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Consistent predictable patterns in the hydrogen and oxygen stable isotope ratios of animal proteins consumed by modern humans in the USA

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Published datasets of proteinaceous animal tissues suggest that co-variation between amino acid hydrogen (δ^2H) and oxygen (δ^{18}O) isotope ratios is a common feature in systems where isotopic variation is driven by geographic or temporal variation in the δ^2H and δ^{18}O values of environmental water. This has led to the development of models relating tissue δ^2H and δ^{18}O values to those of water, with potential application in a number of fields. However, the strength and ubiquity of the influence of environmental water on protein isotope ratios across taxonomic groups, and thus the relevance of predictive models, is an open question. Here we report strong co-variation of δ^2H and δ^{18}O values across a suite of terrestrial and aquatic animal meats purchased in American food markets, including beef, poultry (chicken and turkey), chicken eggs, pork, lamb, freshwater fish, and marine fish. Significant isotope co-variation was not found for small collections of marine bivalves and crustaceans. These results imply that isotopic signals from environmental water were propagated similarly through most of the diverse natural and human-managed foodwebs represented by our samples. Freshwater fish had the largest variation in δ^2H and δ^{18}O values, with ranges of 121 ‰ and 19.2 ‰, respectively, reflecting the large isotopic variation in environmental freshwaters. In contrast marine animals had the smallest variation for both δ^2H (7 ‰ range, crustaceans) and δ^{18}O (3.0 ‰ range, bivalves) values. Known-origin beef samples demonstrated direct relationships between the variance of environmental water isotope ratios and that of collected meats. **Copyright © 2011 John Wiley & Sons, Ltd.**

It is well documented that the hydrogen (δ^2H) and oxygen (δ^{18}O) stable isotope ratios of animal tissues record the isotopic composition of the animal's drinking water. This has been previously demonstrated for several diverse taxa including Aves (quail nails and feathers[1] and house sparrow feathers[2]); beef cattle (muscle tissue,[3] lipids,[4] and meat water[5]); lamb (protein extracted from muscle[6]); and freshwater fish (trout and salmon muscle tissue[7,8]). Some studies demonstrated the link between drinking water and tissue directly by supplying water of known isotopic composition in a controlled laboratory setting and monitoring the δ^2H and/or δ^{18}O values of resultant materials (e.g.,[1,2,7]). Other studies published evidence gathered indirectly, namely through the correlation of measured δ^2H and/or δ^{18}O values for animal tissue of different origin to measured or estimated environmental water δ^2H and δ^{18}O values (e.g.,[3,4,8]). Almost all investigations into the impact of animal drinking water δ^2H and δ^{18}O values on tissue isotopic compositions have presented the relationship using linear regression models to approximate the underlying mechanisms of H- and/or O-atom incorporation.

In most cases where both δ^2H and δ^{18}O values have been measured these isotopic values have exhibited strong co-variant relationships among samples.[2,9–11] Given that isotopic fractionation within the water cycle leads to strong co-variation in water δ^2H and δ^{18}O values over space and time, the co-variation observed in animal tissue δ^2H and δ^{18}O values could be viewed as evidence of the preservation of site- and/or time-specific water isotope signatures in proteins through the fixation of H and O atoms from environmental water. Geographic variation in water isotope ratios largely reflects predictable patterns of rainout from airmasses as they move across the continents,[12,13] which create predictable patterns in environmental water isotope ratios – such as precipitation or tap water δ^2H and δ^{18}O values – that can be displayed graphically in an isotope landscape, or *isoscape*.[14,15] Using established models for isotopic variations in water and applying defined linear relationships between water and tissue δ^2H and δ^{18}O values, it may be possible to predict the original source from the measured isotopic composition of biological materials of unknown origin, as previously demonstrated for animals (including humans),[16,17] plants,[18] microbes,[19] and a variety of food items.[20]

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A semi-mechanistic, process-based model to describe the incorporation of water isotopes into human hair keratin proteins was developed by Ehleringer *et al*.[21] and then subsequently modified and tested using additional data from historic and modern human populations by Bowen *et al*.[22] and Thompson *et al*.,[23] respectively. A key feature of the Ehleringer *et al*.[21] model is the understanding that the δ^2H and δ^{18}O values of keratin reflect the isotopic composition of different pools of dietary and body water H and O. The model predicts that keratin δ^{18}O values will primarily reflect the isotopic composition of water at the sites of peptide digestion in the gut. This is because proteins are cleaved into their constituent amino acids during digestion, functionally exposing nearly every O atom for exchange with body water in the low pH environment of the gut. Once amino acids are absorbed across the gut wall, the neutral pH of blood limits further O-atom exchange until the amino acids arrive at the hair follicles for incorporation into newly synthesized hair. On the other hand, it is suggested that keratin H atoms include a mixture of H atoms from water in the hair follicle and H routed from the diet. Hydrogen atoms bound to carbon within dietary amino acids will not exchange with water, unless the amino acid is synthesized *de novo* prior to its incorporation into hair keratin. Thus, the δ^2H value of the carbon-bound H atoms in synthesized amino acids will reflect the isotopic composition of body water while the δ^2H value of carbon-bound H atoms in essential amino acids will reflect the isotopic composition of diet.

This human hair keratin model offers a theoretical basis for understanding the incorporation of water isotopes into other types of animal proteinaceous tissue because animals digest food and transport amino acids for protein synthesis in a similar manner. While exact model parameters will probably vary among tissues and taxa, the key feature is the same: amino acids incorporated into proteinaceous tissues will reflect the isotopic composition of (1) dietary amino acids and precursors to amino acid synthesis, and (2) drinking water due to isotopic exchange between body water and amino acids during protein cleavage and synthesis. The process-based model approach first described for human hair keratin has also been used (albeit in modified form) to study the impact of drinking water δ^2H and δ^{18}O values on woodrat[24] and house sparrow[2] tissues.

Based on previous studies documenting a link between drinking water and tissue δ^2H and δ^{18}O values,[1–8] we hypothesized that for taxa in which environmental water exerts a strong influence on the δ^2H and δ^{18}O values of proteins, the protein δ^2H and δ^{18}O values should also strongly co-vary for tissues collected from animals living at different locations and drinking water with different isotope ratios. Ubiquitous co-variation between protein δ^2H and δ^{18}O values has previously been predicted based on the process-based model described above, and various studies have suggested that the exact slope of the δ^2H/δ^{18}O relationship should vary for different populations as a function of factors such as the amount of local versus non-local food consumed and various physiological factors.[2,22] We also hypothesized that the range of isotopic values for proteinaceous tissues should vary depending on the range of water isotope values in the environments in which the animals lived and the typical practice by which animals were raised (i.e., local vs. imported food sources). For example, continental animals such as beef cattle and freshwater fish are exposed to large gradients in water isotopes[14,15] and the range in these protein δ^2H and δ^{18}O values should likewise be large. On the other hand, the δ^2H and δ^{18}O values of protein from marine animals, which are exposed to a relatively more homogeneous range of ocean water isotopes,[25] should span a smaller range.

Here we use a survey of proteins commonly available in modern American food markets to document the variation and co-variation of δ^2H and δ^{18}O values in a variety of proteinaceous animal tissues. We examined proteins ('meats') from animals with a variety of distinct dietary, habitat, and physiological characteristics, including terrestrial vertebrate foregut (beef cows and lambs) and hindgut (swine) fermenters; birds (chickens and turkeys, as well as chicken eggs); both freshwater and marine fish; and marine invertebrates (bivalves and crustaceans). Confirmation of our predictions for a diverse range of meats would imply that the propagation of environmental water isotope signals to consumer tissues is generally similar for all animals and a ubiquitous feature in systems where isotopic variation in tissues is driven by variation in drinking water δ^2H and δ^{18}O values. If the expected patterns cannot be confirmed, this would imply that other factors (e.g., physiology) overwhelm the environmental H and O isotopic signal of the water source.

EXPERIMENTAL

Sample collection

A total of 436 meat samples were collected opportunistically and through planned collections[9,11] during the years 2005–2010 from food markets and restaurants in 38 states of the USA, plus Puerto Rico (Table 1). We note that 4 marine fish samples and 2 marine crustacean samples were collected in the Canary Islands and were not purchased from US food markets. The collected samples were a mix of fresh (raw) and processed (cooked) meats and represented a wide range of animal taxa that were categorized into the following groups for subsequent analysis: beef, poultry, chicken eggs, pork, lamb, freshwater fish, marine fish, marine bivalves, and marine crustaceans. The isotopic difference between raw and cooked meats was tested prior to wholesale collection and analysis (see below). The data for some of beef samples included in this survey have been presented previously.[9,11]

With a few exceptions (e.g., the freshwater fish samples, and a subset of the beef samples from small, grass-fed herds) the exact growth location of most sampled animals was unknown (Tables 1 and 2), as is commonly the case in modern American food markets. Because of this lack of origin information for the majority of collected meats we do not know if, for example, the sampled pigs were raised on farms that covered a similar geographic and drinking water isotopic range to that of the sampled beef cows. This uncertainty regarding sample origin must be considered in comparing the range of δ^2H and δ^{18}O values observed for each meat category. Moreover, in the absence of information on the

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Table 1. Total number of meats collected, by state in the USA. Samples of known origin are presented in parentheses and were included in the total number calculated for a state. For example, 4 samples of beef were collected in Florida, of which 2 were known to be from Florida

	Terrestrial animals						Marine animals		
State	Beef	Poultry	Eggs	Pork	Lamb	Fish[a]	Fish[b,*]	Bivalves[c]	Crustaceans[d,**]
Alabama	2	1							
Alaska							2(2)		
Arizona	17	6	7						
California	13	4	4						
Colorado	8	4	2						
Florida	4(2)	2(1)				13(13)			
Hawaii	1								
Idaho	14(8)								
Illinois	2	2	1						
Indiana	3(2)					6(6)			
Iowa	2	1	2						
Kansas	6	2	1						
Louisiana	4	2	2						
Massachusetts	3	1							
Michigan	6	2	3						
Minnesota	1	1							
Mississippi	3	1							
Missouri					1(1)				
Montana	1								
Nebraska	5	2	2						
Nevada	6	1	2						
New Hampshire	5								
New Mexico	7	2							
New York		1							
North Carolina	1						2(2)		
Ohio	2	1	2						
Oklahoma	2	1	1						
Oregon	7(5)								
Pennsylvania	4	2	3						
Puerto Rico	2								
Rhode Island	1								
South Carolina	12(1)		1(1)				6(6)		3(3)
Tennessee	2	3							
Texas	27(1)	5	7	1(1)	1(1)				
Utah	63(3)	13	8(2)	9	2(1)	3(3)	10	5	
Vermont	1								
Virginia	3	1	2						
Washington	2	2							
Wyoming	6	1	3						

[a]Included black crappie, bluegill, catfish, gar, largemouth bass, trout, & unknown species.
[b]Included cod, flounder, haddock, halibut, pigfish, red drum, rockfish, salmon, sheepshead, squirrel fish, surf perch, triggerfish, & unknown species.
[c]Included mussel & oyster.
[d]Included crab & shrimp.
*4 marine fish samples were collected in the Canary Islands, Spain.
**2 marine crustacean samples were collected in the Canary Islands, Spain.

individuals sampled, the data associated with each meat category cannot necessarily be used for an in-depth investigation into specific physiological factors that affect protein δ^2H and δ^{18}O values. Our analysis, however, focused primarily on the panoptic relationships between meat δ^2H and δ^{18}O values from different animal taxa, and as such is not dependent upon detailed information on individual samples.

Sample preparation

Collected meats were stored on wet ice or frozen until arrival in the laboratory, then stored frozen until processing. Subsamples (~5 g) of the thawed meats were freeze-dried, then coarsely ground. Coarsely ground meat samples were loaded into individual cellulose thimbles and delipified for 48 h on a Soxhlet apparatus using a 2:1 mixture of chloroform and

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Table 2. Measured non-exchangeable δ^2H and total δ^{18}O values of beef samples collected from known-origin, grass-fed herds within the continental USA. Also shown are predicted precipitation δ^2H and δ^{18}O values for the source locations

City	State	Latitude	Longitude	Elevation (m)	Measured meat (‰) δ^2H	Measured meat (‰) δ^{18}O	Estimated* precipitation (‰) δ^2H	Estimated* precipitation (‰) δ^{18}O
Soda Springs	ID	42.65	−111.61	1754	−174	9.7	−111	−14.9
Emery	UT	38.92	−111.25	1900	−176	7.8	−100	−13.6
Price	UT	39.60	−110.81	1697	−170	11.3	−100	−13.6
Ashland	OR	42.20	−122.71	624	−148	13.3	−95	−12.4
Central Point	OR	42.38	−122.92	396	−152	12.0	−92	−11.9
Riddle	OR	42.95	−123.36	243	−133	13.8	−89	−11.5
Delphi	IN	40.59	−86.67	170	−118	14.5	−50	−7.5
Lebanon	IN	40.05	−86.47	280	−133	12.6	−49	−7.5
Monticello	MO	40.12	−91.71	168	−133	12.9	−50	−7.4
Vance	SC	33.43	−80.42	37	−105	18.9	−32	−5.1
Grandview	TX	32.27	−97.18	211	−96	18.9	−35	−5.0
Citra	FL	29.41	−82.11	22	−103	18.6	−25	−4.0

*Precipitation estimates from the Online Isotopes in Precipitation Calculator (OIPC[33]).

methanol. The delipified meats were air-dried, then ground a second time to a fine powder,[9,11] and kept at room temperature in 1-dram glass vials open to the atmosphere in the laboratory for at least 5 days prior to weighing.

After exposure to the ambient water vapor, the meats (defatted dry matter) were weighed (0.150 mg ± 10%) in duplicate for hydrogen and oxygen isotope analysis and loaded into pre-baked Ag capsules. Two calibrated[26] keratin reference materials [ground horsehair from Florida (δ^2H = −76 ‰, δ^{18}O = +14.9 ‰) and Utah (δ^2H = −142 ‰, δ^{18}O = +5.7 ‰)] that had been exposed to the ambient laboratory atmosphere alongside the meat samples were also loaded. Loaded capsules were kept under vacuum for a minimum of 5 days prior to analysis.

Stable isotope analysis

The meat samples and reference materials were analyzed for hydrogen and oxygen isotope ratios via isotope ratio mass spectrometry (IRMS) on either a ThermoFinnigan Delta+ XL or a ThermoFinnigan MAT 253 (ThermoFinnigan, Bremen, Germany). Both mass spectrometers were operated in continuous-flow mode with a high-temperature conversion elemental analyzer (ThermoFinnigan) and zero-blank autosampler (Costech Analytical, Valencia, CA, USA) attached. Loaded capsules were pyrolyzed at 1400°C in the presence of glassy carbon to reduce H atoms and to convert O atoms into gaseous H_2 and CO, respectively. The gases were separated on either a 0.6- or 1.0-m 0.25" (o.d.) molecular sieve 5Å gas chromatography column (Costech Analytical) held at 95°C.

The stable isotope abundances are expressed in 'delta' (δ) notation in parts per thousand (‰), calculated as:

$$\delta = \left[\left(R_{\text{sample}}/R_{\text{standard}}\right) - 1\right] * 1000$$

where R_{sample} and $R_{standard}$ are the ratios of rare (^{2}H or ^{18}O) to abundant (^{1}H or ^{16}O) isotopes in a sample and an international standard, respectively. The international standard for both H and O is Vienna Standard Mean Ocean Water.

A fraction of the H atoms in meats is labile and can exchange with H atoms in ambient water vapor.[27,28] To control for this, the total measured δ^2H values of the two horsehair reference materials that had been exposed to the atmosphere were compared with the previously determined δ^2H values of their non-exchangeable H atoms and the difference used to calculate the non-exchangeable H isotopic composition of the analyzed meats exposed to the same ambient conditions.[26,29] Thus, the δ^2H values presented throughout the text are for the non-exchangeable H atoms only.

Most analyses were completed at the Stable Isotope Ratio Facility for Environmental Research (SIRFER) on the University of Utah campus in Salt Lake City, UT, USA over the course of 6 years (2005–2010). Some samples were analyzed at IsoForensics Inc. in Salt Lake City in 2010. The analytical precision, calculated as 1σ of the measured hydrogen and oxygen isotope ratios of a commercially available powdered keratin included in all analyses (n = 187), was 1.7 ‰ for H and 0.29 ‰ for O. The reported hydrogen and oxygen isotope ratios are the averages of duplicate sample capsules.

Experimental study: raw vs. cooked meats

Some beef, poultry, pork, and lamb samples as well as all chicken egg samples were cooked at the time of, or immediately following, collection. Most freshwater fish and marine meat samples were collected and processed raw. Prior to analyzing the collected samples, the effect of cooking on the measured hydrogen and oxygen isotope ratios was examined using samples of raw beef (steak) and raw chicken (breast). Sub-samples of the beef and chicken were cooked in separate pans on the stovetop without oil until cooked through. Raw and cooked sub-samples (n = 4 for each meat and type) were dried and delipified, then analyzed for H and O isotopic composition. There was no difference in the measured δ^2H (raw beef: –143 ‰, cooked beef: –144 ‰, unpaired Student's t-test t = 0.65, P = 0.65; and raw chicken: –118 ‰, cooked chicken: –118 ‰, t = 0.17, P = 0.87) or the δ^{18}O (raw beef: 13.0 ‰, cooked beef: 12.9 ‰, unpaired Student's

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t-test t = 0.38, P = 0.71; and raw chicken: 14.4 ‰, cooked chicken: 14.2 ‰, t = 0.71, P = 0.50) values of the raw and cooked meats. Thus, no distinction was made between raw or cooked collected meats during processing, isotope ratio measurements, or statistical analyses.

Statistical analysis

Correlations between the measured δ^2H and δ^{18}O values of the meat categories with >5 samples were analyzed using Pearson correlation (r) with significance level set to α = 0.05; correlation coefficients were calculated in Prism 5 for Mac OS X (GraphPad Software Inc., La Jolla, CA, USA). Relationships between measured meat δ^2H and δ^{18}O values were analyzed by reduced major axis (RMA) linear regression analysis using R[30] with the 'lmodel2' (Model II Regression) package, version 1.6-3 (authored by Pierre Legendre, 2008).

Traditionally, relationships between the measured δ^2H and δ^{18}O values of biological materials have been analyzed using ordinary least-squares (OLS) linear regression analysis, which assumes an asymmetric relationship between the two variables. These OLS regression models typically assign oxygen isotope ratios as x (as for the Global Meteoric Water Line[12]). In contrast, RMA analysis is symmetric; that is, a single line can describe the relationship between the δ^2H and δ^{18}O variables, regardless of which is assigned as x. When there is no *a priori* reason to assume a particular dependence of one variable on the other, RMA regression is the recommended method for analyzing a bivariate relationship.[31] The slopes of the calculated RMA lines for each meat category were compared in a pairwise fashion using a test statistic (T_{12}) described by Clarke[32] with a significance level set to α = 0.01. These calculations were performed using Excel® 2008 for Mac (Microsoft Corporation).

Drinking water δ^2H and δ^{18}O values for known-origin beef samples were predicted using the Online Isotopes in Precipitation Calculator (OIPC[33]) and coordinates for the town/city nearest the farm. Coordinates were estimated using the Global Gazetter version 2.2.[34] Although deviations between the isotopic composition of water resources used by in residential and agricultural systems are not uncommon,[14] the precipitation estimates provide a first-order representation of the large-scale patterns in water resource isotope ratios that is a reasonable approximation here given the large range of values encompassed by our sampling sites. Relationships between measured known-origin beef tissue δ^2H and δ^{18}O values and predicted drinking water δ^2H and δ^{18}O values, respectively, were analyzed using OLS regressions. The relationship between known-origin beef tissue δ^2H and δ^{18}O values was also analyzed using OLS regression to allow for direct comparison with previously published datasets.

RESULTS

The measured δ^2H and δ^{18}O values of the collected meats spanned wide isotope ranges from −188 ‰ (a freshwater fish) to −65 ‰ (a marine fish) for δ^2H and from 5.3 ‰ (a freshwater fish) to 24.5 ‰ (a freshwater fish) for δ^{18}O (Table 3). Freshwater fish exhibited the largest variation in δ^2H and δ^{18}O values (range = 121 ‰ and 19.2 ‰, respectively). Marine crustaceans had the smallest variation in δ^2H values (range = 7 ‰), while marine bivalves had the smallest variation in δ^{18}O values (range = 3.0 ‰).

Table 3. Statistics for the measured non-exchangeable δ^2H (top) and total δ^{18}O (bottom) values of collected meats

	Measured non-exchangeable δ^2H, ‰				
Meat	mean ± SD	median	maximum	minimum	n
Beef	−129 ± 19	−124	−96	−181	248
Poultry	−122 ± 8	−120	−103	−154	64
Chicken eggs	−116 ± 11	−113	−96	−140	53
Pork	−131 ± 13	−130	−112	−163	10
Lamb	−119 ± 31	−111	−93	−162	4
Freshwater fish	−107 ± 36	−89	−67	−188	22
Marine fish	−91 ± 13	−91	−65	−123	25
Marine bivalves	−143 ± 6	−144	−136	−151	5
Marine crustaceans	−102 ± 4	−100	−99	−107	5
	Total measured δ^{18}O, ‰				
Meat	mean ± SD	median	maximum	minimum	n
Beef	13.6 ± 2.4	13.8	20.8	6.9	248
Poultry	13.9 ± 1.8	14.1	20.6	10.1	64
Chicken eggs	14.2 ± 1.9	14.5	17.3	10.1	52
Pork	12.3 ± 2.4	12.0	16.6	7.5	9
Lamb	16.7 ± 5.1	18.4	20.8	9.2	4
Freshwater fish	16.8 ± 6.0	19.8	24.5	5.3	22
Marine fish	19.2 ± 1.3	19.4	21.3	16.3	24
Marine bivalves	20.4 ± 1.2	20.8	21.4	18.4	5
Marine crustaceans	20.4 ± 1.4	20.7	21.8	18.4	5

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The measured δ^2H and δ^{18}O values of the beef, poultry, chicken eggs, pork, marine fish, and freshwater fish samples were significantly and strongly positively correlated (Pearson correlation coefficient, r; P <0.001 in all cases; calculated r-values presented below). The RMA regression line slopes and intercepts (±95% confidence intervals) for meat categories with >5 samples are described by the equations (Fig. 1):

$$\delta^2\text{H} = 7.76(\pm 0.44) * \delta^{18}\text{O} - 234(\pm 6)‰ (r = 0.89) \text{for beef,}$$
$$\delta^2\text{H} = 4.57(\pm 0.57) * \delta^{18}\text{O} - 186(\pm 8)‰ (r = 0.87) \text{for poultry,}$$
$$\delta^2\text{H} = 5.84(\pm 0.59) * \delta^{18}\text{O} - 199(\pm 8)‰ (r = 0.93) \text{for chicken eggs,}$$
$$\delta^2\text{H} = 5.56(\pm 1.40) * \delta^{18}\text{O} - 200(\pm 17)‰ (r = 0.96) \text{for pork,}$$
$$\delta^2\text{H} = 10.68(\pm 3.36) * \delta^{18}\text{O} - 296(\pm 65)‰ (r = 0.70) \text{for marine fish, and}$$
$$\delta^2\text{H} = 5.90(\pm 0.67) * \delta^{18}\text{O} - 206(\pm 11)‰ (r = 0.97) \text{for freshwater fish.}$$

We note that because these line equations were calculated using RMA regression, the slopes and intercepts presented above cannot be directly compared with those in previous publications (e.g.,[2,9,11]). However, due to the high r-values, the slopes and intercepts calculated using OLS regression analysis were similar to those calculated by RMA regression analysis (Table 4). The marine fish dataset was the exception; the slope of the OLS regression line was lower (7.49) and the intercept was higher (−235 ‰) than that of the RMA regression line (10.68 and −296 ‰, respectively; Table 4).

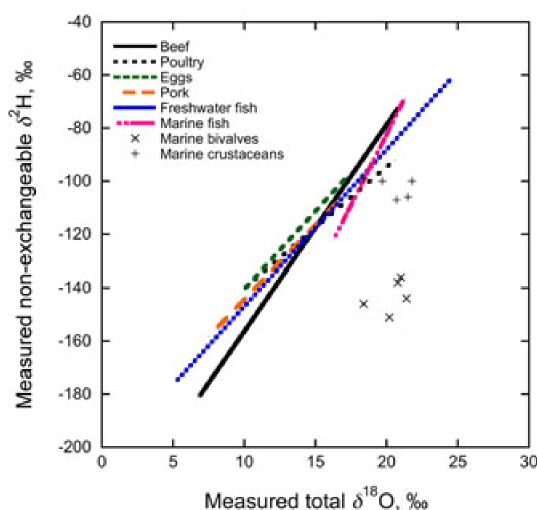

Figure 1. Hydrogen and oxygen isotope co-variation within different meat categories. Reduced major axis regression lines were fitted to the hydrogen and oxygen isotope data for categories with >5 samples. See text for equations describing the lines.

Table 4. Comparison of calculated RMA and OLS regression equations for meat categories with >5 samples

		Slope		Intercept (‰)	
Meat	n	RMA	OLS	RMA	OLS
Beef	248	7.76	6.92	−234	−223
Poultry	64	4.57	3.99	−186	−178
Chicken eggs	52	5.84	5.45	−199	−193
Pork	9	5.56	5.33	−200	−197
Marine fish	22	10.68	7.49	−296	−235
Freshwater fish	24	5.90	5.73	−206	−203

The slopes of the beef and poultry RMA lines were significantly different, as tested using the method of Clarke[32] (T_{12} = 3.413, degrees of freedom =233, P <0.001). At α = 0.01, no other pairwise RMA slope comparison was statistically significant. The values for almost all the samples clustered together in a relatively limited area of δ^2H/δ^{18}O 'space', as illustrated by the overlapping regression lines (Fig. 1). The clear exceptions to this pattern are the marine bivalve samples, which cluster distinctly from the other samples and consistently have lower δ^2H values, relative to their δ^{18}O values, than samples from any of the other meat groups.

A subset of the beef samples was collected from known-origin herds (Table 2), allowing us to directly investigate the relationship between local environmental water and proteinaceous tissue isotopic compositions. The measured δ^2H and δ^{18}O values of the defatted meat from these known-origin herds were significantly and strongly positively correlated (Fig. 2; r = 0.95, P <0.0001). The OLS regression line between the beef tissue δ^2H and δ^{18}O values is described by the equation δ^2H = 7.45(±0.79)*δ^{18}O − 239(±11) ‰; values in parentheses are 95% confidence intervals. The slope of the known-origin beef OLS line was not significantly different from the slope of the OLS line (Table 4) fitted to all the beef samples collected in this survey.

We also observed significant and strong positive correlations between measured beef meat δ^2H and δ^{18}O values and estimated drinking water δ^2H and δ^{18}O values (r = 0.93, P <0.0001 and r = 0.87, P <0.001, respectively). The relationships were described by the OLS regression line equations (Fig. 3):

$$\delta^2\text{H}_{\text{beef}} = 0.83(\pm 0.11) * \delta^2\text{H}_{\text{water}} - 80(\pm 8)‰ \text{ and}$$
$$\delta^{18}\text{O}_{\text{beef}} = 0.81(\pm 0.14) * \delta^{18}\text{O}_{\text{water}} + 21.4(\pm 1.5)‰.$$

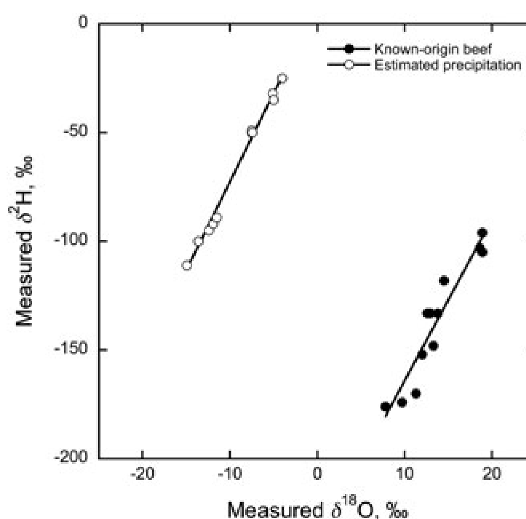

Figure 2. Hydrogen and oxygen co-variation of known-origin beef samples and precipitation estimated for beef source locations. The beef and water δ^2H and δ^{18}O values were positively correlated (beef: δ^2H = 7.45*δ^{18}O − 239 ‰, r^2 = 0.90, P <0.0001; water: δ^2H = 8.13*δ^{18}O + 9 ‰, r^2 = 0.99, P <0.0001).

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Rapid Communications in Mass Spectrometry

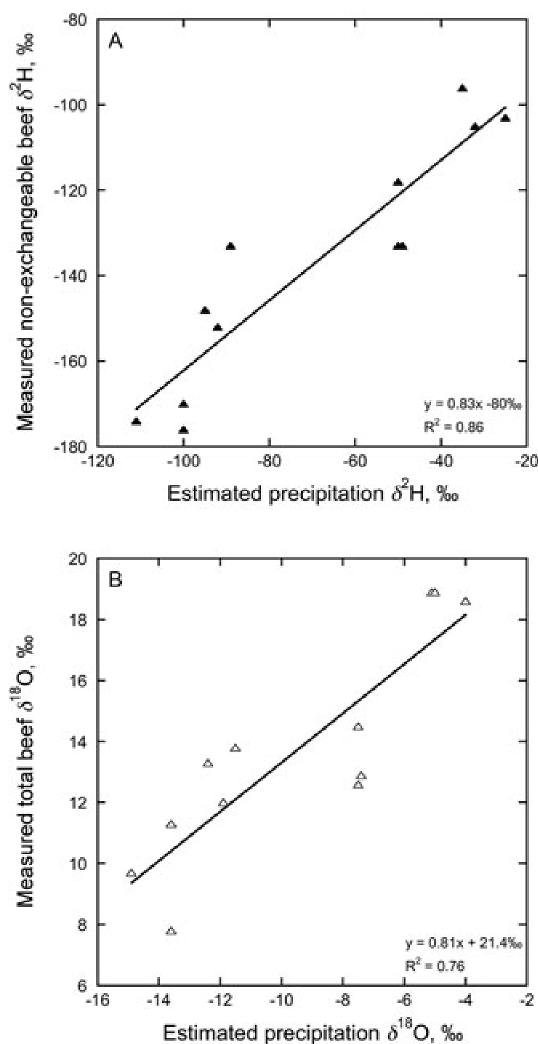

Figure 3. The δ^2H (A) and δ^{18}O (B) values of known-origin beef samples and estimated water values paired by source location. The data were positively correlated ($\delta^2H_{beef} = 0.83*\delta^2H_{water} - 80$ ‰, $r^2 = 0.86$, $P < 0.0001$; and $\delta^{18}O_{beef} = 0.81*\delta^{18}O_{water} + 21.4$ ‰, $r^2 = 0.76$, $P < 0.001$).

DISCUSSION

Continental meats

In this study we collected meats from a number of different animals, ranging in size from small (chickens) to large (beef cows). The animals under consideration used a variety of behavioral and physiological cooling mechanisms; they also digested and metabolized food using very different pathways (e.g., foregut vs. hindgut fermentation). In addition, we compared animals that lived in extremely different habitats (land vs. water). Despite these differences the coupled δ^2H and δ^{18}O values of all meats from continental animals drinking or living in freshwater (beef, poultry, chicken eggs, pork, and freshwater fish) generally displayed remarkably similar patterns, with a mean (± SD) slope for the RMA relationships between δ^2H and δ^{18}O of 5.9 ± 1.2 (Fig. 1) and for the OLS relationships of 5.5 ± 1.0 (Table 4).

The strong positive correlations observed between the measured δ^2H and δ^{18}O values of the meats from continental animals fit our expectation that the propagation of environmental water isotope signals into proteinaceous tissues is a ubiquitous feature. As discussed, the link between the isotopic composition of water and animal tissues has been previously documented both directly[3,5] and indirectly[4,35] for beef cattle. In addition, there have been studies directly documenting relationships between water and avian tissue δ^2H and δ^{18}O values[1,2] as well as between water and freshwater fish muscle δ^2H values.[7,8] Our results extend this work and provide the broadest evidence to date that the incorporation of environmental water isotopes into vertebrate proteinaceous tissues is similar across a wide variety of animal taxa.

However, there were some differences in the relationships between δ^2H and δ^{18}O values among the meats collected from animals drinking or living in freshwater, namely the beef and poultry groups. We discuss two potential explanations for this disparity. First, differences in diet (e.g., local vs. non-local feed) can influence the relationships between the δ^2H and δ^{18}O values of the proteinaceous tissues as demonstrated using a set of hair samples from historic human populations.[22] Consumption of local food is predicted to increase the slope of the line describing the co-variation between tissue δ^2H and δ^{18}O values because of the greater contribution of dietary sources to keratin H relative to O. The higher slope for the beef meat group could be due to beef cattle consuming more local feed while grazing in pasture whereas chickens and turkeys were provided more non-local feed.

Second, although drinking water is the dominant source of H and O to the body water of most terrestrial vertebrates, the production of metabolic water from the digestion of food can influence body water δ^2H and δ^{18}O values.[36] Sweating and panting can also affect body water δ^2H and δ^{18}O values, by increasing the amount of water leaving an individual's body water pool. The impact of water addition and water loss depends upon the relative size of each input and output to the pool and their rate of introduction and loss (e.g., total water flux, TWF). Animals with higher TWF typically have body water δ^2H and δ^{18}O values that are more similar to those of drinking water.[36,37] If we assume the animals in this survey were provided water with isotopic compositions that fit the Global Meteoric Water Line (GMWL), we would expect animals with higher TWF to have tissue δ^2H vs δ^{18}O slopes similar to the GMWL's slope of ~8.[12] Thus, the higher slope for the beef meat group could be due to the increased flux of water through beef cattle compared with poultry. Finally, we acknowledge that the disparity in sample sizes for the different meat categories (Table 3) may have had an effect on the calculated H vs. O relationships.

Aquatic meats

As hypothesized, the large isotopic range in terrestrial environmental waters[14,15] translated into a large isotopic range in proteins from continental vertebrates (Table 3). This was especially evident for the freshwater fish category that contained many known-origin samples (Table 1). This group had the largest ranges in both measured δ^2H and δ^{18}O values, which reflect the isotopic variation of freshwater rivers and lakes in the USA from which these samples originated. For example, the fish with the lowest

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measured hydrogen and oxygen isotope ratios was caught in a Utah reservoir, where the environmental water hydrogen and oxygen isotope ratios are typically low (i.e., δ^2H: –120 to −110 ‰, δ^{18}O: –16 to −14 ‰[38,39]). The highest δ^2H and δ^{18}O values were measured for fish caught in a central Florida reservoir, where the isotope ratios of environmental waters are higher (i.e., δ^2H: > −20 ‰, δ^{18}O: > −2 ‰[40]).

Given the relatively more homogeneous isotope ratios of marine waters[25] compared with those of meteoric freshwaters,[14,15,40] we expected smaller ranges in the measured isotope ratios for marine meats than for continental meats. The results were consistent with this expectation for the measured δ^{18}O values (Table 3). All marine meats (fish, bivalves, and crustaceans) had similar ranges in measured δ^{18}O values (ca. 16 % to 22 ‰, Table 3). In comparison, the measured δ^2H values displayed a proportionally larger range (ca. –145 ‰ to −65 ‰, Table 3).

In the context of the modified protein-isotope model for historic human populations described by Bowen *et al.*,[22] the larger range in δ^2H values than in δ^{18}O values for the marine fish samples is consistent with dietary heterogeneity, which is predicted to influence H isotope ratios more strongly than O isotope ratios. Considering that our marine fish collection includes fish that feed at a range of trophic levels, estuarine and open-water species, and potentially some farm-raised individuals (Table 1), the suggestion of large ranges in dietary H isotopes is not surprising. On the other hand, the smaller range in δ^{18}O values observed for the marine meats suggests that relatively homogeneous isotopic sources, such as ocean water and molecular O_2, are the main determinants for the δ^{18}O values of marine animal tissues.

While it was possible to fit a RMA regression line to the marine fish samples, it was not possible to fit a line to the marine bivalves or marine crustaceans (Fig. 1), suggesting that factors affecting the isotopic composition of these marine meats may be even more variable than for marine fish. Moreover, the observation that the δ^2H and δ^{18}O values for our limited set of marine invertebrate samples occupied a completely distinct area of δ^2H/δ^{18}O 'space' from the other samples suggests distinct environmental or physiological controls on the isotope ratios of these animals. Bivalves are largely sedentary and must rely on food filtered from the surrounding environment. While more mobile, marine crustaceans are typically scavengers, consuming food items as they become available. Dietary heterogeneity for these animals is therefore probably even larger than for marine fish, which could affect the tissue δ^2H values to such a degree that the contribution of environmental water to the tissue δ^2H and δ^{18}O values is overwhelmed and the co-variation in δ^2H and δ^{18}O values is not propagated from water to protein. Bowen and colleagues[22] have previously attributed human hair samples occupying anomalous regions of δ^2H/δ^{18}O 'space' to the consumption of allochthonous dietary resources, and it is possible that this model also could apply to the bivalve samples measured here. A larger and better-controlled sample set of marine bivalves and crustaceans may allow us to further investigate the impact of dietary heterogeneity and provenance on the H and O isotope ratios of proteins from these animals. At present, we are unaware of other, published datasets of marine animal tissue δ^2H and δ^{18}O values that could be included in such an investigation.

Known-origin beef

The slope of the known-origin beef δ^2H vs. δ^{18}O OLS regression line (7.45) was the second highest after that of the marine fish group and was not significantly different from the slope of all beef data (6.92; Table 4). The high slope observed for the known-origin beef samples collected from grass-fed herds is probably due to a combination of the consumption of locally derived feed and the high water turnover (e.g., TWF) in the cattle, as discussed previously. Using the OLS regression lines describing the relationships between tissue and water δ^2H and δ^{18}O values for the known-origin samples as a proxy for drinking water contribution to beef tissue, we find that ~83% of the H-atoms in beef muscle tissue are derived from water while ~81% of the O-atoms come from water. The O contribution is higher than the ~60% observed for Japanese beef cattle by Nakashita *et al.*[3]. The contributions are also much higher than those previously observed for a variety of other proteinaceous animal tissues, including birds (~15–30%[1,2]), freshwater fish (~50%[8]), and humans (~30–40%[21,41]). In fact, the percentages for the known-origin beef samples are more similar to the isotopic contribution of drinking water to dairy cow milk water (~90%[37]) than to any other published protein data.

If the slopes between the tissue and water δ^2H and δ^{18}O values are reasonable proxies for the contribution of drinking water to tissue isotopic composition, we could predict the slope between tissue δ^2H and δ^{18}O values for known-origin beef samples *a priori* using the method of Wolf *et al.*[2] For a complete description of the calculation derivation based on the semi-mechanistic model of Ehleringer *et al.*,[21] see the text of Wolf *et al.*[2] Briefly, the slope of the OLS regression line between the tissue δ^2H and δ^{18}O values is equal to the slope between the drinking water δ^2H and δ^{18}O values multiplied by the ratio of the drinking water H contribution to the O contribution for the tissue. Using the estimated drinking water values for our known-origin beef samples (Table 2), we find that the predicted slope between the δ^2H and δ^{18}O values = [(0.83/0.81)*8.2] = 8.4, which is similar to the observed value of ~7.5.

Implications

Alongside previous publications,[1–8] the patterns that we observed between the δ^2H and δ^{18}O values of the surveyed animal meats from multiple taxa support the hypothesis that proteinaceous animal tissues record the isotopic composition of drinking water in a similar, systematic manner across a wide range of taxonomic diversity. Several recent taxon-specific studies in which there was greater control on sample origin and dietary and drinking water intake have calibrated detailed process-based models describing the incorporation of H and O atoms from diet and water into the proteinaceous tissues.[2,21] Although we lack the necessary level of information to develop such models for each of the animal groups sampled here, the common patterns of δ^2H and δ^{18}O values observed in this survey suggest that the basic framework applied in the earlier studies could be applicable across a wide range of animal taxa. An expanded collection of known-origin samples to develop the appropriate model parameters and fully define the relationship between drinking water and animal tissues δ^2H and δ^{18}O values would allow for future modeling and mapping applications for these groups.

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Despite a lack of origin information for many of the samples in our dataset, we were able to test a method described by Wolf *et al.*[2] to estimate the slope of the relationship between tissue δ^2H and δ^{18}O values from the slope of the relationship between the water δ^2H and δ^{18}O values and the proportional contributions of drinking water to tissue H and O content. The calculated slope agreed well with the observed slope for a subset of known-origin beef samples. The calculated slope also agreed well with the slope that we observed for all beef samples collected in this survey, which likely means that the proportional contributions of drinking water to the tissue δ^2H and δ^{18}O values observed for the known-origin samples were similar to those for the other beef samples. The slopes between the tissue δ^2H and δ^{18}O values for the other surveyed meats were lower than those observed for the beef samples. This suggests: (1) the contribution of H from water to tissue for the other meats was lower than for beef, or (2) the contribution of O from water to tissue for the other meats was higher than for beef.

Hydrogen and oxygen stable isotope analysis has previously proven helpful when reconstructing the movement history of humans based on tissues that do not turn over after production, such as hair or nail keratin. When predicting human hair δ^2H and δ^{18}O values from location and drinking water (or vice versa) using process-based models, modelers include some estimation of the δ^2H and δ^{18}O values of dietary inputs for the individual.[21–23] Previous studies have estimated diet in one of two ways: by assigning average δ^2H and δ^{18}O values for a continental supermarket diet,[21] or by including a local food component, which is linked to local water δ^2H and δ^{18}O values.[22,23] In either case, understanding the natural ranges in food (especially meat) δ^2H and δ^{18}O values will enable modelers to better estimate the isotope ratios of continental supermarket and local dietary inputs.

One of the potential applications of meat hydrogen and oxygen stable isotope analysis is in the determination of food and animal origins.[20,42] Outbreaks of food-borne illnesses often trigger large food recalls because it is difficult to trace the origin and distribution of individual food items. Given the relationship between the δ^2H and δ^{18}O values of meats and the water available to animals raised for meat, it may be possible to broadly source the regions-of-origin of foods via stable isotope analysis. Describing where a food may have originated while excluding other geographical regions has important economic implications. Beyond food safety, origin assessment using stable isotope analysis could also be useful for verifying claims of foods that producers market as originating from a particular region or country, for verifying that imported foods are truly imported, and for assuring consumers who purchase locally grown foods that they are buying local products.

CONCLUSIONS

We report measured δ^2H and δ^{18}O values for a diverse collection of ~430 meat tissue samples. Despite the absence of specific provenance information for most samples, we were able to show that the samples exhibit several patterns indicative of the ubiquity and systematic nature with which environmental water δ^2H and δ^{18}O values are recorded in proteinaceous tissues. Co-variation in the δ^2H and δ^{18}O values of meats collected from continental animals drinking or living in freshwater was generally similar, suggesting that these animals recorded the isotopic composition of water inputs in a similar fashion. Marine proteins exhibited proportionally smaller variation in δ^{18}O values and larger variation in δ^2H values, and occupied a broader range of δ^2H/δ^{18}O 'space' than did other samples, likely due to dietary heterogeneity within this group. The relationships between the δ^2H and δ^{18}O values of tissue and environmental water for the samples of known-origin beef suggested that water is a large contributor to tissue isotopic composition (~80%). Using the known-origin beef samples, we were able to test a method for predicting the slope of the relationship between tissue δ^2H and δ^{18}O values. The results could be used to understand in a general sense the difference in water contribution to tissue δ^2H and δ^{18}O values for the other meats collected in this survey. The implications of these results are far-reaching, with applications in migration studies, modeling, and food sourcing.

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