0.409	0.028
$\delta^{15}$ N vs. household income	29	-0.175	0.365
$\delta^{15}$ N vs. adjusted gross income	29	-0.297	0.117

Table 4. Multiple linear relationship of carbon isotope ratios in human hair across ZIP codes (n = 29) in the SLV as related to SES measures

	Coefficient	SE	Р
Cost of living	-0.14436	0.06103	0.026
Household income	0.01017	0.00534	0.068
Adjusted gross income	-0.01437	0.0057	0.019
<i>R</i> <sup>2</sup>		0.648	<0.001

sugars derived from corn or sugarcane) could contribute to syntheses of nonessential amino acids, reducing the likelihood of a significant correlation between these two stable isotopes (23–25, 43).

# Hair Isotopes: A Proxy for Community-Level Dietary Differences

Observations of hair  $\delta^{13}$ C and  $\delta^{15}$ N values (Fig. 1) are not new, as similar data have been presented in other studies. Yet previous studies have not placed hair  $\delta^{13}$ C and  $\delta^{15}$ N values in the context of evaluating population-scale variations in the proportions of different dietary protein sources contributing to an individual's diet. Furthermore, previous studies have not considered the population-scale context of an enriched  $\delta^{13}$ C diet and the importance to understanding corn-fed, animal-derived proteins in our diets.

We now explore the calculated magnitudes of corn-fed animal-protein contributions to the diets of individual observations for the United States and in the SLV metropolitan region. While the actual percentage of corn-fed animal-protein contribution to an individual's diet is influenced by the  $\delta^{13}C$ values of the end members, the relative differences among individuals are not. Using hair  $\delta^{13}$ C values of -24.8% and -11.4% as estimates of 0% and 100% corn-fed, animal-derived protein end members (Materials and Methods), respectively, the calculated maximum utilization of corn-fed, animal-derived proteins by an individual in the United States was 75% (SI Appendix). The average corn-fed, animal-derived animal protein contributions to diets of United States individuals in different cities and towns ranged from 37 to 65%, with an average national value of 57% across the 65 cities and towns sampled (SI Appendix). As fast foods are well established as a significant source of corn-fed, animal-derived proteins (36, 62), it is not unreasonable to assume that a significant portion of the cornfed, animal-derived animal proteins come from meals eaten outside the home. Recent surveys show that at least one-fourth of the children and adults across the United States consume fast foods on a daily basis (73, 74).

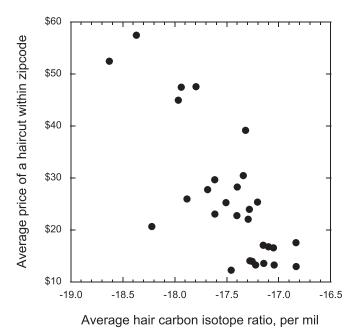
The average dietary contribution of corn-fed, animalderived proteins across SLV ZIP codes varied between 46% and 60%, spanning highest to lowest cost-of-living ZIP code areas (Fig. 6). As a result, we estimated that corn-fed, animalderived protein consumption in the SLV averaged 55%, similar to the national average. Again, the highest corn-fed, animal-derived protein rates were measured in lower-income, lower cost-of-living areas of the SLV than in comparison to ZIP codes having the highest cost of living and highest incomes (SI Appendix).

Within the SLV, measured variations in hair  $\delta^{13}$ C values were also significantly correlated with human health. Using body mass index (BMI) estimates calculated from driver's licenses within a ZIP code as a measure of obesity, hair  $\delta^{13}$ C values were significantly correlated with the calculated percent obesity (Table 5) (r = 0.716, n = 29, P < 0.001) (see *SI Appendix* for individual values). Translating measured hair estimates of corn-fed, animal-derived protein provides insights into possible linkages among diet, SES factors, and health. Both calculated utilization of corn-fed, animal-derived proteins and obesity rates exhibited similar trends with cost of living across ZIP codes (Fig. 6). The incidence of obesity was estimated to be highest in the lowest cost-of-living area of this metropolitan region, a well-documented SES trend of higher obesity rates in economically disadvantaged areas (75-78). As the correlation of obesity rates with utilization of corn-fed, animal-derived proteins was highly significant (r = 0.717, n =29, P = 0.001), this trend suggests that consumption of cornfed, animal-derived proteins is linked to poorer overall health, as has been suggested previously (79). Furthermore, the positive correlation of hair  $\delta^{15}$ N values across ZIP codes with obesity rates ties obesity rates to variations in consumption of animal-sourced foods (r = 0.363, n = 29, P = 0.052). By inference, the results associate obesity rates with consumption of animal-derived proteins.

There have been a number of recent long-term studies evaluating the impacts of animal- vs. plant-derived proteins on human health (80–83). Similarly, there have been many studies that show diet quality follows socioeconomic gradients (84). The common conclusions from these surveys are that 1) diet quality and SES are correlated and 2) the negative healthrelated impacts of animal-derived protein on human health are manifested through increased incidences of cardiovascular disease and increased mortality rates. As hair  $\delta^{13}$ C values offer a quantifiable metric of the proportions of animal vs. plant proteins in individual diets, it is possible that hair  $\delta^{13}$ C values could provide an approach for regional assessments of diet and health risks in communities across the United States.

# **Materials and Methods**

Sampling Locations. We sampled human hair at national and regional geographic scales within the central and intermountain portions of the United States. For national-scale analyses, we sampled discarded hair in trash bins from barbershops and salons in 65 small-to-large cities (representing 20 states) across the United States; discarded hair was identifiable at the individual level. For a regional assessment, we similarly sampled



**Fig. 5.** A plot of the correlation between average carbon isotope ratio of human scalp hair and the average cost of a haircut in ZIP codes within the SLV (r = -0.767, n = 29; P < 0.001).

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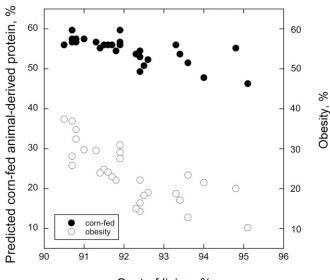
discarded hair from barbershops and salons from 29 different ZIP codes across the SLV with a population of 1+ million. The national survey spanned a geographical range that was expected to represent a broad cultural and economic spectrum but was primarily within the central states and did not include coastal states (68, 69). In a complementary fashion, the SLV survey was designed to explore economic-based variation within a contiguous megapolitan region. In designing the national and regional sampling schemes, we anticipated that heterogeneity of hair stable isotope ratio values should occur, since large differences in hair  $\delta^{13}$ C and  $\delta^{15}$ N values have been previously shown within the United States (42, 60).

Hair Sampling. In this study human scalp hair was collected as trash from bins in barbershops and salons, as previously described by Ehleringer et al. (85) and Covarrubias (86). All hair samples were placed in individual paper envelopes at the time of collection. As samples were all discarded hair from trash bins, no information was known regarding the age, gender, diet, health, or travel history of the individuals associated with the discarded hair. We assumed that hair samples represented individuals local to the collection site for purposes of SES and analyses.

For the United States national survey, three to five hair samples were collected per city and the ZIP code for those collections was recorded. The selected towns and cities were generally small with population ranges of 5,000 to 100,00 individuals (85). For the SLV surveys, the sampling was more intensive and hair was collected from each of three to four barbershops or salons per ZIP code within the SLV. Within each barbershop or salon in the SLV, three to four individual hair samples were collected. Here barbershop and salon names were recorded to facilitate subsequent comparisons of national (chain) versus locally owned (nonchain) store operations.

**Stable Isotope Analyses.** Prior to stable isotope analysis, all hair samples were washed twice in a 2:1 chloroform:methanol mixture to remove lipids and other surface contaminants and then were ground to a fine powder using a ball mill (Retsch). For  $\delta^{13}C$  and  $\delta^{15}N$  analysis, 500  $\mu g$  (±10%) of ground material was loaded into tin capsules.

Hair samples were analyzed using an isotope ratio mass spectrometer (Thermo Delta V) operated in continuous flow mode. Tin capsules were loaded into a zero-blank autosampler (Costech Analytical) interfaced with an elemental analyzer (Carlo Erba) where capsules were flash-combusted to produce CO<sub>2</sub> and N<sub>2</sub> for carbon and nitrogen isotope analyses, respectively. Hair samples were analyzed alongside a set of three internal laboratory



Cost of living, %

**Fig. 6.** Plots of the correlation between the cost of living and the calculated corn-fed, animal-derived protein (gray circles) (r = -0.727, n = 29, P < 0.001) and the ZIP-code average obesity (open circles) (r = -0.759, n = 29, P < 0.001) across 29 ZIP codes within the SLV.

### Table 5. Correlations among carbon and nitrogen isotope ratios in human hair sampled across the SLV and the percent obesity (BMI > 30) within a ZIP code

	n	r	Р
$\delta^{13}$ C vs. percent obesity	29	0.716	<0.001
$\delta^{15}$ N vs. percent obesity	29	0.364	0.052

reference materials (powdered keratins) that had been previously calibrated against international standards. Results for  $\delta^{13}$ C values are presented on the Vienna Pee Dee Belemnite scale; for  $\delta^{15}$ N values, on the atmospheric AIR scale. The analytical precisions (1  $\sigma$ ), based on long-term measurements of internal laboratory reference materials for  $\delta^{13}$ C and  $\delta^{15}$ N values, were 0.1 % and 0.2 %, respectively. Stable isotope ratios are reported using the standard  $\delta$ -notation relative to an international standard in units per mil (‰):  $\delta = (R_{sample}/R_{standard} - 1)$ , where  $R_{sample}$  and  $R_{standard}$  are the molar ratios of the heavy to light isotopes (e.g.,  ${}^{13}C/{}^{12}$ C) of the sample and standard, respectively.

**Socioeconomic Analyses.** For SES analyses, stable isotope data are presented as means  $\pm 1$  SD per ZIP code unless stated otherwise. Cost of living, mean household income, and adjusted gross income data per ZIP code were used as SES measures. For the national surveys, we used 2016 SES data downloaded from www.city-data.com as the primary quantitative measures of SES. These data are based on US Census data. We assumed that SES rankings among cities had not changed between 2005 (year samples were collected) and 2016 (earliest year SES data downloadable). For the SLV surveys, we downloaded 2012 cost-of-living data from www.city-data.com, but used 2016 mean household income and adjusted gross income data from www. city-data.com since those were the earliest downloadable datasets. A comparison of 2012 and 2016 cost-of-living data showed a very high and significant correlation (r = 0.876, P < 0.00001), suggesting that combining SES data across years was valid here for comparative purposes.

The 2012 average price of a haircut within a barbershop or salon within each ZIP code in the SLV was also obtained, providing a fourth SES metric. These data were collected by recording haircut prices listed in a barbershop or salon and allowed us to evaluate 1) whether or not there were differences in the dietary proportions of corn-fed, animal-derived proteins among salons charging different prices for a haircut and 2) whether or not there were differences in the dietary proportions of corn-fed, animalderived proteins in clients visiting national versus locally owned barbershops and salons.

**Obesity Measures.** Obesity data were obtained from the Utah Department of Health, using their Utah Behavioral Risk Factor Surveillance System (UBRFSS). The UBRFSS estimated average obesity percentages based on average ZIP code BMI data, which relies on height and weight data reported on Utah driver licenses.

Estimating CAFO-Derived Animal Protein. Estimates of average CAFO feed were derived from the literature, suggesting input feed diets of 85% corn and 15% soybean (49, 61, 87). Based on population surveys of different commercial lines, we used average carbon isotope ratio values of corn and soybean of -13.2% and -29.0%, respectively, to estimate CAFO feed input carbon isotope ratio values (88, 89). The estimated carbon isotope ratio of beef, assuming these parameters, is similar to averaged beef values measured in national surveys (37, 62). Converting between feed input at the CAFO and ultimately human scalp hair carbon isotope was a two-step process, first incorporating the +1.5% isotopic enrichment between feed input (85% corn, 15% soybean) and animal muscle carbon isotope ratio values (90), and then second incorporating the +2.7% isotopic enrichment between animal tissue in the diet and human hair carbon isotope ratio values (91, 92).

Data Availability. All data associated with this report are provided in *SI Appendix*.

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