

Variation of Hydrogen, Carbon, Nitrogen, and Oxygen Stable Isotope Ratios in an American Diet: Fast Food Meals

LESLEY A. CHESSON,^{*,†,§} DAVID W. PODLESAK,[†] ALEXANDRA H. THOMPSON,^{†,§}
THURE E. CERLING,^{†,§,#} AND JAMES R. EHLERINGER^{†,§}

Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, Utah 84112; IsoForensics, Inc., Suite 205, 423 Wakara Way, Salt Lake City, Utah 84108; and Department of Geology and Geophysics, University of Utah, 135 South 1460 East, Salt Lake City, Utah 84112

The stable isotopes of hydrogen, carbon, nitrogen, and oxygen provide insights into a heterotrophic organism's diet and geographic origin. Although the contribution of food $\delta^2\text{H}$ and $\delta^{18}\text{O}$ to the final tissue signal will not vary for constrained diets, it will for animals eating varied diets, that is, humans. This study surveyed the isotopic range in one portion of the American diet, fast food meals. Hamburger patties, buns, and French fries from national chain restaurants across the United States and from local restaurants (Salt Lake City, UT, and Charleston, SC) were analyzed for $\delta^2\text{H}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ (patties only) and $\delta^{18}\text{O}$ values. Patties and buns from local Utah restaurants were more depleted for $\delta^2\text{H}$, $\delta^{13}\text{C}$, and $\delta^{18}\text{O}$ values than samples from other restaurants. There were no significant differences in δ values among French fries. All three components of the fast food meal displayed significant linear $\delta^2\text{H}$ versus $\delta^{18}\text{O}$ relationships ($\delta^2\text{H} = 7.8\delta^{18}\text{O} - 237\text{‰}$, $\delta^2\text{H} = 5.9\delta^{18}\text{O} - 258\text{‰}$, and $\delta^2\text{H} = 3.3\delta^{18}\text{O} - 231\text{‰}$ for patties, buns, and fries, respectively). The findings show that significant predictable variation exists in the stable isotopic composition of fast food meals. It is proposed that the variation in $\delta^{13}\text{C}$ values of hamburger (beef) patties is indicative of differences in cattle-rearing practices, whereas $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values are evidence of geographic variation in food sources. Although the patterns support the concept of a "continental" supermarket diet, there appears to be a strong regional component within the diet.

KEYWORDS: Stable isotope; hydrogen; carbon; nitrogen; oxygen; American diet; fast food; beef; bread; potato

INTRODUCTION

The adage "you are what you eat" holds true isotopically. The stable isotopes of hydrogen ($\delta^2\text{H}$), carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$), and oxygen ($\delta^{18}\text{O}$) record signals from the environment in the tissues of both plants and animals (1). Carbon is a conservative recorder, reflecting the diet of a heterotrophic organism (2–5) due to the dependence of heterotrophic organisms on dietary carbon inputs. The original sources of carbon in any land-based food webs are plants, most of which follow one of two main photosynthetic pathways: C_3 or C_4 (6). Each pathway fractionates against ^{13}C differently, resulting in characteristically depleted $\delta^{13}\text{C}$ values for C_3 plants (wheat, temperate grasses) and enriched $\delta^{13}\text{C}$ values for C_4 plants (corn). On the other hand, nitrogen is enriched from diet to organism, approximately 3‰ per trophic "step" (7) associated with

fractionation events during metabolism. Despite this enrichment, the relationship between nitrogen in diet and tissue persists throughout a trophic system and thus both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values can be used to trace the flow of organic matter in animal food webs.

Hydrogen and oxygen stable isotopes of precipitation and tap water vary predictably on the basis of differences in geography (8, 9). The general pattern of spatial variation in $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values is one of decreasing values from low-latitude, low-elevation coastal regions toward inland, high-latitude, and mountainous regions. Plants incorporate the isotopic signal from local precipitation, which is then propagated throughout subsequent steps of the food web. Thus, $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values have utility in identifying the geographic origin of animal materials such as bird feathers (10, 11), butterfly wings (12), microbial spores (13), and human hair (14, 15) and fingernails (16).

Whereas the sole source of carbon and nitrogen in animal tissues is diet, there are multiple sources for hydrogen and oxygen including drinking water; food water; dietary proteins, carbohydrates, and lipids; and diatomic oxygen ($\delta^{18}\text{O}$ values

* Corresponding author [telephone (801) 581-8917; fax (801) 581-4665; e-mail chesson@biology.utah.edu].

[†] Department of Biology, University of Utah.

[§] IsoForensics, Inc.

[#] Department of Geology and Geophysics, University of Utah.

only). The effect each of these sources has on the final isotopic composition of a tissue depends on geographic location, as well as dietary choices. Ehleringer et al. (15) proposed that the strong correlation seen in the relationship of $\delta^2\text{H}$ versus $\delta^{18}\text{O}$ for modern U.S. hair samples is evidence that a “supermarket” diet does not mask a geographically derived water signal. Their model predicted hair stable isotopic composition based on drinking water and assumption of constant values for $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of food. The authors noted that the slopes of the hair versus water relationships were sensitive to the fraction of locally derived food in an individual’s diet.

In this study we set out to begin to isotopically measure a “continental supermarket” diet (15, 17). The standard American diet has come under increasing scrutiny as the rates of obesity and disease incidence in the United States continue to climb (18, 19). Almost 20% of the daily caloric (i.e., energy) intake in an average U.S. citizen’s diet comes from nutrient-poor sweets, soft drinks, and alcohol (18). The increased intake of nutrient-poor food coincides with a decrease in energy expenditure; driving a car is responsible for ~11% of the daily energy used by Americans. It should then come as no surprise that more than one-third of all food purchased and eaten by American households is consumed away from the home (20). With these statistics in mind, we began our survey with the staple of quick, prepared foods: the fast food meal.

MATERIALS AND METHODS

Fast food meals were collected from national chain and local restaurants in the metropolitan areas of Salt Lake City, UT, and Charleston, SC. Additionally, samples were collected from national fast food restaurant outlets across the contiguous United States. All samples were collected by ordering meals from the restaurant menu, and thus all samples were cooked as per restaurant specifications. Any effects on stable isotopic composition related to cooking, baking, or frying processes are assumed to be consistent from sample to sample. Upon purchase, a ~5 g piece of a plain beef patty (hereafter, hamburger patty) was either submerged in isopropyl alcohol within a glass vial or stored in a zip-top plastic bag. A single French fry was stored in a second plastic bag. For a subset of restaurants, a second French fry was also collected to compare the effect of cooking oil on carbon isotope ratios. A ~5 g piece of plain hamburger bun was collected in a manila coin envelope.

Hamburger and fry samples in plastic bags were stored frozen until freeze-dried. Hamburger bun samples were dried in an oven at 60 °C and then stored at room temperature. Once dry, all samples were ground to a homogeneous powder using a mortar and pestle. Hamburger samples in isopropyl alcohol were stored at room temperature. While in storage, some lipids from the meat sample were extracted and visible in the supernatant. Thus, isopropyl storage was considered a “pre”-delipidation step when frozen storage was not practical. All hamburger samples and one French fry from each collection location were loaded into cellulose thimbles and delipidated on a Soxhlet apparatus for 48 h using a 2:1 mixture of chloroform and methanol. Due to the length of the extraction period, there should be no difference in the degree of delipidation between samples. Delipidated samples were dried, ground a second time, and stored in glass vials. All other French fries and hamburger buns were not processed further.

Bun and fry samples analyzed for carbon only and hamburger patties analyzed for both carbon and nitrogen were loaded (2.00 mg \pm 10%) into tin capsules. Samples were analyzed on a Finnigan-MAT Delta S isotope ratio mass spectrometer (IRMS, Bremen, Germany) with an Elemental Analyzer (Carlo Erba, Milan, Italy) and zero-blank autosampler (Costech Analytical, Valencia, CA) attached. Stable isotope ratios for laboratory reference materials used during CN analysis were calibrated using NBS-19 and atmospheric air (AIR) for C and N, respectively. The standard deviations (SD) of repeated measurements of the same commercially produced powdered keratin reference material throughout all protein analyses were 0.2‰ for C and 0.1‰ for N. The

SD for the same cellulose reference material throughout all carbohydrate (buns and fries) analyses was 0.1‰.

Ground hamburger samples analyzed for hydrogen and oxygen stable isotopes were allowed to equilibrate with ambient water vapor for 48 h alongside laboratory reference materials prior to being loaded (150 μg \pm 10%) into prebaked silver capsules. We assumed there is partial isotopic exchange of hydrogen atoms in meat with water (either in liquid or vapor form), similar to keratin (21). Therefore, all protein samples were analyzed together with reference materials for which the $\delta^2\text{H}$ value of nonexchangeable H had been previously determined. The measured values for the reference materials were used to determine the nonexchangeable H isotope ratios of the samples from the measured values using isotope mass balance. Ground carbohydrate samples were analyzed along with an internal laboratory cellulose reference material that was stored under vacuum prior to loading. The stable isotope ratios for all laboratory reference materials used during HO analysis were calibrated using IAEA-CH-7 and NBS-19 for H and O, respectively.

All samples for HO analysis were stored under vacuum for at least 5 days before analysis. Samples were analyzed in duplicate on a ThermoFinnigan-MAT Delta Plus XL IRMS with a Thermo Chemical Elemental Analyzer (TC/EA) and zero-blank autosampler attached. High-temperature reduction at 1400 °C ensured complete conversion of organic H and O to gaseous H_2 and CO , respectively (22). Resultant gases were separated on a 1-m, 0.25 in. (outer diameter) molecular sieve 5A column (Costech Analytical). Baseline separation on masses 28, 29, and 30 between the N_2 and CO peaks was >40 s. Standard deviations for a powdered keratin quality control material included in all protein analyses were 2‰ for H and 0.2‰ for O. For carbohydrate samples, the SDs of the cellulose reference material were 2‰ for H and 0.1‰ for O.

Stable isotope contents are expressed in “delta” notation as δ values in parts per thousand (‰), where $\delta\text{‰} = (R_A/R_{\text{std}} - 1) \times 1000$ and R_A and R_{std} are the molar ratios of the rare to abundant isotope (e.g., $^{13}\text{C}/^{12}\text{C}$) in the sample and standard, respectively. The international standard for carbon is PeeDee Belemnite (V-PDB) and that for nitrogen, atmospheric air (AIR). The international standard for both hydrogen and oxygen is Standard Mean Ocean Water (V-SMOW).

Calculation of Predicted Wheat Cellulose Values. Cellulose values for the major wheat-growing regions of the contiguous United States were predicted using a model developed by Roden et al. (23). Average yearly precipitation values for the regions were calculated from the Online Isotopes in Precipitation Calculator (www.waterisotopes.org 8). Average growing season (defined here as May through September), afternoon relative humidity values, and maximum temperatures were calculated with data downloaded from the National Climatic Data Center (<http://lwf.ncdc.noaa.gov>). Wheat-growing regions were selected from maps published by the U.S. Department of Agriculture, National Agricultural Statistics Service (24). For a given wheat-growing region, the largest cities within the region were selected to calculate average relative humidity, maximum temperature, and precipitation.

Statistical Analysis. Statistical analyses were performed using InStat version 3.0a (GraphPad Software, Inc., San Diego, CA). Before analysis, samples purchased from national restaurant outlets around the United States were compared to samples purchased from national restaurant outlets in Utah. Among the three meal components and four isotope species measured, only $\delta^2\text{H}$ values were statistically different between hamburger patties and buns purchased in the United States and Utah ($t_{27} = 2.58$, $P < 0.05$; and $t_{30} = 3.13$, $P < 0.01$, respectively). Therefore, we decided to split samples into three groups based on location and type of restaurant: local restaurants in South Carolina (SC) and Utah (UT), plus all lumped national fast food chain restaurants (national). A single local restaurant sampled in Idaho (ID) is included in figures but was not used in any statistical analyses. The relationships between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and between $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of various components of a fast food meal were tested using linear regression. The difference in stable isotope composition between separate meal components (patty, bun, and fry) was tested using analysis of variance (ANOVA). Differences between group locations were tested using ANOVA and Tukey’s HSD post hoc test to identify significant differences among food items.



Figure 1. Locations of fast food restaurants within the contiguous United States and Puerto Rico sampled in this survey.

Table 1. Stable Isotopic Composition of the Three Components of a Fast Food Meal

component	N	stable isotopic composition			
		$\delta^2\text{H}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{18}\text{O}$ (‰)
hamburger patty	48				
mean		-131 ± 21	-15.7 ± 2.8	6.4 ± 0.5	13.5 ± 2.4
max		-103	-11.6	8.3	19.0
min		-181	-23.3	5.3	9.3
hamburger bun	49				
mean		-85 ± 10	-23.7 ± 0.7		29.4 ± 1.4
max		-61	-22.2		32.8
min		-120	-25.3		24.0
French fry	49				
mean		-147 ± 11	-26.0 ± 0.8		24.7 ± 1.6
max		-100	-23.2		28.8
min		-164	-27.8		21.8

RESULTS

We collected hamburger meals (patty, bun, and fry) from 48 restaurants in 21 locations throughout the contiguous United States and Puerto Rico (**Figure 1**). Results of the analyses are shown in **Table 1** and **Figure 2**. Components from one fast food meal, purchased from a local chain in Salt Lake City, were analyzed 10 times each to determine homogeneity within samples; standard deviations are shown in **Table 2**. To further explore isotopic variation, useful for interpreting data, we visited multiple outlets of a single national fast food chain within the Salt Lake City area, collecting meals (a) from six geographically dispersed outlets on a single day and (b) every day for a week at a single outlet. Additionally, we collected a single meal from an outlet of the same fast food chain and subsampled each meal component six times. Standard errors are shown in **Table 3**.

Carbon and Nitrogen. All but three hamburger patty samples fit within a narrow range of $\delta^{15}\text{N}$ values, from 5.7 to 7.0‰ (**Figure 3**). The maximum $\delta^{15}\text{N}$ values, 8.2 and 8.3‰, were samples purchased at a national fast food chain in South Carolina and a local restaurant in Utah, respectively. A second hamburger sample collected from the local Utah restaurant fell within the narrow range of $\delta^{15}\text{N}$ values. The minimum value of 5.3‰ was sampled from a local restaurant in Idaho. The range in $\delta^{13}\text{C}$ values for the patties was larger than that of $\delta^{15}\text{N}$ values (**Figure 2**). The most enriched $\delta^{13}\text{C}$ value (-11.6 ‰) was a sample purchased from a national chain in San Juan, PR, whereas the most depleted sample (-23.3 ‰) was purchased at a local restaurant in Salt Lake City, UT.

There was a significant difference between the mean $\delta^{13}\text{C}$ values of hamburger patties purchased from local restaurants in Utah and samples purchased from either local restaurants in South Carolina or national chains ($F_{2,44} = 16.10$, $P < 0.0001$). In general, Utah hamburger samples were more depleted in $\delta^{13}\text{C}$

values than those from elsewhere in the United States. There was no significant difference between the mean $\delta^{15}\text{N}$ values of patties from Utah, South Carolina, or national restaurants.

There was a significant linear correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for all hamburger samples ($r^2 = 0.094$, $P < 0.05$; equation: $\delta^{15}\text{N} = -0.1\delta^{13}\text{C} + 5.5$), although the percentage of the variance explained was low. However, the correlation is spurious, as removal of the three patty samples that did not fit in the narrow $\delta^{15}\text{N}$ range caused the correlation to become insignificant ($r^2 = 0.009$, $P < 0.05$; equation: $\delta^{15}\text{N} = -0.01\delta^{13}\text{C} + 6.2$).

Hamburger buns purchased from local chains in both Utah and South Carolina were significantly more depleted in $\delta^{13}\text{C}$ values than those purchased at national chains ($F_{2,45} = 17.64$, $P < 0.0001$). There was a lower range in $\delta^{13}\text{C}$ values for buns than delipidified French fries (**Figure 2**). Delipidified fries were significantly more depleted than buns ($t_{98} = 14.89$, $P < 0.0001$). Carbon values for delipidified French fries were not statistically different between Utah, South Carolina, and national restaurants. A sample of raw baking potato from one author's (L.C.) pantry analyzed for comparison had a $\delta^{13}\text{C}$ value indistinguishable from the group mean (**Table 1**). Comparing $\delta^{13}\text{C}$ values for French fries collected from 21 restaurants both before and after lipid extraction showed an average 0.8‰ enrichment after delipidification. This difference was statistically significant ($t_{40} = 2.22$, $P < 0.05$).

Hydrogen and Oxygen. Mean values of hamburger patties from Utah restaurants were significantly more depleted than patties from South Carolina and national restaurants for both hydrogen ($F_{2,43} = 68.94$, $P < 0.0001$) and oxygen ($F_{2,43} = 44.12$, $P < 0.0001$). There was a significant linear correlation between $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values for all hamburger samples (**Figure 4**; $r^2 = 0.822$, $P < 0.0001$; equation: $\delta^2\text{H} = 7.8\delta^{18}\text{O} - 237$ ‰). The slope of the line (7.8, with 95% confidence intervals of 6.7 and 8.9) was statistically indistinguishable from the slope of the global meteoric water line (GMWL, $m = 8$).

There was a significant linear relationship between $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values for hamburger buns (**Figure 5**; $r^2 = 0.62$, $P < 0.0001$; equation: $\delta^2\text{H} = 5.9\delta^{18}\text{O} - 258$ ‰). The linear relationship for modeled cellulose ($r^2 = 0.70$, $P < 0.0001$; equation: $\delta^2\text{H} = 8.5\delta^{18}\text{O} - 344$ ‰) is graphed alongside hamburger bun data for reference in **Figure 5**. The two lines were not significantly different from one another, with overlapping 95% confidence intervals. Hamburger buns purchased from Utah restaurants were significantly more depleted in $\delta^2\text{H}$ values than those bought from South Carolina and national restaurants ($F_{2,45} = 5.43$, $P < 0.05$); there was no difference in $\delta^{18}\text{O}$ values among the groups.

There was also a significant linear relationship between $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values for delipidified French fries (**Figure 6**; $r^2 = 0.24$, $P < 0.001$; equation: $\delta^2\text{H} = 3.3\delta^{18}\text{O} - 231$ ‰). Mean hydrogen and oxygen values for fries purchased from local restaurants in either Utah or South Carolina were statistically indistinguishable from one another and from national chain samples. The baking potato included for reference fell within the range of observed hydrogen and oxygen values. The slope of the $\delta^2\text{H}$ versus $\delta^{18}\text{O}$ relationship for French fries (3.3, with 95% confidence intervals of 1.6 and 5.2) was not statistically different from the slope for buns (5.9, with 95% confidence intervals of 4.5 and 7.2). However, fries were significantly more depleted for both hydrogen and oxygen than buns ($t_{98} = 28.75$, $P < 0.0001$; and $t_{98} = 15.42$, $P < 0.0001$, respectively).

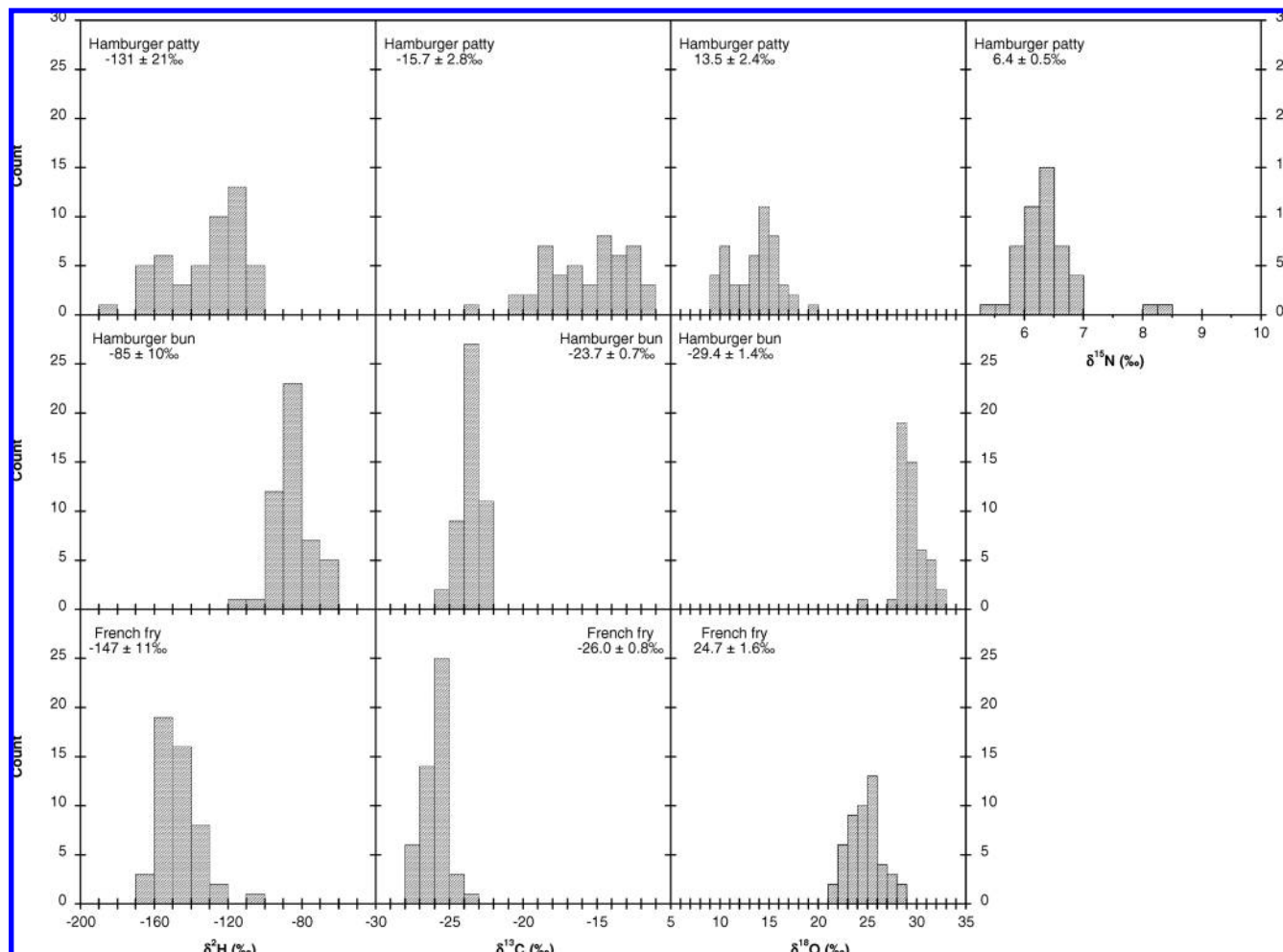


Figure 2. Histograms for $\delta^2\text{H}$, $\delta^{13}\text{C}$, and $\delta^{18}\text{O}$ values of hamburger patties, hamburger buns, and French fries collected in this survey, as well as $\delta^{15}\text{N}$ values of hamburger patties only.

Table 2. Standard Deviations for 10 Replicate Analyses of the Three Components of a Fast Food Meal

component	standard deviation			
	$\delta^2\text{H}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{18}\text{O}$ (‰)
hamburger patty	1.60	0.41	0.07	0.33
hamburger bun	1.36	0.06		0.13
French fry ^a	before	3.61	0.12	0.55
	after	2.08	0.09	0.38

^a French fries have been analyzed both before and after delipification.

DISCUSSION

Hamburger sandwiches (patty and bun) purchased from Utah restaurants were more depleted for carbon, hydrogen, and oxygen stable isotopes than sandwiches from South Carolina and national restaurants, which might reflect a local signal in a national fast food diet. Sandwiches from South Carolina restaurants were different from national samples only when the $\delta^{13}\text{C}$ values of the buns were considered. However, there were no differences among French fry samples from the three groups. The sole Idaho sample collected in this survey fell within the ranges of the Utah group, except the $\delta^{15}\text{N}$ value of the hamburger patty.

Variability within Samples. There was little variation among carbon and nitrogen stable isotope ratios in a hamburger patty, hamburger bun, and serving of French fries from one meal. Thus, the single values reported here are sufficient to character-

Table 3. Standard Errors from the Analysis of the Three Fast Food Meal Components Collected from Multiple Outlets of a National Fast Food Chain^a

collection	component	N	standard error	
			$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
6 outlets (1 day)	patty	6	0.52	0.16
	bun	6	0.03	
	fry	6	0.16	
1 outlet (7 days ^b)	patty	6	0.59	0.15
	bun	7	0.05	
	fry	6	0.35	
1 meal (6 subsamples)	patty	6	0.21	0.10
	bun	6	0.02	
	fry	6	0.20	

^a Samples were obtained in three separate collection efforts; see text for a full description of each collection. ^b Consecutively.

ize a sample. It appears neither our subsampling technique nor physical processing of samples before analysis introduced significant isotopic heterogeneity. Conversely, there was greater variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between meals purchased from multiple outlets on a single day and between meals purchased from a single outlet over seven consecutive days than the single, subsampled meal. However, we believe the variation was not large enough to affect the patterns presented above and discussed below.

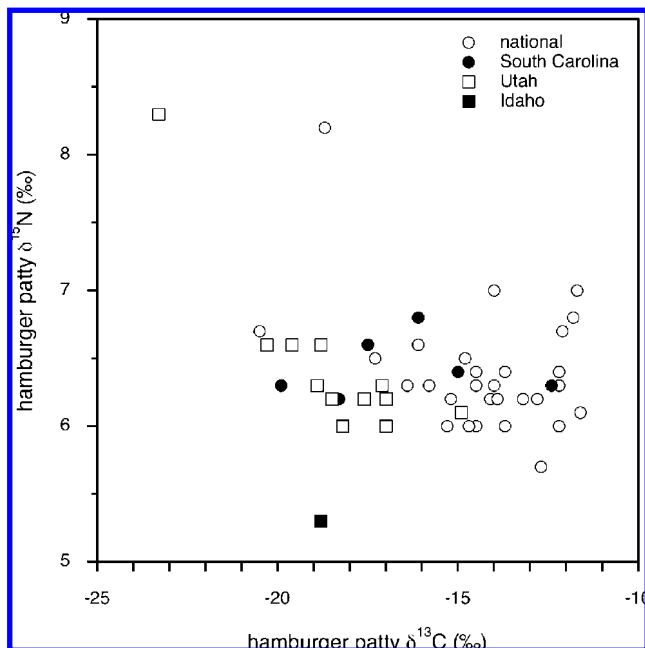


Figure 3. Delipified hamburger patty $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from national fast food chains (open circles) and local restaurants in South Carolina (solid circles), Utah (open squares), and Idaho (solid square).

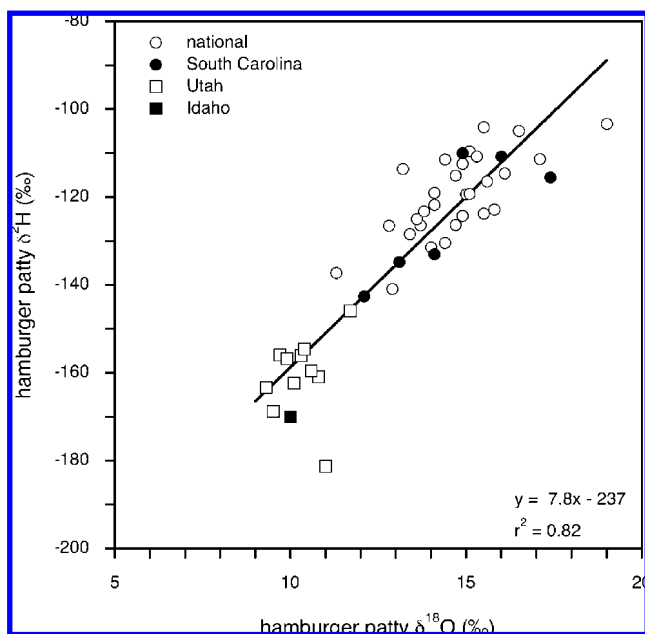


Figure 4. Delipified hamburger patty average $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values from national fast food chains (open circles) and local restaurants in South Carolina (solid circles), Utah (open squares), and Idaho (solid square). Solid line is the simple linear regression line for all samples.

Hamburger Patties. The mean $\delta^{13}\text{C}$ value (-15.7‰) for this study was slightly more depleted than values reported previously for beef samples from the United States (25–27). However, the variation observed by Schmidt et al. ($\text{SD} = 0.1\text{‰}$; 25) was much smaller than we measured ($\text{SD} = 2.7\text{‰}$), suggesting more homogeneous sources or limited sampling in that study. Cows eating a pure C_3 diet would have mean $\delta^{13}\text{C}$ values near -26.7‰ , whereas cows on a C_4 diet would be near -12.5‰ (6). It appears several hamburger patties came from cows whose diets included a significant C_3 fraction, contributing to the wide variation in $\delta^{13}\text{C}$ values observed. Even if all hamburger patties supplied to fast food restaurants in the United

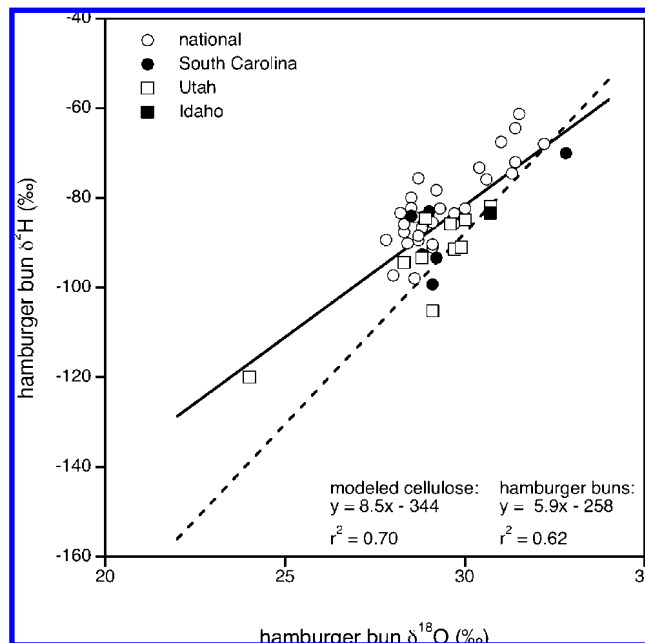


Figure 5. Hamburger bun average $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values from national fast food chains (open circles) and local restaurants in South Carolina (solid circles), Utah (open squares), and Idaho (solid square). Solid line is the simple linear regression line for all samples. Dashed line is the simple linear regression line for modeled cellulose from the major wheat-growing regions of the United States.

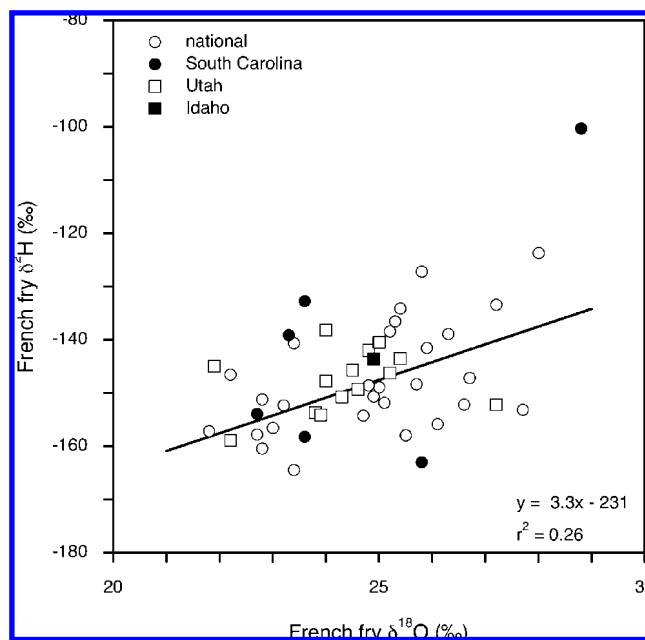


Figure 6. Delipified French fry average $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values from national fast food chains (open circles) and local restaurants in South Carolina (solid circles), Utah (open squares), and Idaho (solid square). Solid line is the simple linear regression line for all samples.

States come from a few national supply companies, the meat is a mix of cattle on various feeds. For example, patties bought from local Utah restaurants had the most depleted $\delta^{13}\text{C}$ values, which we interpret as cattle raised primarily on pasture forage. However, the lowest $\delta^{13}\text{C}$ value observed in hamburger patties in this survey (-20.3‰) is not as depleted as a pure C_3 diet (6) or as depleted as beef samples from the United Kingdom (27, 28), the Republic of Ireland (27, 29), or Scotland (27).

Interestingly, a non-U.S. patty from Windsor, ON, Canada, had a $\delta^{13}\text{C}$ value (-19.0‰) more than 5‰ lower than a hamburger sampled from the same national fast food chain in Detroit, MI, just across the U.S.–Canada border. The depleted $\delta^{13}\text{C}$ value in the Canadian hamburger is more similar to European beef (27–29). However, it is unlikely the Canadian hamburger contained a significant fraction of imported European beef. Most Canadian beef imports arrive from Australia, New Zealand, and the United States with Australian and New Zealand meats used mainly for grinding (30). Whereas countries in the European Union (EU) do export meat worldwide, EU beef and veal accounted for only 4.3% of the world trade in 2004; Canada imports only 2.7% of the total EU beef and veal exported (31).

There was no difference in $\delta^{15}\text{N}$ values from patty samples purchased from different restaurants. This is similar to data from Manca et al. (32), who found $\delta^{15}\text{N}$ values could not be used to differentiate local dairy, factory, and imported cheeses in Sardinia. Our data showed some slight variation in $\delta^{15}\text{N}$ values for hamburger patties, but there was no clear pattern based on restaurant chain size and/or distribution.

The majority of the $\delta^{15}\text{N}$ values of the hamburger patties agreed with data published for beef samples in the United States, the United Kingdom, the Republic of Ireland, and Scotland (27, 29), although in some cases our samples were slightly depleted (26) or enriched (25, 33). The single Canadian patty sample had a $\delta^{15}\text{N}$ value within the data range observed for U.S. hamburger patties. A seasonal survey of organically and conventionally reared beef in Ireland showed very little variation in nitrogen isotopic composition of either type throughout the year; mean values matched values we observed in American hamburgers (29). The tight range of $\delta^{15}\text{N}$ values for the majority of the U.S. hamburger samples could be due to the relative stability of the nitrogen inputs into the cattle feed and subsequently the animal. Presumably, most managed feed crops are heavily fertilized with Haber–Bosch nitrogen. Because the median commercial fertilizer nitrogen isotopic composition is 0.0‰ , with a tight range (34, 35), and most fertilizers commercially produced are chemically similar, the nitrogen input to soils would not vary over time. Thus, the $\delta^{15}\text{N}$ values of a feed crop (for example, corn) should be fairly constant over time as well. Assuming an average trophic level enrichment of 3‰ for N, the feed for cows in the American beef cattle industry should be approximately $3\text{–}4\text{‰}$. Indeed, a review of the literature finds values of approximately 3‰ for corn meal and corn silage (26, 33, and 36, but see also data reviewed in refs 37 and 38).

The most depleted component of the fast food meal for both hydrogen and oxygen was the hamburger patty, similar to findings of O'Brien and Wooller (39), who observed lower values for $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in meat and eggs than in fruits, vegetables, and carbohydrate-based mixed foods. Similar to $\delta^{13}\text{C}$ values, $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values for the sole Canadian patty sample were more depleted (-150 and 8.5‰ , respectively) than its counterpart sampled in Detroit, MI (-114 and 13.2‰ , respectively).

The large range in $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values for hamburger patties sampled is indicative of variation in source water for the animal tissue, that is, variation in the geographic origin for beef cattle. An inventory of cows within the United States shows the greatest amount of beef cattle production in Texas, Missouri, Oklahoma, Nebraska, and South Dakota (24), a “cattle belt.” The large geographic range in production sites translates into a large range for precipitation $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values (8), which are then recorded into hamburger patties. For local Utah restaurants, the meat potentially comes from a relatively depleted

region, for example, South Dakota, which is arguably the most “local” (to Utah) of the cattle-belt states.

The similarity between the slopes of the GMWL and $\delta^2\text{H}$ versus $\delta^{18}\text{O}$ relationship of hamburger patties is indicative of the influence local precipitation has on two trophic levels: primary producers and primary consumers. A study of beef samples collected in Germany by Boner and Förstel (40) demonstrated a strong relationship between $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values from cattle muscle tissue water. The slope of the relationship (5.3) was significantly different from the GMWL, a result they attributed to fractionation during evapotranspiration by plants the cattle were eating. However, the authors found a seasonal trend in tissue water—in winter months, tissue water was almost identical to groundwater for the region. If tissue water mimics groundwater and groundwater is an amalgamation of local precipitation values, tissue water should thus fall along the GMWL in the winter. Assuming $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of tissue solid are the same as tissue water values, it should be no surprise that the hydrogen and oxygen isotope ratios of meat fall along the GMWL. Heaton et al. (27) recently sampled raw beef from several cattle-producing countries around the world, including the United States, and found a $\delta^2\text{H}$ versus $\delta^{18}\text{O}$ relationship for extracted lipids with a slope of 8.8, similar to both the relationship found for U.S. delipidated solids in this study and the GMWL.

The relationship of $\delta^2\text{H}$ versus $\delta^{18}\text{O}$ for patty samples purchased from national restaurants has a significantly different, non-overlapping slope from the data set as a whole (equation: $\delta^2\text{H} = 4.7\delta^{18}\text{O} - 188\text{‰}$). The slope of the national hamburger patties relationship was more similar to the observed relationship between hydrogen and oxygen for human hair in the United States ($\delta^2\text{H} = 5.7\delta^{18}\text{O} - 166\text{‰}$; 15) and human hair from four Asian countries ($\delta^2\text{H} = 5.4\delta^{18}\text{O} - 168\text{‰}$; 41).

Hamburger Buns. The range we observed in hamburger bun $\delta^{13}\text{C}$ values was 5 times greater than the range seen in American flours (42) and 3 times larger than the range seen in Italian flours (43). In addition, the average value for hamburger buns was more enriched than American (26, 42) or Italian (43) flours. This may be due to the inclusion of high-fructose corn syrup (HFCS) in the ingredients list for commercially produced hamburger buns. Brescia et al. (43) demonstrated there was no significant difference in the $\delta^{13}\text{C}$ value of flours that were then processed into dough and finally baked to produce bread. These breads were collected from local bakers in Italy and baked according to traditional recipes, which very likely do not include HFCS.

Almost all hamburger buns analyzed clustered between -105 and -61‰ for hydrogen and between 27.8 and 32.2‰ for oxygen. These values are similar to modeled plant cellulose values in Kansas, Nebraska, western Oklahoma, northern Texas, and the Ohio and Mississippi River valleys. Two exceptions—a hamburger bun collected from a local restaurant in South Carolina and a bun collected from a local chain in Utah—fell outside the main cluster of modeled cellulose hydrogen and oxygen values. It appears the suppliers of these samples did not use wheat grown in the “bread basket” of the United States, where the majority of wheat flour originates. The Canadian hamburger bun sample had $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values (-94 and 25.9‰ , respectively) similar to those of the depleted local Utah hamburger bun outlier (-120 and 24.0‰ , respectively).

French Fries. The average $\delta^{13}\text{C}$ value for delipidated French fries collected in this survey was indistinguishable from the $\delta^{13}\text{C}$ value of a raw baking potato purchased from the grocery store (but see ref 26, where the $\delta^{13}\text{C}$ value for potato was found to

be higher). Lipid within a French fry can come from one of two sources: oil added prior to frying and oil absorbed during frying. The national chains we surveyed published ingredients lists for French fries on company Websites; the second ingredient in all lists was partially hydrogenated soybean oil. Literature $\delta^{13}\text{C}$ values for soybean oil are near -30‰ (26, 44). Among the fries we analyzed the $\delta^{13}\text{C}$ value after delipification was 0.8‰ heavier than before, suggesting frying in a C_3 cooking oil. Morrison et al. (28) analyzed several common fats and oils and found that all have a C_3 signal: -30.6 to -27.7‰ . Despite the opportunity to use corn oil for frying, it appears from both the published ingredients list and then isotopic evidence that fast food restaurants are using soybean oil, or some other C_3 cooking oil, when frying.

Is There a Supermarket Diet? From this survey of fast food meals in the United States, we have shown that there exists significant variation in the stable isotopic composition of common fast food items. The variation in $\delta^{13}\text{C}$ values of hamburger patties may be indicative of differences in cattle-rearing practices (corn versus pasture feed), whereas $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of the meat and bread in the sandwich indicate that food comes from isotopically (i.e., geographically) diverse locations. Understanding the isotopic variation within different dietary items could be useful in investigating region-of-origin claims or screening for adulteration. Heaton et al. (27) caution on the limitations of stable isotope analysis, warning that it is far easier to answer a region-of-origin claim when the field of possibilities is limited than when it is wide open. At present we do not feel we can use the data presented here in a predictive manner; we would not attempt to analyze a hamburger patty or French fry from an unknown location and then predict a region of origin. However, the variations in stable isotope ratios presented here do have broader implications in the definition of a “continental supermarket” diet for the U.S. population. An individual may actually be consuming a “regional supermarket” diet that varies through time, from season to season, as the region of origin for specific foods changes. As the regional nature of the diet shifts, an individual could isotopically appear to move, without actually moving location. However, turnover rates for isotopes in tissue can be quite slow (45), so the consumer never reaches carbon and nitrogen isotope equilibrium with one regional diet before it changes. Thus, the regional signals averaged by the individual may be distinguishable from a “continental” diet with respect to $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The mean $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values used by Ehleringer et al. (15) when modeling human hair isotopic composition (-115 and 26.0‰ , respectively) are similar to the mean values for the combined fast food meals samples we surveyed (-121 and 22.5‰ , respectively). On the other hand, the model predicts that $\delta^{18}\text{O}$ values of carbohydrates influence the isotope ratio of hair proteins only indirectly through their effects on body water $\delta^{18}\text{O}$ values. Characterizing the range found in food items would help when presented with an individual whose diet varies consistently from the average “continental” diet. Sampling fast food meals seasonally at geographically disparate regions of the United States may help in teasing apart a regional versus a continental diet in that regard.

ACKNOWLEDGMENT

We thank Corrie Brown, Mary Chesson, Patricia Draper, Sarah Fitch, H. B. Harpending, Jennifer Hunter, Scott McWilliams, Emily Powers, Kevin Uno, and the entire Ehleringer lab for sampling aid. We thank Michael Lott, W. Ike, and A. May for analytical help. Jason West helped with modeling cellulose

values. Janet Hurley and John Howa substantially edited an early manuscript draft. We are also grateful for the input of four anonymous reviewers, which improved this publication.

LITERATURE CITED

- (1) West, J.; Bowen, G. J.; Cerling, T.; Ehleringer, J. Stable isotopes as one of nature's ecological recorders. *Trends Ecol. Evolution* **2006**, *21*, 408–414.
- (2) O'Connell, T. C.; Hedges, R. E. M. Investigations into the effect of diet on modern human hair isotopic values. *Am. J. Phys. Anthropol.* **1999**, *108*, 409–425.
- (3) Macko, S. A.; Lubec, G.; Teschler-Nicola, M.; Andrushevich, V.; Engel, M. H. The Ice Man's diet as reflected by the stable nitrogen and carbon isotopic composition of his hair. *FASEB J.* **1999**, *13*, 559–562.
- (4) Bol, R.; Pflieger, C. Stable isotope (^{13}C , ^{15}N and ^{34}S) analysis of the hair of modern humans and their domestic animals. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 2195–2200.
- (5) Passey, B.; Robinson, T.; Ayliffe, L.; Cerling, T.; Sponheimer, M.; Dearing, D.; Roeder, B.; Ehleringer, J. Carbon isotope fractionation between diet, breath CO_2 , and bioapatite in different animals. *J. Archaeol. Sci.* **2005**, *32*, 1459–1470.
- (6) Cerling, T.; Harris, J.; MacFadden, B.; Leakey, M.; Quade, J.; Eisenmann, V.; Ehleringer, J. Global vegetation change through the Miocene/Pliocene boundary. *Nature* **1997**, *389*, 153–158.
- (7) Post, D. Using stable isotopes to estimate trophic position: models, methods and assumptions. *Ecology* **2002**, *83*, 702–718.
- (8) Bowen, G. J.; Revenaugh, J. Interpolating the isotopic composition of modern meteoric precipitation. *Water Resour. Res.* **2003**, *39*, 1299–1311.
- (9) Bowen, G. J.; Ehleringer, J.; Chesson, L.; Stange, E.; Cerling, T. Stable isotope ratios of tap water in the contiguous United States. *Water Resour. Res.* **2007**, *43*, W03419.
- (10) Chamberlain, C.; Blum, J.; Holmes, R.; Feng, X.; Sherry, T.; Graves, G. The use of isotope tracers for identifying populations of migratory birds. *Oecologia* **1997**, *109*, 132–141.
- (11) Hobson, K. A.; Atwell, L.; Wassenaar, L. I. Influence of drinking water and diet on the stable-hydrogen isotope ratios of animal tissues. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 8003–8006.
- (12) Wassenaar, L. I.; Hobson, K. A. Natal origins of migratory monarch butterflies at wintering colonies in Mexico: new isotopic evidence. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15436–15439.
- (13) Kreuzer-Martin, H.; Lott, M.; Dorigan, J.; Ehleringer, J. Microbe forensics: oxygen and hydrogen stable isotope ratios in *Bacillus subtilis* cells and spores. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 815–819.
- (14) Sharp, Z.; Atudorei, V.; Panarello, H.; Fernández, J.; Douthitt, C. Hydrogen isotope systematics of hair: archeological and forensics applications. *J. Archaeol. Sci.* **2003**, *30*, 1709–1716.
- (15) Ehleringer, J.; Bowen, G. J.; Chesson, L.; West, A.; Podlesak, D.; Cerling, T. Hydrogen and oxygen isotope ratios in human hair are related to geography. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 2788–2793.
- (16) Fraser, I.; Meier-Augenstein, W.; Kalin, R. M. The role of stable isotopes in human identification: a longitudinal study into the variability of isotopic signals in human hair and nails. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 1109–1116.
- (17) Nardoto, G.; Silva, S.; Kendall, C.; Ehleringer, J.; Chesson, L.; Ferraz, E.; Moreira, M.; Ometto, J.; Martinelli, L. Geographical patterns of human diet derived from stable-isotope analysis of fingernails. *Am. J. Phys. Anthropol.* **2006**, *131*, 137–146.
- (18) Block, G. Foods contributing to energy intake in the US: data from NHANES III and NHANES 1999–2000. *J. Food Compos. Anal.* **2004**, *17*, 439–447.
- (19) Cordain, L.; Eaton, S.; Sebastian, A.; Mann, N.; Lindeberg, S.; Watkins, B.; O'Keefe, J.; Brand-Miller, J. Origins and evolution of the Western diet: health implications for the 21st century. *Am. J. Clin. Nutr.* **2005**, *81*, 341–354.

- (20) Blisard, N.; Stewart, H. *Food spending in American households, 2003–04*; Economic Information Bulletin 829; U.S. Department of Agriculture: Washington, DC, 2007; 115 pp.
- (21) Bowen, G. J.; Chesson, L.; Nielson, K.; Cerling, T. E.; Ehleringer, J. R. Treatment methods for the determination of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of hair keratin by continuous-flow isotope-ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 2371–2378.
- (22) Leuenberger, M.; Filot, M. Temperature dependencies of high-temperature reduction on conversion products and their isotopic signatures. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 1587–1598.
- (23) Roden, J.; Lin, G.; Ehleringer, J. A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose. *Geochim. Cosmochim. Acta* **2000**, *64*, 21–35.
- (24) U.S. Department of Agriculture National Agricultural Statistics Service, Census of Agriculture, 2002; available at <http://www.agcensus.usda.gov/>.
- (25) Schmidt, O.; Quilter, J.; Bahar, B.; Moloney, A.; Scrimgeour, C.; Begley, I.; Monahan, F. Inferring the origin and dietary history of beef from C, N and S stable isotope ratio analysis. *Food Chem.* **2005**, *91*, 545–549.
- (26) Schoeller, D.; Minagawa, M.; Slater, R.; Kaplan, I. Stable isotopes of carbon, nitrogen and hydrogen in the contemporary North American human food web. *Ecol. Food Nutr.* **1986**, *18*, 159–170.
- (27) Heaton, K.; Kelly, S. D.; Hoogewerff, J.; Woolfe, M. Verifying the geographical origin of beef: the application of multi-element isotope and trace element analysis. *Food Chem.* **2008**, *107*, 506–515.
- (28) Morrison, D.; Dodson, B.; Slater, C.; Preston, T. ^{13}C natural abundance in the British diet: implications for ^{13}C breath tests. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 1321–1324.
- (29) Bahar, B.; Schmidt, O.; Moloney, A.; Scrimgeour, C.; Begley, I.; Monahan, F. Seasonal variation in the C, N and S stable isotope composition of retail organic and conventional Irish beef. *Food Chem.* **2008**, *106*, 1299–1305.
- (30) Young, L.; Barber, D.; Fetterly, G. Report on Canadian beef supply and disposition with beef quality categorization; Economic and Policy Analysis Directorate; Agriculture and Agri-Food Canada Publication 1941E; 1997; 35 pp.
- (31) European Commission Agriculture and Rural Development, Agricultural trade statistics, 2006; available at http://ec.europa.eu/agriculture/agrista/index_en.htm.
- (32) Manca, G.; Franco, M.; Versini, G.; Camin, F.; Rossman, A.; Tola, A. Correlation between multielement stable isotope ratio and geographical origin in Peretta cows' milk cheese. *J. Dairy Sci.* **2006**, *89*, 831–839.
- (33) Bahar, B.; Monahan, F.; Moloney, A.; O'Kiely, P.; Scrimgeour, C.; Schmidt, O. Alteration of the carbon and nitrogen stable isotope composition of beef by substitution of grass silage with maize silage. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 1937–1942.
- (34) Vitória, L.; Otero, N.; Soler, A.; Canals, A. Fertilizer characterization: isotopic data (N, S, O, C, and Sr). *Environ. Sci. Technol.* **2004**, *38*, 3254–3262.
- (35) Shearer, G.; Kohl, D.; Commoner, B. The precisions of determinations of the natural abundance of nitrogen-15 in soils, fertilizers and shelf chemicals. *Soil Sci.* **1974**, *118*, 309–316.
- (36) Masud, Z.; Vallet, C.; Martin, G. Stable isotope characterization of milk components and whey ethanol. *J. Agric. Food Chem.* **1999**, *47*, 4693–4699.
- (37) Piasentier, E.; Valusso, R.; Camin, F.; Versini, G. Stable isotope ratio analysis for authentication of lamb meat. *Meat Sci.* **2003**, *64*, 239–247.
- (38) Knobbe, N.; Vogl, J.; Pritzkow, W.; Panne, U.; Fry, H.; Lochotzke, H.; Preiss-Weigert, A. C and N stable isotope variation in urine and milk of cattle depending on the diet. *Anal. Bioanal. Chem.* **2006**, *386*, 104–108.
- (39) O'Brien, D.; Wooller, M. Tracking human travel using stable oxygen and hydrogen isotope analyses of hair and urine. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 2422–2430.
- (40) Boner, M.; Förstel, H. Stable isotope variation as a tool to trace the authenticity of beef. *Anal. Bioanal. Chem.* **2004**, *378*, 301–310.
- (41) Thompson, A.; Chesson, L.; Podlesak, D.; Cerling, T.; Ehleringer, J. Stable isotope analysis of modern human hair collected along a geographic transect across Asia: $\delta^2\text{H}$, $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ data reveal geography and diet. in preparation.
- (42) Branch, S.; Burke, S.; Evans, P.; Fairman, B.; Wolff Briche, C. A preliminary study in determining the geographical origin of wheat using isotope ratio inductively coupled plasma mass spectrometry with ^{13}C , ^{15}N mass spectrometry. *J. Anal. At. Spectrom.* **2003**, *18*, 17–22.
- (43) Brescia, M.; Sacco, D.; Sgaramella, A.; Pasqualone, A.; Simeone, R.; Peri, G.; Sacco, A. Characterisation of different typical Italian breads by means of traditional, spectroscopic and image analyses. *Food Chem.* **2007**, *104*, 429–438.
- (44) Bréas, O.; Guillou, C.; Reniero, F.; Sada, E.; Angerosa, F. Oxygen-18 measurement by continuous flow pyrolysis/isotope ratio mass spectrometry of vegetable oils. *Rapid Commun. Mass Spectrom.* **1998**, *12*, 188–192.
- (45) Ayliffe, L. K.; Cerling, T. E.; Robinson, T.; West, A. G.; Sponheimer, M.; Passey, B. H.; Hammer, J.; Roeder, B.; Dearing, M. D.; Ehleringer, J. R. Turnover of carbon isotopes in tail hair and breath CO_2 of horses fed an isotopically varied diet. *Oecologia* **2004**, *139*, 11–22.

Received for review November 16, 2007. Revised manuscript received March 12, 2008. Accepted March 14, 2008. This study was supported by IsoForensics, Inc.

JF0733618