



## Analytical Methods

Isotopic consequences of consumer food choice: Hydrogen and oxygen stable isotope ratios in foods from fast food restaurants *versus* supermarkets

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## ABSTRACT

We investigated geographic trends in the isotopic composition of the modern American diet, purchasing paired food items from fast food restaurants and supermarkets across the USA. We observed large ranges in  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values, suggesting variation in the region-of-origin for beef, wheat, and potatoes. Mean restaurant meal  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values ( $-114$  and  $22.6\%$ , respectively) were similar to supermarket values ( $-111$  and  $22.1\%$ , respectively). There were no correlations between restaurant beef and local tap water isotope values but significant correlations between supermarket beef and water ( $\delta^2\text{H}_{\text{beef}} = \delta^2\text{H}_{\text{water}} * 0.19 - 115\%$  and  $\delta^{18}\text{O}_{\text{beef}} = \delta^{18}\text{O}_{\text{water}} * 0.17 + 14.8\%$ ) suggesting regionality in the source of beef available to supermarket patrons. We observed no correlations between the stable isotopic composition of carbohydrates and local tap water. Understanding regional differences observed in some foods but not others will help refine parameters in models used to explore human movements in anthropological, archaeological, and forensic studies.

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## 1. Introduction

Modern Americans dine at a continental table, eating nationally distributed foods which are often produced in non-local (to the consumer) regions of the USA (Leff, Ramankutty, & Foley, 2004; Monfreda, Ramankutty, & Foley, 2008). The result can be a “food footprint” which is larger than that of a generation ago (Gerbens-Leenes & Nonhebel, 2002). For example, residents of North Carolina and Utah can have simultaneous access to California strawberries and Florida oranges, despite the large geographic distances between the two purchase locations and between the purchase locations and food production regions. While recent popular literature has championed locally-produced foods (Kingsolver, Hopp, & Kingsolver, 2007), the convenience and price of mass-produced items widely available in fast food restaurants and supermarkets (Jekanowski, 1999; Jekanowski, Binkley, & Eales, 2001) are likely to prevent any real change in American dining habits in the near future.

Recent advances in stable isotope analysis have made it possible to investigate the region-of-origin claims of goods and materials. These investigations exploit predictable variations in the stable

isotope ratios of hydrogen ( $\delta^2\text{H}$ ) and oxygen ( $\delta^{18}\text{O}$ ) within the global water cycle, which are subsequently incorporated into the plants and animals that we eat each day. The stable isotopes of water vary across continents, with high latitude, inland, and cooler regions being relatively depleted in the heavy isotopes of hydrogen and oxygen; in contrast, low latitude, coastal, and warmer regions tend to have water that is enriched in these heavy stable isotopes (Bowen, Ehleringer, Chesson, Stange, & Cerling, 2007). As plants incorporate local water isotopes, these signals are then propagated through subsequent trophic levels in the food web. The spatial distributions of water  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values have thus been successfully used to discern the geographic origin of goods such as beef (Heaton, Kelly, Hoogewerff, & Woolfe, 2008; Nakashita et al., 2008), cheese (Camin et al., 2004), lamb (Camin et al., 2007), and wine (West, Ehleringer, & Cerling, 2007).

Due to consolidation within American food supply chains, food purchased in two geographically disparate regions of the USA could have similar  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values, as seen in supermarkets in Alaska and New York (O'Brien and Wooller, 2007). Ehleringer et al. (2008) capitalised on the stability of US food sources, assuming constant  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values for dietary input in a process-based model predicting drinking water stable isotope ratios from modern American human hair  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values. The authors noted, however, the model was sensitive to the fraction of continentally- versus locally-derived food in an individual's diet and

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predicted a stronger relationship between water and hair stable isotope ratios as more local foods were consumed. Unlike many animals, humans have the latitude to make a wide range of food choices. Larger, philosophical choices can relate to the relative trophic level of a consumer (omnivore *versus* vegan, for example) and consequently have large impacts on the final isotopic composition of the consumer (Birchall, O'Connell, Heaton & Hedges, 2005). Humans also make a myriad of smaller choices on a daily basis (a #1 *versus* #4 combo meal, for example), which may also have both isotopic and caloric impacts.

We surveyed one facet of the American diet across a continental transect to explore the potential disconnect between the location food is purchased and the region of its origin by examining the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of common food items. One-third of all food purchased by Americans is consumed away from the home (Blisard & Stewart, 2007). Thus, we began our survey with the model of convenience food, the fast food meal. We also examined isotopic variation in analogous food items that can be purchased in supermarkets and prepared at home to explore the impacts of food choice on dietary stable isotope ratios.

## 2. Materials and methods

### 2.1. Sample acquisition and preparation

Children's meals were purchased from multiple outlets of a national fast food restaurant chain in 18 states in the contiguous USA during February 2008. Analogous food items – ground beef, loaf bread, and a baking potato – were also purchased from a traditional grocery retailer (“supermarket”) in each city from which a fast food meal was collected. Raw ground beef was purchased from the supermarket's meat counter when possible; otherwise, samples were collected through purchase of the least expensive container of prepackaged ground beef. When available, the store brand of white loaf bread was purchased. If no store brand was offered, the least expensive package of white loaf bread was instead collected. Potatoes were purchased from the produce section.

Upon purchase, 5–10 g of the plain beef patty (“hamburger”) or raw ground beef was subsampled in a 15-ml conical polypropylene centrifuge tube. A single French fry was collected in a separate tube. A ~5-g piece of the hamburger bun was subsampled in a manila coin envelope. A whole slice of loaf bread was collected in a manila 6 × 8” envelope. All meat and fry samples were stored on wet ice until frozen, then freeze-dried. Bun and bread samples were dried at 60 °C, then stored at room temperature. Potatoes were stored intact at room temperature until a piece was cut from the interior of the potato, immediately frozen, and then freeze-dried. Dried samples were ground to powder using a mortar and pestle. Meat and fry samples were loaded into cellulose thimbles and defatted with a mixture of 2:1 chloroform:methanol for 48 h on a Soxhlet apparatus. Defatted samples were dried and ground a second time. All other carbohydrate samples collected in this survey (buns, bread, and potatoes) were not processed further.

In every city tap water samples were collected from three separate locations among fast food restaurants, supermarkets, hotels, rest stops, and gas stations. Samples were collected in either 30-ml conical polypropylene centrifuge tubes or 1-dram glass vials by allowing the tap to run for 5–10 s before filling the collection tube/vial. Samples were sealed with Parafilm® M and stored in a cool, dark location prior to analysis.

### 2.2. Stable isotope analysis

Prior to analysis, ground meat samples were equilibrated for at least 48 h with ambient water vapour alongside three powdered

keratin laboratory reference materials. Because labile H atoms within the samples can exchange with H atoms from the atmosphere, the non-exchangeable H isotopic composition of the unknowns was calculated using the co-equilibrated reference materials, for which the non-exchangeable  $\delta^2\text{H}$  value had been previously determined (Bowen, Chesson, Nielson, Cerling, & Ehleringer, 2005). There was a difference between the fraction of H atoms available for exchange in meat tissue and the ground horse-hair reference materials. However, the difference was slight (~3%) and thus did not introduce a large error in the final calculated non-exchangeable  $\delta^2\text{H}$  values (Chesson, Podlesak, Cerling, & Ehleringer, 2009). Ground carbohydrate samples were also equilibrated with ambient water vapour then analysed alongside a laboratory cellulose reference material that was stored under vacuum prior to loading.

Food samples and reference materials were loaded (150  $\mu\text{g} \pm 10\%$ ) in silver capsules and stored under vacuum for a minimum of 5 days prior to analysis. Water samples and reference materials were loaded (400  $\mu\text{l}$ ) into GC vials and sealed. Samples were analysed in duplicate on a ThermoFinnigan-MAT Delta Plus XL isotope ratio mass spectrometer (Bremen, Germany) with a high temperature conversion elemental analyser (TC/EA) attached. Samples were pyrolysed at 1400 °C, producing  $\text{H}_2$  and CO gas. Gases were separated on a 1-m, 0.25 in (outer diameter) molecular sieve 5 Å gas chromatography column (Costech Analytical, Valencia, CA). Solid samples were introduced to the pyrolysis column *via* a zero-blank autosampler (Costech Analytical). Water samples were injected using a PAL autosampler (LEAP Technologies, Carrboro, NC). The  $\sigma$  values for a powdered keratin quality control material included in all meat analyses were  $\pm 1.4\%$  and  $\pm 0.4\%$  for H and O, respectively. For carbohydrate analyses, the  $\sigma$  values for the laboratory cellulose reference material were  $\pm 1.2\%$  and  $\pm 0.3\%$  for H and O, respectively. The analytical precision for water samples was  $\pm 1.55\%$  and  $\pm 0.17\%$  for H and O, respectively.

Stable isotopic compositions are reported in “delta” notation as  $\delta$ -values expressed as

$$\delta = (R_A/R_S - 1) \times 1000\%$$

where  $R_A$  and  $R_S$  are the ratios of heavy to light isotopes (e.g.  $^2\text{H}/^1\text{H}$ ) in the sample and standard, respectively. The international standard for H and O is Vienna Standard Mean Ocean Water (VSMOW).

### 2.3. Statistical analysis

Correlations between  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values were tested using ordinary least square regression analysis in Excel® 2004 for Mac (Microsoft® Corporation) with the Analysis Toolpak add-in. The 95% confidence intervals for correlations were calculated using InStat® Version 3.0a (GraphPad Software, Inc., San Diego, CA). Residuals of the regressions were calculated in Excel® then plotted using a box plot in Kaleidagraph version 4.02 (Synergy Software, Reading, PA) to identify outliers. Differences between matched samples (e.g., baking potatoes and French fries) were tested using a paired *t*-test in InStat®.

## 3. Results and discussion

We hypothesised that consolidation within agriculture (Jekawonski, 1999; Leff et al., 2004; Monfreda et al., 2008) and livestock processing (Drabenstott, Henry, & Mitchell, 1999; Herath, Weersink, & Carpentier, 2004; MacDonald & Ollinger, 2005) in the USA would result in limited range in region-of-origin – and therefore, limited range in isotopic composition – of food items in the American market. We assumed this would be especially evident for food purchased from outlets of a single national fast food chain, which

often contracts with food suppliers (Jekanowski, 1999) through vertical integration to ensure stability in supply (Barkema, Drabbenstott, & Welch, 1991). Results of the stable isotope analyses of fast food meals and analogous supermarket food items from 32 cities throughout the contiguous USA (Fig. 1) to test this hypothesis are summarised in Table 1. The large range in  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values for all three types of food, regardless of source (restaurant versus supermarket) or city, suggested there is likewise large variation in the regions cattle are raised and wheat or potatoes are grown.

### 3.1. H and O isotopic variation in water samples

The correlation between the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values for the collected tap water samples, averaged by city, is described by the equation

$$\delta^2\text{H} = \delta^{18}\text{O} * 8.1 + 6\text{‰}$$

(Figs. 2 and 3;  $r^2 = 0.986$ ,  $p < 0.0001$ ). Statistically, this was indistinguishable from the Global Meteoric Water Line, defined as

$$\delta^2\text{H} = \delta^{18}\text{O} * 8.0 + 10\text{‰}$$

(Craig, 1961). The  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of the tap water samples spanned a large range, from  $-128\text{‰}$  to  $-7\text{‰}$  for H and from  $-17.0\text{‰}$  to  $-1.0\text{‰}$  for O (Table 1). The highest values were from samples collected in Oklahoma City, OK while the lowest values were from Evanston, WY. This was expected based on maps of USA tap water stable isotope ratios, which predict high tap water  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values in the central portions of TX and OK and low values along the Rocky Mountains in the country's interior (Bowen et al., 2007).

### 3.2. H and O isotopic variation in fast food meals

Hamburger patties had the largest range in  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of the fast food meal items (Table 1). This is in agreement with an earlier survey of meals purchased at a mix of local- and national-chain American fast food restaurants (Chesson, Podlesak, Thompson, Cerling, & Ehleringer, 2008). However, the absolute H and O isotope ranges reported by Chesson et al. (2008) are slightly

larger than those measured in this study. We hypothesise this is due to the inclusion of local fast food restaurants in their data set, which may source more locally-produced beef from regions with very low or high water  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values. Despite sampling from a single national fast food restaurant in this survey, we still observed large variations in  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values among the hamburger patties purchased in different cities. There was a single outlier in the dataset, collected in Lake Charles, LA. With the outlier excluded, the hamburger patties displayed a significant linear correlation between  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values with the equation

$$\delta^2\text{H} = \delta^{18}\text{O} * 6.5 - 213\text{‰}$$

(Fig. 2;  $r^2 = 0.911$ ,  $p < 0.0001$ ). The hamburger patty collected in Laramie, WY had the highest  $\delta^2\text{H}$  value ( $-107\text{‰}$ ) while the hamburger patty from Grand Junction, CO had the highest  $\delta^{18}\text{O}$  value ( $16.2\text{‰}$ ). The sample from Riverside, CA had the lowest  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values ( $-148\text{‰}$  and  $10.2\text{‰}$ , respectively).

Hamburger buns had the smallest range in  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values among the fast food samples. Wheat is grown in a relatively confined region of intensive agriculture in the USA, the central Great Plains (Leff et al., 2004). The limited geographic variation in source translates into a more limited isotopic range in wheat products such as hamburger buns. Despite the smaller overall range in  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values, the buns also displayed a significant linear correlation between H and O isotopes with the equation

$$\delta^2\text{H} = \delta^{18}\text{O} * 5.3 - 237\text{‰}$$

(Fig. 2;  $r^2 = 0.772$ ,  $p < 0.0001$ ). The hamburger bun collected in El Paso, TX had the highest  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values ( $-66\text{‰}$  and  $31.4\text{‰}$ , respectively); the hamburger bun from Riverside, CA had the lowest values ( $-99\text{‰}$  and  $27.0\text{‰}$ , respectively).

Four French fry samples were identified as outliers, collected in Iowa City, IA; Las Cruces, NM; and Austin and Houston, TX. Excluding outliers, the highest  $\delta^2\text{H}$  value in the data set was a French fry collected in Cleveland, OH ( $-127\text{‰}$ ); the highest  $\delta^{18}\text{O}$  value was a sample collected in Lake Charles, LA ( $26.3\text{‰}$ ). The lowest values were fries collected in San Luiz, AZ ( $-152\text{‰}$  for H) and Phoenix, AZ ( $21.7\text{‰}$  for O). Potatoes are grown in an area of intense agriculture even more limited in geographic range than wheat, mainly Idaho (Leff et al., 2004). We again assumed the lack of geographic

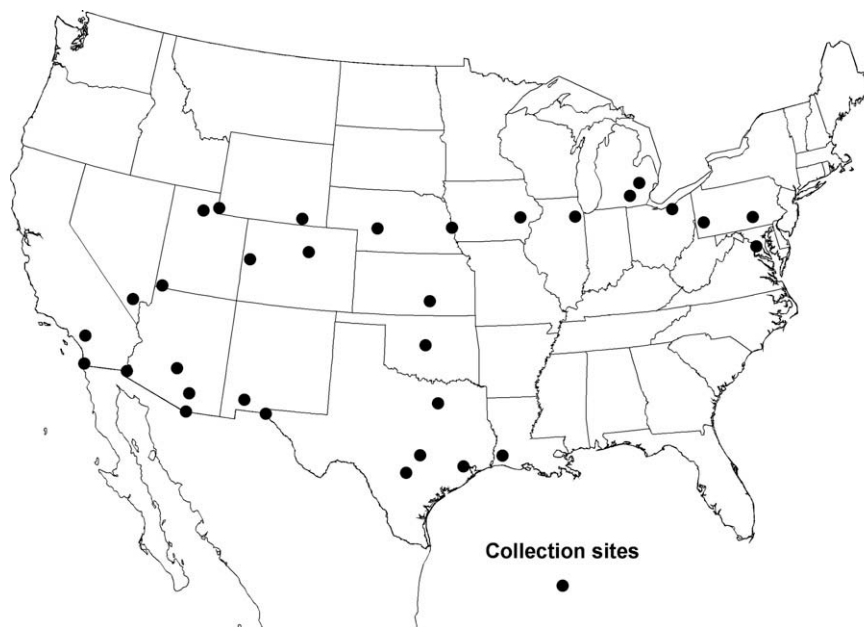
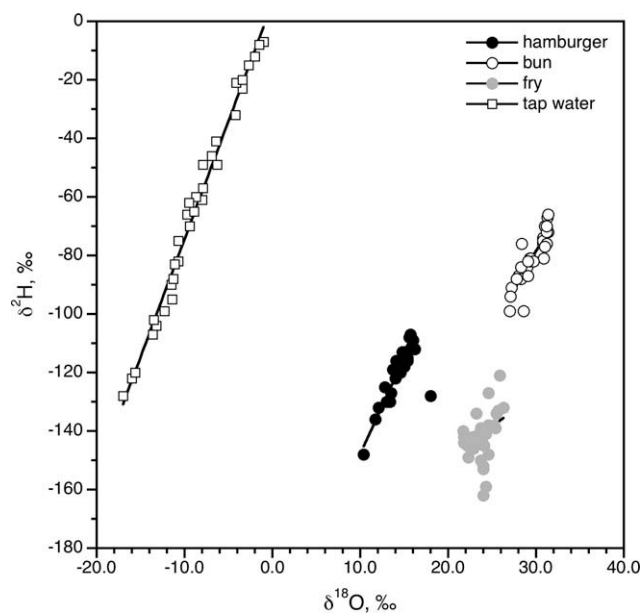


Fig. 1. Locations of the fast food restaurants and supermarkets within the contiguous USA sampled in this survey.

**Table 1**

Stable isotopic composition of the food items and tap water samples collected in this survey. Meat samples and French fries have been defatted.

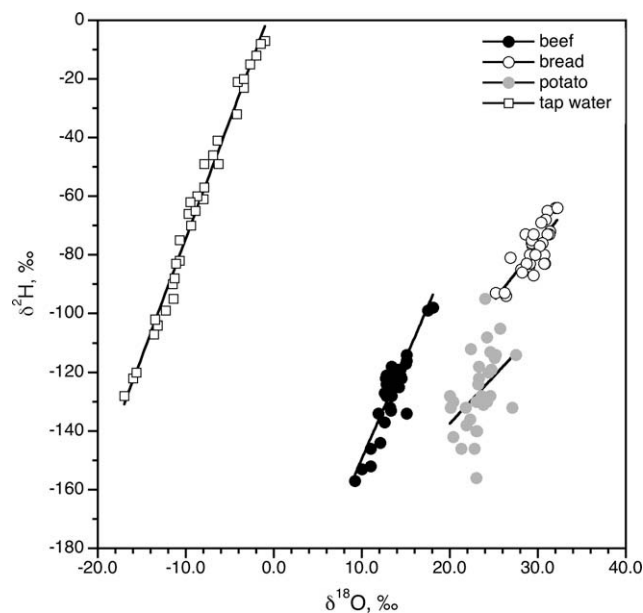
Item	n	$\delta^2\text{H}$ (‰)			$\delta^{18}\text{O}$ (‰)		
		Mean $\pm$ $\sigma$	Min	Max	Mean $\pm$ $\sigma$	Min	Max
Hamburger patty	32	$-120 \pm 10$	-148	-107	$14.4 \pm 1.5$	10.4	18.0
Ground beef	32	$-127 \pm 13$	-157	-98	$13.4 \pm 1.8$	9.2	18.1
Hamburger bun	32	$-80 \pm 9$	-99	-66	$29.7 \pm 1.4$	27.0	31.4
Loaf bread	32	$-79 \pm 8$	-94	-64	$29.5 \pm 1.7$	25.2	32.2
French fry	32	$-142 \pm 8$	-162	-121	$23.8 \pm 1.2$	21.7	26.3
Baking potato	31	$-127 \pm 13$	-156	-95	$23.4 \pm 1.8$	20.0	27.5
Tap water	32	$-64 \pm 35$	-128	-7	$-8.7 \pm 4.4$	-17.0	-1.0

**Fig. 2.** Mean  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values for the three food items purchased from fast food restaurants in this survey. Hamburger and fry samples have been defatted. Average tap water values for each city are shown for reference. Solid lines represent the ordinary least squares regression for each group; see text for equations.

source variation would result in a small isotopic variation but observed a relatively large range in defatted fry  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values. There was a significant linear correlation between hydrogen and oxygen described by the equation

$$\delta^2\text{H} = \delta^{18}\text{O} * 2.2 - 94\text{‰}$$

(Fig. 2;  $r^2 = 0.240$ ,  $p < 0.01$ ). The large range in  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values for French fries may be explained by two or more processes. First, potatoes are a water-stress-sensitive crop and must be irrigated regularly during growth (Shock, Flock, Eldredge, Pereira, & Jensen, 2006). The temporal and geological source of water can impact its isotopic composition, as seen in high-elevation water, seasonal precipitation, and pre-Holocene groundwater (Bowen et al., 2007). If one potato field in Idaho is irrigated with Snake River water while a nearby field is irrigated with local groundwater, the differences in the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of river water and groundwater will impact the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of the resulting potato tubers, despite the fact the plants were grown in the same geographic area. Second, potatoes are essentially storage vessels and the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of water stored in the tuber as the growing season progresses could be different from water used during daily irrigation. The impact of water on the isotopic composition of plant tissue is dependent upon the relationship between the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of source water (i.e. stored water) and the final product (i.e. starch). While the relationship between

**Fig. 3.** Mean  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values for the three food items purchased from supermarkets in this survey. Beef samples have been defatted. Average tap water values for each city are shown for reference. Solid lines represent the ordinary least squares regression for each group; see text for equations.

source water and cellulose is well documented (Roden, Lin, & Ehleringer, 2000), the relationship between water and storage tissues may be different, affecting the final potato  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values.

All fast food items clustered in non-overlapping groups on a dual isotope plot, despite the outliers within the data set (Fig. 2). This corroborated the non-overlapping groups in samples collected from a variety of American fast food chains by Chesson et al. (2008). If we treat each fast food item (hamburger patties, hamburger buns, and French fries) as a dietary input in a mixing model (Phillips & Gregg, 2001), an American who ate equal proportions of each would consume an average isotope value of  $-114\text{‰}$  for H and  $22.6\text{‰}$  for O in the meal.

### 3.3. H and O isotopic variation in supermarket food items

Ground beef samples had the greatest range in  $\delta^{18}\text{O}$  values (Table 1). The ground beef sample collected in St. George, UT was identified as an outlier in the dataset. With that sample excluded, there was a significant linear correlation between  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of ground beef described by the equation

$$\delta^2\text{H} = \delta^{18}\text{O} * 7.0 - 219\text{‰}$$

(Fig. 3;  $r^2 = 0.796$ ,  $p < 0.0001$ ). Ground beef collected in San Antonio, TX had the highest  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values ( $-98\text{‰}$  and  $18.1\text{‰}$ , respectively) while the sample from Evanston, WY had



the lowest ( $-157\text{‰}$  and  $9.2\text{‰}$ , respectively). The high and low values from TX and WY, respectively, correlate well with high and low tap water  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values sampled from those regions (see Section 3.1, above). This suggests a link between purchase location and supermarket food region-of-origin when using tap water isotopic composition as a proxy for geography. A similar trend was also seen in beef lipid collected around the world, with low lipid  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values from countries in cooler regions with low water  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values, and higher values from countries in warmer regions (Heaton et al., 2008).

The loaf bread collected in Tucson, AZ had the highest  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values ( $-64\text{‰}$  and  $32.2\text{‰}$ , respectively). The lowest bread  $\delta^2\text{H}$  value was collected in Cleveland, OH ( $-94\text{‰}$ ); the lowest  $\delta^{18}\text{O}$  value was collected in Iowa City, IA ( $25.2\text{‰}$ ). Similar to hamburger buns, the loaf bread samples had the smallest range in  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of the supermarket food items, most likely again due to the limited range of wheat agriculture in the USA (Leff et al., 2004). Despite having the smallest isotope range, loaf bread  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values were significantly correlated (Fig. 3;  $r^2 = 0.643$ ,  $p < 0.0001$ ; equation:  $\delta^2\text{H} = \delta^{18}\text{O} * 3.8 - 191\text{‰}$ ).

Baking potatoes had the greatest range in  $\delta^2\text{H}$  values. Two baking potatoes were identified as outliers (Iowa City, IA and Fenton, MI); without those samples, there was a significant correlation between  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of baking potatoes with the equation

$$\delta^2\text{H} = \delta^{18}\text{O} * 3.2 - 202\text{‰}$$

(Fig. 3;  $r^2 = 0.293$ ,  $p < 0.01$ ). As with defatted French fries, there was more variation in baking potato stable isotope compositions than expected based on limited growing region (Leff et al., 2004). Again, this is mostly likely due to source differences in the water used for irrigation and the impact of water stored in the potato tubers on final tissue  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values. Excluding the outliers, the potato from San Antonio, TX had the highest  $\delta^2\text{H}$  value ( $-105\text{‰}$ ) while the sample from Salt Lake City, UT had the lowest ( $-146\text{‰}$ ). The potato from Riverside, CA had the highest  $\delta^{18}\text{O}$  value ( $27.5\text{‰}$ ) and the potato from Lake Charles, LA had the lowest ( $20.0\text{‰}$ ).

Despite the outliers within the data sets, the food items clustered in distinct groups in a dual isotope plot (Fig. 3), similar to the individual items from a fast food meal (Fig. 2). Grouping the three food items purchased at supermarkets throughout the USA as a meal, an American who ate equal proportions of ground beef, loaf bread, and potato would consume average values of  $-111\text{‰}$  and  $22.1\text{‰}$  for H and O, respectively.

### 3.4. Comparison of paired food samples

The 95% confidence intervals for the slopes of the linear correlations between  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of the hamburger patties, hamburger buns, and French fries overlapped those of the ground beef samples, loaf bread, and baking potatoes, respectively. The similarities between the slopes of the  $\delta^2\text{H}$  versus  $\delta^{18}\text{O}$  relationships for hamburger patties and ground beef samples, hamburger buns and loaf bread, and French fries and baking potatoes in this survey suggest that the isotopic composition for matched restaurant and supermarket samples were also correlated. However, there was a significant difference in both the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of paired meat samples purchased at fast food restaurants and supermarkets across the 32-city sampling transect ( $t_{31} = 3.184$ ,  $p < 0.01$  and  $t_{31} = 3.092$ ,  $p < 0.01$ , respectively). For both H and O, the isotope ratios of fast food samples were on average higher than their supermarket counterparts. Thus, the choices an American citizen makes at dinner when deciding to cook at home or dine out could have

very real, significant ramifications for the isotopic composition of the meat he or she consumes.

Paired bread samples (hamburger buns versus loaf bread) were not statistically different when considering either  $\delta^2\text{H}$  or  $\delta^{18}\text{O}$  values.

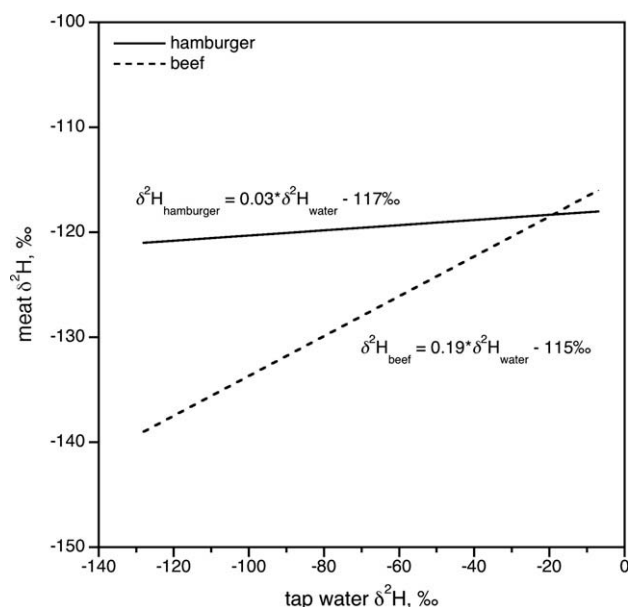
Hydrogen and oxygen are linked during photosynthesis (Roden et al., 2000) and thus should exhibit similar patterns in plant tissues. However, we found a significant difference in paired French fry and baking potato samples for H ( $t_{30} = 6.009$ ,  $p < 0.0001$ ) but no difference for O ( $t_{30} = 0.9378$ ,  $p > 0.05$ ). Baking potato samples had higher  $\delta^2\text{H}$  values than their analogous fry samples. We do not believe the uncoupling of H and O is an artifact of sample processing, as the mean weight percent ratios (%O)/%H) in defatted, cooked French fries and raw baking potatoes were indistinguishable (data not shown). The national restaurant chain visited in this survey publishes an ingredients list for French fries. Unlike the baking potatoes, French fries include two additional plant-derived ingredients: dextrose, a simple sugar, and “beef flavour,” made from a wheat derivative. While the hydrogen and oxygen weight percent ratios for dextrose and wheat are not likely to be significantly different from the potato, the hydrogen and oxygen isotopic composition of the additives could be different, affecting final  $\delta^2\text{H}$  values in particular.

### 3.5. Using stable isotopes to investigate region-of-origin

As stated previously, both precipitation and tap water  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values vary spatially across continents. The patterns observed in water isotopes are affected by latitude, altitude, and climate (“continentality”) at a location (Bowen et al., 2007). Thus, we used our collected tap water data as a proxy for location (i.e., geography) when investigating continental trends.

We found the lowest ground beef  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values in Evanston, WY, where we also collected tap water with the lowest  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values. We observed the highest ground beef  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values in TX, geographically near the city with the highest tap water  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values. These examples suggest a link between purchase location and food region-of-origin, suggesting a food footprint that is more regional than continental for modern Americans (Chesson et al., 2008). However, hamburger patties were not consistent with the above pattern. The highest hamburger  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values were from Laramie, WY, geographically near the lowest ground beef values collected in Evanston. A closer examination of the complete hamburger patty and ground beef datasets detected no statistically significant correlation between the  $\delta^2\text{H}$  value of a delipidated hamburger sample purchased in a city and that city's tap water (Fig. 4 and Table 2). There was, however, a significant correlation between the  $\delta^2\text{H}$  values of supermarket ground beef and tap water (Fig. 4 and Table 2;  $r^2 = 0.25$ ,  $p < 0.01$ , equation:  $\delta^2\text{H}_{\text{beef}} = \delta^2\text{H}_{\text{water}} * 0.19 - 115\text{‰}$ ). There was also no significant correlation between the  $\delta^{18}\text{O}$  values of hamburger patties and local tap water (Table 2) but a significant correlation between the  $\delta^{18}\text{O}$  values of ground beef and tap water (Table 2;  $r^2 = 0.17$ ,  $p < 0.05$ , equation:  $\delta^{18}\text{O}_{\text{beef}} = \delta^{18}\text{O}_{\text{water}} * 0.17 + 14.8\text{‰}$ ). Though the proportion of variation in supermarket beef  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values explained by tap water isotope ratios was modest ( $\sim 20\%$  for each), grocery retailers appear to source more locally-produced meat than their restaurant counterparts.

Studies have shown the isotopic composition of cattle drinking water is highly correlated with final tissue  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values, as demonstrated in a north-south transect through Japan (Nakashita et al., 2008). The relationship between tissue and drinking water  $\delta^{18}\text{O}$  values observed by Nakashita et al. (2008; equation:  $\delta^{18}\text{O}_{\text{beef}} = \delta^{18}\text{O}_{\text{water}} * 0.59 + 15.56\text{‰}$ ) had a greater slope than the relationship we observed for supermarket beef samples in the USA. However, we were constrained to use the tap water in a



**Fig. 4.** Plot of the ordinary least squares regressions between the mean  $\delta^2\text{H}$  values of delipidated meat samples and the local tap water, paired by purchase location. See Table 2 for goodness-of-fit measures.

**Table 2**

The slope, intercept, and goodness-of-fit measures for the ordinary least squares regressions between different food items and tap water. The correlations between the  $\delta^2\text{H}$  values of food and water are given on the left; correlations between  $\delta^{18}\text{O}$  values are given on the right.

Item	Versus tap water $\delta^2\text{H}$			Versus tap water $\delta^{18}\text{O}$		
	Slope	Intercept (‰)	$r^2$	Slope	Intercept (‰)	$r^2$
Hamburger patty	0.03	-117	0.01	0.03	14.6	0.01
Ground beef	0.19	-115	0.25**	0.17	14.8	0.17*
Hamburger bun	0.08	-75	0.10	0.09	30.5	0.08
Loaf bread	0.05	-75	0.05	-0.01	29.4	0.00
French fry	0.03	-139	0.05	0.04	24.1	0.02
Baking potato	0.03	-125	0.01	-0.05	23.0	0.01

\*  $p < 0.05$ .

\*\*  $p < 0.01$ .

purchase city as a proxy for actual drinking water, which may have contributed to our somewhat weaker correlation coefficient. Despite this constraint, we observed a geographic trend in our ground beef dataset, which may be evidence of regionality in the source of beef available to supermarket patrons.

There appeared to be limited regionality in the source of bread and potatoes in the American food market. There were no significant correlations between the stable isotope ratios of hamburger buns or loaf bread and local tap water (Table 2). We present two potential explanations for this observation. First, wheat is grown in a limited few regions of the USA (Leff et al., 2004), which results in a more limited isotopic range in plant  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values. Second, the structure of the American food supply system for carbohydrates may divorce the region-of-origin of the raw material (e.g., wheat) from the purchase location of the final product (e.g., a hamburger bun). This is extremely likely in the vertically-integrated food supply chains used by fast food restaurants (Jekanowski, 1999).

There were also no significant correlations between the stable isotope ratios of French fries or baking potatoes and local tap water (Table 2). Again, we present two possible explanations for this phenomenon. First, two (or more) isotopically distinct water sources

could be used for irrigation during the potato plant growth period. If the switch between water sources occurred randomly or was not documented, the potato tuber  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values are no longer predictably linked to region-of-origin. Second, plant-derived additives to products like French fries may be sourced from regions far distant from the origin of the potato. The additional isotopic signal imparted by the additives may mask any link to potato region-of-origin.

#### 4. Conclusions and implications

Overall, the differences in isotopic composition of food items collected in this survey were not significant when grouping items by meal. The mean  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values for an American fast food meal ( $-114\text{‰}$  and  $22.6\text{‰}$ , respectively; see Section 3.2) were not statistically different from an analogous supermarket meal ( $-111\text{‰}$  and  $22.1\text{‰}$ , respectively; see Section 3.3). The fast food and supermarket meal values calculated in this survey were very similar to mean meal values previously reported for fast food purchased throughout the USA (Chesson et al., 2008) and supermarket food items purchased in AK and NY (O'Brien and Wooller, 2007). Our mean meal  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values were also reasonably consistent with the average dietary inputs ( $-115\text{‰}$  and  $26.0\text{‰}$ , respectively) previously used in a process-based model describing the incorporation of hydrogen and oxygen into hair collected from modern Americans (Ehleringer et al., 2008).

In the model of Ehleringer et al. (2008) there are multiple sources of H and O in hair, including diet. Amino acids from protein in the diet can be routed straight to the hair follicle and thus will directly impact both  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values in hair keratin. Conversely, carbohydrates in the diet impact the  $\delta^{18}\text{O}$  values in hair only indirectly, by affecting the oxygen isotopic composition of body water [e.g., through metabolic water (Ehleringer et al., 2008; Podlesak et al., 2008)]. The original model uses constant  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values for all food items in the modern American diet; it does not allow for isotopic differences among types of foods (i.e., protein, carbohydrate, and lipid), due to a consumer's geographic location. Recent studies examining the hydrogen and oxygen stable isotopes ratios of human hair from indigenous (Bowen et al., 2009) and modern Asian (Thompson et al., in press) populations have modified the original Ehleringer et al. (2008) model to include a "local food" parameter. This parameter acknowledges the impact of the consumer's geographic location on the isotopic composition of diet and allows that some proportion of diet can originate from the same isotopic region as the consumer.

However, the additional local food parameter still does not consider the possibility of differences among food types due to geography, instead using a single relationship for all food items regardless of the actual consumer's dietary macronutrient intake. Based on our work, we believe additional dietary parameters may be needed. Using one parameter to estimate the impact of local food on final tissue  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values is not applicable for every food type. For example, we found no geographic trend in the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of carbohydrates we collected (hamburger bun, loaf bread, French fry or baking potato). Americans consume fast food and supermarket carbohydrates with similar isotopic compositions, regardless of location. A single parameter is likely to be adequate when modelling the impact of dietary carbohydrates on the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of tissue. On the other hand, we observed a trend between purchase location and isotopic composition for ground beef purchased from supermarkets. Thus, the impact of geographic location on meat  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values must be considered in models predicting tissue isotopic composition from that of diet through the inclusion of additional protein-specific parameters.

As demonstrated in this survey and recent anthropological investigations (Bowen et al., 2009; Thompson et al., in press), the link between geography and the isotopic composition of diet is extremely relevant when investigating modern human movements and when identifying the region-of-origin of subjects in anthropological, archaeological, or forensic studies. Continued surveys of the geographic trends in carbohydrates and proteins in the modern American diet, especially over seasonal timescales, will help in refining the model parameters used in such studies. It will also allow future modelling efforts to incorporate the impact of geographic location on the isotopic composition of some food items but not others.

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