Chapter 33
Light-Element Isotopes (H, C, N, and O) as Tracers of Human Diet: A Case Study on Fast Food Meals

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Abstract Tracking the production and movement of foods from farm to fork has become increasingly complex in recent years due to changes in modern food markets, including the globalization of food sources and their distribution. Dietary habits have changed as well, with meals eaten outside of the home representing an ever-increasing fraction of the modern human diet. Stable isotope analysis has proven to be a reliable technique for characterizing biological substances, such as food, because stable isotopes record aspects of photosynthesis, nutrition, and source water. Here we present stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, and $\delta^{18}O$) measured for one ubiquitous example of modern convenience food, the fast food meal. In a series of paired observations among cities in the U.S., we compared the isotopic composition of components of a fast food meal to similar foods purchased from grocery stores. Isotope analyses revealed several interesting patterns in both food production and food origin within the modern American food market. Carbon isotope ratio analysis of proteins highlighted the impact of consumer choice: patrons in a fast food restaurant consumed beef containing a lower proportion of C_4 plants than beef purchased from a grocery store. The relative trophic-level position of beef available in both fast food restaurants and grocery stores was similar based on $\delta^{15}N$ values. The $\delta^{2}H$ and $\delta^{18}O$ values of the beef, bread, and milk samples were independently and positively correlated, suggesting that both plants and animals recorded the isotopic composition of local environmental water and, therefore, geographic source. Overall, these results demonstrated the promise and potential of food stable isotope analyses to broad areas of societal interest: food safety, food authenticity, and food traceability.

33.1 Introduction

Food sources and eating habits are changing rapidly in our increasingly globalized societies. Tracing the production and movement of food from farm to fork is not necessarily an easy task in the modern food market because the diet of today's global citizen often includes foods from other countries or continents. Maintaining tracking records as foods are moved across the globe is difficult and poor or lost records often prevent investigators from easily tracing food source. Despite the globalization of food markets, there is an emerging interest in buying local foods. Even so consumers lack a method to verify that foods labeled as locally grown are truly produced locally. Stable isotope analysis can be useful for food tracing and has proven to be a powerful tool for investigations into the diet of both ancient (Macko et al. 1999a, b; O'Connell and Hedges 1999a; Sponheimer and Lee-Thorp 1999; White et al. 2004) and modern (O'Connell and Hedges 1999b; O'Connell et al. 2001; Nardoto et al. 2006) man.

Historically, applications of stable isotope analysis in dietary studies have focused on understanding the

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impact of different inputs on the final isotopic composition of the consumer's tissue(s) [e.g., (Nardoto et al. 2006; O'Brien and Wooller 2007; Ehleringer et al. 2008)]. However, stable isotope analysis can also be used to trace the origin of and determine the production method for individual food items (Rossmann 2001; Kelly et al. 2005). To date, most food tracer studies have focused on the analysis of specialty items, such as cheese (Pillonel et al. 2003; Camin et al. 2004; Manca et al. 2006), olive oil (Bontempo et al. 2009; Camin et al. 2010) or wine (West et al. 2007) that have relatively few and well understood origins and methods of production. Stable isotope analyses can be applied more broadly to the protein, carbohydrate, and lipid components of human diets.

Stable isotopes act as natural recorders of the biological, chemical, environmental, and physiological processes at work on organic materials (West et al. 2006b). The analysis of a plant or animal samples for a suite of elemental isotope concentrations can therefore provide information on the previous conditions experienced by those tissues. The information provided by each of the elements hydrogen (H), carbon (C), nitrogen (N), and oxygen (O) varies; together, the analysis and interpretation of these light-element isotopes often provides unambiguous information in tracing studies of human dietary food items.

33.1.1 Carbon Isotope Ratios Are Ultimately Linked to Photosynthesis

The measured $\delta^{13}C$ value of a food is ultimately linked to plant photosynthesis because plants discriminate against $^{13}CO_2$ in the atmosphere during carbon assimilation (Ehleringer et al. 1993). The carbon isotopic composition of atmospheric CO_2 is relatively constant across the globe, with an approximate average value of −8‰ (Keeling et al. 2005), but see (Peck and Tubman 2010) for a discussion of changing carbon dioxide $\delta^{13}C$ values. Discrimination against ^{13}C in carbon dioxide is larger in plants that use the C_3 (Calvin cycle) photosynthetic pathway than in plants that use the C_4 (Hatch-Slack cycle) pathway. Thus, C_3 plants, which include temperate grasses (e.g., wheat, barley, rye, rice) and most fruit and vegetables as important human dietary components, are characterized by $\delta^{13}C$ values ranging from −30 to −22‰ (Cerling et al. 1997). On the other hand, C_4 plants are characterized by relatively higher $\delta^{13}C$ values, ranging from −14 to −10‰ (Cerling et al. 1997). Examples of C_4 plants important in human diet include corn (maize), sugar cane, and other tropical grains such as millet, teff, and fonio.

Due to the large differences in the $\delta^{13}C$ values of C_3 and C_4 plants, it is possible to develop two-source mixing models to calculate the relative proportions of different plant sources to a mixture (Phillips and Gregg 2001). For example, a two-source mixing model can be used to detect the introduction of a C_4-plant derived adulterant (cane sugar; $\delta^{13}C = -12.5‰$) to C_3-plant derived food item (maple syrup; $\delta^{13}C = -23.6‰$) (Martin et al. 1996). This approach has also been used to detect the adulteration of wildflower honey, a C_3 plant-based food, with C_4 sugars (White et al. 1998).

The $\delta^{13}C$ values of animal-derived food items like beef, chicken, or milk will reflect the carbon stable isotope ratios of the animal's dietary inputs (Bahar et al. 2005; Schmidt et al. 2005; Camin et al. 2008; Rhodes et al. 2010). Similar to the adulteration detection examples, mixing models can also be used to calculate the fraction of C_3- or C_4-plants fed to an animal during its growth (Schwarcz and Schoeninger 2011). However, because of a small fractionation during the incorporation of carbon atoms from diet to tissue, the $\delta^{13}C$ value of the carbon source may not exactly match that of the tissue (Peterson and Fry 1987).

33.1.2 Nitrogen Isotopes Trace the Flow of Organic Matter Through Food Webs

Ecosystem nitrogen influxes and effluxes influence soil and plant baseline $\delta^{15}N$ values. The nitrogen cycle – and its impact on soils and plants – has been previously reviewed (Högberg 1997; Amundson et al. 2003; Pardo and Nadelhoffer 2010), so we present only a summary here. Inputs to the ecosystem include atmospheric N deposition (soils) and N fixation (plants). Nitrogen outputs include gaseous losses and hydrologic leaching from soils. Examples of internal fluxes and transformation within the ecosystem are N taken up from soil by plants via nitrogen fixation or mycorrhizae; alterations within plants via transport,

assimilation, and translocation; and then deposited to soil via litter fall.

In general, nitrogen stable isotope ratios are increased ~3.2‰ per trophic step in a food web (Peterson and Fry 1987). It is therefore possible to broadly estimate the functional trophic level (TL) of many consumers within a food web as

$$TL_{consumer} = TL_{base} + (\delta^{15}N_{SC} - \delta^{15}N_{base})/3.2,$$

where TL_{base} is the assumed TL of the organism used to estimate the food web's baseline nitrogen isotope ratio (for a primary consumer, $TL_{base} = 1$) and $\delta^{15}N_{base}$ and $\delta^{15}N_{SC}$ are the measured nitrogen isotope ratios of the web's baseline and a secondary consumer, respectively [equation modified from (Post 2002)]. However, nitrogen discrimination between diet and consumer can vary about the value of 3.2‰ due to many factors, including the amount of nitrogen (as protein) in the diet and the quality of the protein source (Robbins et al. 2005, 2010). For example, nitrogen discrimination factors published in the literature range from 1.4‰ for nursing coyote offspring (Jenkins et al. 2001) to 5.8‰ for grizzly bears on controlled diets fed apples [(Felicetti et al. 2003); see also (Robbins et al. 2005)].

Despite the multiple pathways for N movement in an ecosystem, nitrogen isotope ratios can be used to construct food web assemblages by estimating organisms' trophic levels (Cabana and Rasmussen 1996; Hansson et al. 1997; Bösl et al. 2006). Nitrogen stable isotope analysis is also a powerful tool for investigations into the flow of organic matter and energy through a food web (Peterson et al. 1985; Vander Zanden et al. 1999). The nitrogen isotope analysis of human hair has been used to successfully trace the source (animal vs. plant) of protein in the consumer's diet (O'Connell and Hedges 1999b; Petzke et al. 2005a, b).

33.1.3 Hydrogen and Oxygen Isotope Ratios Record Geolocation Information

The majority of water in the Earth's water cycle is found in the oceans, which have defined hydrogen and oxygen stable isotope ratios of 0‰ for each (Gat 1996). As water from the surface of the ocean is evaporated into the atmosphere, the water vapor in clouds is depleted in ^{2}H and ^{18}O relative to the ocean. In turn, water condensation from the cloud mass is enriched in ^{2}H and ^{18}O relative to the cloud. After a precipitation event the residual cloud mass is further depleted in ^{2}H and ^{18}O relative to both the original cloud and the ocean. The systematic fractionation within a cloud mass during successive precipitation events results in a predictable depletion of ^{2}H and ^{18}O across landscapes (Gat 1996). This simplified model does not capture all the processes taking place in the hydrologic cycle, but it explains a large portion of the isotope gradients observed across the Earth's surface that are related to the isotope ratios of meteoric water.

The predictable geographic pattern in meteoric water $\delta^{2}H$ and $\delta^{18}O$ values can be spatially presented in an isotopic landscape, or *isoscape* (West et al. 2010b). In general, higher $\delta^{2}H$ and $\delta^{18}O$ water values are measured near coasts and in low latitudes or low elevation regions. Lower $\delta^{2}H$ and $\delta^{18}O$ values are measured in high latitude, high altitude, and inland regions. The tissues of both plants and animals record the stable isotope ratios of local environmental water (West et al. 2006a, 2008, 2010b). Thus, the stable isotope ratios of a plant- or animal-derived food item will vary with geography and these values can be used to investigate its region-of-origin.

Two concepts serve as the basis for understanding the relationship between animal or plant tissue and water. First, (Craig 1961) described the relationship between the $\delta^{2}H$ and $\delta^{18}O$ values of meteoric water collected across the globe. Known as the Global Meteoric Water Line (GMWL), it is described as

$$\delta^{2}H = 8 * \delta^{18}O + 10‰.$$

Because the hydrogen and oxygen isotope ratios of meteoric water are correlated, we expect that the $\delta^{2}H$ and $\delta^{18}O$ values of animal and plant tissues will also be related and mimic the spatial distribution of water isotopes. However, the water in organisms is subject to evaporation, which differentially impacts the abundance of ^{2}H and ^{18}O in tissues. In animals, metabolic water will additionally influence the $\delta^{2}H$ and $\delta^{18}O$ values of body water. The foundation for understanding the isotopic enrichment measured in animal and plant tissue $\delta^{2}H$ and $\delta^{18}O$ values associated with

evaporation is the Craig-Gordon model (Craig and Gordon 1965). Mechanistic studies on the δ^2H and δ^{18}O values of animal and plant tissues for food tracing are all necessarily based on these two fundamental concepts.

33.1.4 Fast Food Stable Isotope Analysis Characterizes the Average American Diet

One defining characteristic of today's American diet is the inclusion of prepared foods obtained away from the home. In 1970, the average US citizen spent 26% of his total yearly food budget on food prepared away from home (Lin and Frazão 1999). By 1999, the proportion had roughly doubled to ~50% (Clauson 2000). Fast food restaurants are the single largest supplier of away-from-home food for the average American, accounting for approximately 12% of daily caloric (i.e., energy) intake (Guthrie et al. 2002). The "meat and potatoes" fast food meal leads sales, with consumers eating hamburgers and French fries more often than any other restaurant menu item offered (Jekanowski 1999; French et al. 2000).

Americans increased spending on foods prepared outside the home from $263 billion in 1992 to $415 billion in 2002, an increase of 23% when corrected for inflation (Stewart et al. 2004). In an effort to continue increasing profit margins, the food industry has begun to change the face of agriculture in the USA. Key to this change is consolidation of supply chains, allowing a company to control all aspects of food production from feedlot to meat counter and thereby cutting costs in the process. Between 1975 and 2000, there was increased geographic concentration in the cattle, hog, and poultry industries (Drabenstott et al. 1999; Herath et al. 2004). By 1999, approximately 60% of all cattle were raised in three states, while two-thirds of chickens were raised in five (Drabenstott et al. 1999), creating the most concentrated manufacturing sector in the USA (Herath et al. 2004).

Ongoing consolidation in the food industry should reduce isotopic variation for different food items. We investigated patterns in meat production methods through the stable isotope analysis of carbon and nitrogen. We used hydrogen and oxygen stable isotope ratios to investigate the potential relationship between local environmental conditions at food growing regions and the measured δ^2H and δ^{18}O values of food. Because of its importance to the modern American diet, we concentrated our survey on the most ubiquitous example of food prepared away-from-home: the fast food meal.

We focused on the three basic components of a fast food meal: (a) protein (beef patty), (b) carbohydrates (hamburger bun and French fries), and (c) beverage (milk). Dietary protein is the source of essential amino acids used for protein synthesis in the consumer and thus, the analysis of proteinaceous tissues can be used to infer something of a consumer's protein intake. Carbohydrates (and, though not discussed in this text, fat) represent the energy source for metabolism. For example, the stable isotope analysis of breath CO_2 is used to indicate the carbohydrate fuel of a consumer (Ayliffe et al. 2004). The liquid intake of a consumer will impact his body water pool, which in turn influences the isotopic composition of amino acids and proteins in the consumer's other tissues.

33.2 Materials and Methods

We collected the hamburger (beef) patty, hamburger bun, French fries, and milk drink from Hamburger Happy Meals™ purchased from McDonald's® restaurants in 36 cities distributed across the contiguous USA in February 2008 (Fig. 33.1). To allow food choice comparisons, ground beef (and chicken), loaf bread, and milk were also purchased in a supermarket in each city. Details of sample collection, preparation, and analysis can be found in (Chesson et al. 2008, 2010a, b).

In stable isotope analysis of foods to calculate dietary inputs or for region-of-origin assignment, it is important that samples are lipid-free as the carbon and hydrogen isotope ratios of lipids are typically light and inconsistent amounts of lipids in samples can differentially affect measured δ^{13}C and δ^2H values. Thus, all meat samples and French fries were delipified prior to analysis to remove fat and cooking oils. In order to assure sample homogeneity, delipified samples were then ground to a fine powder to minimize heterogeneity. No special procedures were required to process the hamburgers buns or loaf breads prior to grinding. We extracted the water from milk

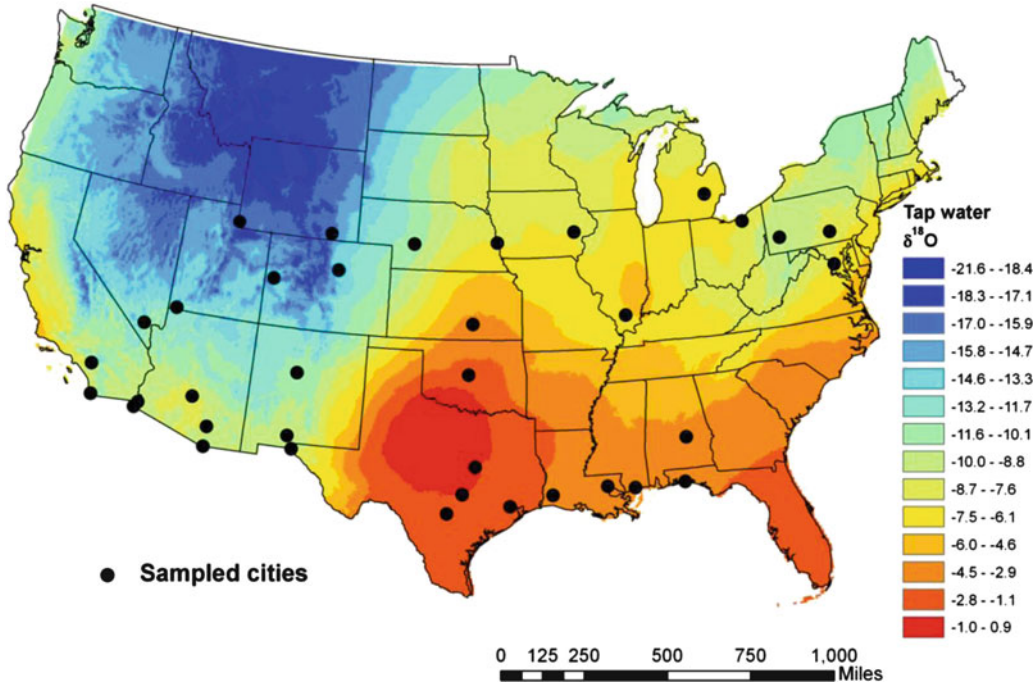

Fig. 33.1 Locations of fast food restaurants (McDonald's®) and supermarkets visited in this American food survey, shown on a predicted tap water oxygen isoscape for the USA (Bowen et al. 2007)

samples for stable isotope ratio analysis (West et al. 2006a).

Measured H, C, N, and O isotope ratios are reported vs. Vienna Standard Mean Ocean Water (VSMOW), Pee Dee Belemnite (PDB), atmospheric air, and VSMOW, respectively. We recognized that some proportion of the H atoms in organic molecules is exchangeable (DeNiro 1981). Therefore, the H exchange factors were controlled for meats during sample preparation and analysis (Bowen et al. 2005; Chesson et al. 2009). Atom exchange with the environment was not a concern for C, N, or O stable isotope ratio measurements.

33.3 Results and Discussion

33.3.1 *Supermarket Beef Cattle Eat More Corn than Fast Food Cattle in the USA*

As a result of consolidation in meat production and supply chains in the USA, we hypothesized that beef cattle raised for the fast food and for the grocery retail markets would be fed similar feed and thus display similar $\delta^{13}C$ values. As expected, we found that the mean carbon isotope ratios of beef were similar, whether purchased as McDonald's hamburgers or as ground beef from supermarkets. However, the distributions of measured $\delta^{13}C$ values within each dataset were markedly different, as seen in a comparison of the cumulative frequency distributions for hamburger and ground beef carbon isotope ratios (Fig. 33.2). The supermarket dataset contained samples with (on average) higher measured $\delta^{13}C$ values than the hamburger dataset; yet it also contained the two lowest measured $\delta^{13}C$ values. As shown in Fig. 33.2, the distribution of carbon isotope ratios in the supermarket beef dataset was not normal, as compared to the $\delta^{13}C$ values of restaurant hamburgers.

Several recent studies have investigated the impact of diet on the carbon isotope ratios of ruminants (De Smet et al. 2004; Bahar et al. 2005; Schmidt et al. 2005; Bahar et al. 2008). Two of these present two-source mixing models for calculating the incorporation of C_4-based diet into cow tissue (De Smet et al. 2004; Bahar et al. 2005). Using a similar approach as that presented by De Smet et al. (2004) and Bahar et al. (2005), we calculated the percentage of C_4-based plant

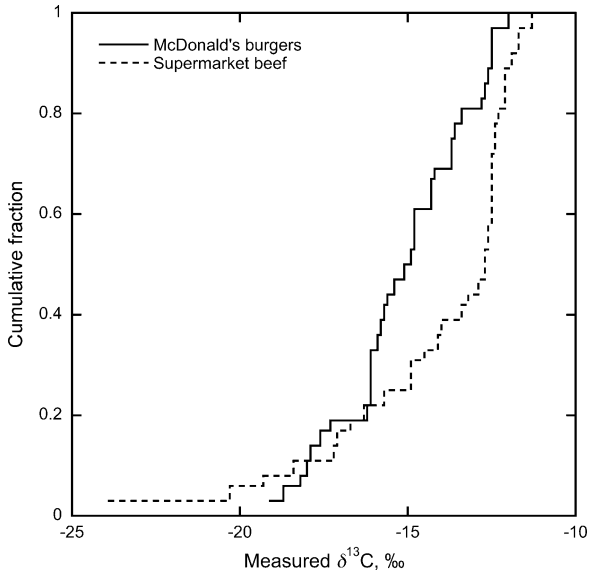

Fig. 33.2 The distribution of measured carbon isotope ratios in the fast food hamburger (*solid line*) and supermarket ground beef (*dashed line*) samples collected in this survey. Data are presented as cumulative fractions to highlight the different distribution in measured $\delta^{13}C$ values of each dataset

material in the diets of the cows that served as the source of beef for McDonald's restaurants and supermarkets. (We assume that mostly corn and not C_4 grass represents this dietary biomass.)

In the mixing model we used a value of −24.0‰ to represent cattle eating only C_3 biomass, which was calculated from the analysis of beef from a ranch raising grass-fed cattle in Idaho ($1\sigma = 0.4$‰, $n = 8$; unpublished data). We used a carbon isotope ratio of −11.4‰ to represent cattle eating solely C_4 biomass, which was calculated using hamburgers collected from McDonald's restaurants in Brazil ($1\sigma = 0.8$‰, $n = 10$; unpublished data.) The calculated proportion of corn in the diet of cattle that served as the source of meat for McDonald's hamburgers ranged from 0.39 to 0.95. The range for fractional contribution of corn in the diet calculated for supermarket beef was larger and spanned from 0.01 to 1.0.

From the available beef datasets and the dietary corn proportion calculations we observed a previously undocumented difference between the measured $\delta^{13}C$ values of lipid-free beef samples collected in the same location but from different sources: fast food restaurants and supermarkets. In addition, the proportional contribution of C_4 plants to the diets of beef cattle sourced by supermarkets spanned a larger range than the contribution of C_4 plants to cattle sourced by restaurants. Based on this observation, a consumer choosing to eat dinner at McDonald's could incorporate a significantly different carbon isotope ratio from his hamburger patty than a consumer who chose to grill her own burger at home using beef from the supermarket meat counter. Interestingly, it appears beef cattle from American supermarkets were generally fed more corn prior to slaughter than beef cattle served by McDonald's restaurants, contrary to popular perception (Dotinga 2008; Pickert 2008).

33.3.2 There Are No Trophic Level Differences Between Restaurant and Supermarket Beef

Beef cattle are primary consumers and in the USA are likely consuming mostly corn prior to slaughter (see Sect. 33.3.1, above). Thus, we expected that the measured $\delta^{15}N$ values of beef cattle collected from fast food restaurants and supermarkets would be similar. If we knew or could estimate the $\delta^{15}N$ value of the baseline for the beef food web, we hypothesize that we would be able to calculate the functional trophic level of beef cattle.

There was no difference between the mean $\delta^{15}N$ values of beef in the two datasets (Fig. 33.3). All beef appear to occupy the same relative trophic level. While this is not surprising since these animals are primary consumers, it does suggest that the nitrogen isotope values of the feed available to beef stock (e.g., corn, grasses) were grown on nitrogen sources that had generally the same nitrogen isotope ratio value. The measured $\delta^{15}N$ values of all the beef samples covered a small range, from 5.1 to 7.8‰ for hamburgers and from 5.3 to 7.9‰ for ground beef from supermarkets.

We observed a difference in the mean measured $\delta^{15}N$ values of beef and chicken available to the modern American consumer (Fig. 33.3); nitrogen stable isotope ratios were significantly lower for chicken than for McDonald's hamburgers or supermarket ground beef (ANOVA with Tukey's post-hoc test; $F_{2,104} = 814.9$, $P < 0.0001$). The range in $\delta^{15}N$ values for chicken samples was 1.6–3.5‰. Our fast food meat nitrogen isotope data agreed well with a survey of fast food meals purchased from different

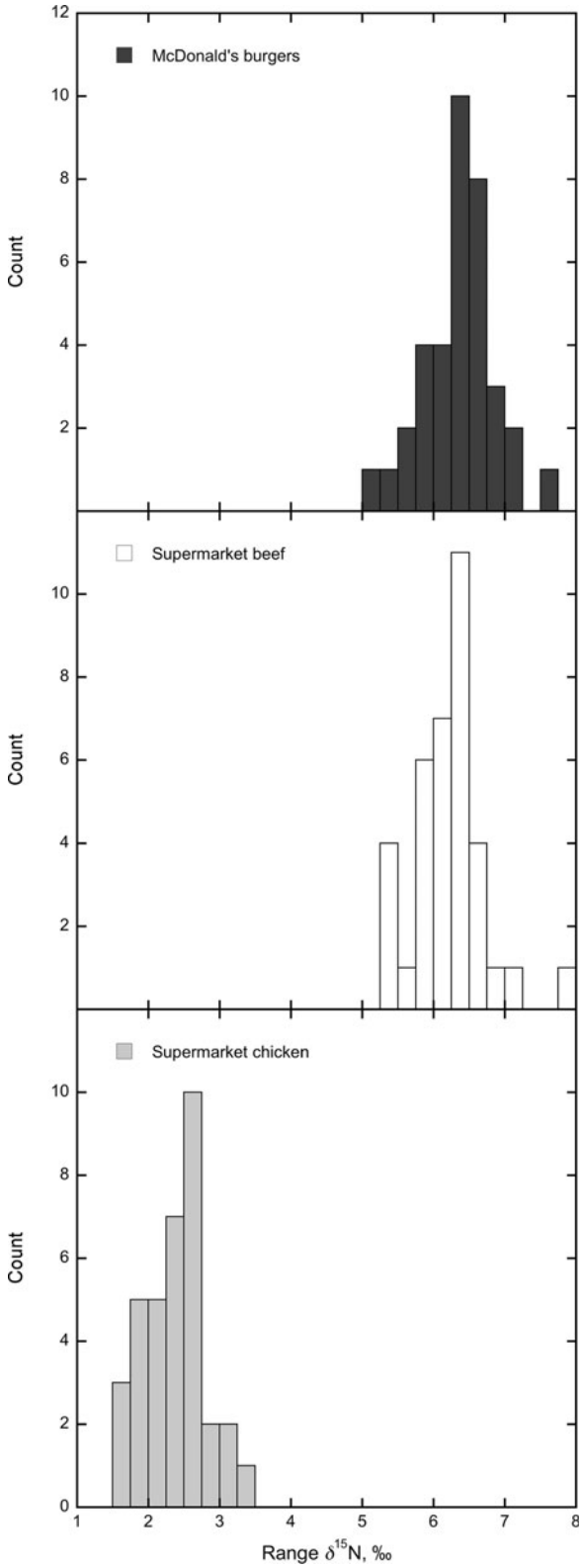

Fig. 33.3 The distribution of measured nitrogen isotope ratios of collected fast food hamburgers (*top*), supermarket ground beef (*middle*), and supermarket chicken (*bottom*)

restaurant chains in selected cities in the USA by Jahren and Kraft (2008).

In this survey of American fast food items we assumed, as did Jahren and Kraft (2008), that the majority of feed for animals raised for sale to the food industry is corn. We therefore used a δ^{15}N values for the nitrogen input of corn fed to both chickens and cows of ~3‰ (Schoeller et al. 1986; Masud et al. 1999; Bahar et al. 2005). Using the modified equation of Post (2002) and a diet-consumer nitrogen discrimination factor of 3.2‰, we calculated the mean functional TL of beef cattle and chickens where corn was the food web baseline so that $TL_{base} = 0$ and $\delta^{15}N_{base} = 3‰$. We calculated mean TL for beef purchased at both McDonald's restaurants and supermarkets as 1.0; the calculated TL of chicken from supermarkets was 0.0.

While a calculated TL of ~1 was expected for beef cattle, we did not expect to find that the calculated trophic level of chickens was the same as that of plants (i.e., TL = 0). Chickens are omnivorous and would naturally occupy a functional trophic level greater than one. We present two possibilities to explain the low calculated TL of chickens. First, our estimates of feed δ^{15}N values may be incorrect for chicken. In other words, chickens raised for distribution to fast food restaurants are fed a nitrogen source with a δ^{15}N value near 0‰. Alternatively, the fractionation between diet and tissue nitrogen isotope ratios, assumed to be 3.2‰ here, is not the same for beef cattle and chickens, leading us to underestimate the functional TL of chickens. For commercially raised chicken that cannot forage, pre-mixed feeds include nitrogen in the form of fish or feather meals. A chicken consuming a diet with large quantities of crude protein – protein from an animal-derived source – may have a dietary nitrogen discrimination factor much lower than an average value of 3.2‰ (Robbins et al. 2005, 2010).

33.3.3 Beef Hydrogen and Oxygen Ratios May Be Useful for Region-of-Origin Assignment

If cattle production is consolidated in the USA, the range in measured δ^{2}H and δ^{18}O values observed in beef samples may be limited due to the limited geographic (and thus, isotopic) variation of the production regions. Independent of the amount of beef isotopic

variation the relationship between δ^2H and δ^{18}O values of beef tissue should mimic the GMWL because beef cattle incorporate environmental water during growth. We expected to see a positive correlation between measured beef hydrogen and oxygen isotope ratios. However, due to the impact of metabolic water on an organism's body water pool and/or the evaporation of water within an organism's tissues we did not necessarily expect to find that the slope or intercept of the relationship between beef δ^2H and δ^{18}O values would exactly match those of the meteoric water line.

The measured δ^2H and δ^{18}O values of beef purchased from McDonald's restaurants and supermarket meat counters spanned a large range, from −157 to −98‰ for hydrogen and from 9.2 to 18.1‰ for oxygen. The hydrogen and oxygen isotope ratios of beef co-varied (Fig. 33.4) and the ordinary least squares regression (OLS) lines fitted to the separate beef datasets were described by the equations

$$\delta^2\mathrm{H} = 6.4{*}\delta^{18}\mathrm{O} - 211‰ \left(\mathrm{r}^2 = 0.91\right)$$

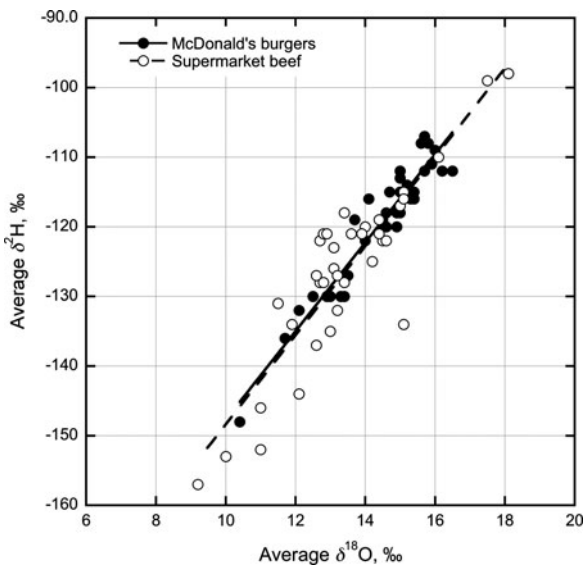

Fig. 33.4 A bi-plot of the measured hydrogen and oxygen isotope ratios of fast food hamburgers (*closed circles*) and supermarket ground beef (*open circles*). The measured δ^2H and δ^{18}O values for each data set were correlated. Ordinary least squares regression lines fitted to the data are shown in *solid black* for burgers and *dashed black* for ground beef and described by the equations $\delta^2\mathrm{H} = 6.4{*}\delta^{18}\mathrm{O} - 211‰$ ($r^2 = 0.91$) and $\delta^2\mathrm{H} = 6.4{*}\delta^{18}\mathrm{O} - 213‰$ ($r^2 = 0.80$), respectively

for fast food restaurant hamburgers and

$$\delta^2\mathrm{H} = 6.4{*}\delta^{18}\mathrm{O} - 213‰ \left(\mathrm{r}^2 = 0.80\right)$$

for ground beef from supermarket meat counters. Both the lines were different from the GMWL. However, neither of the slopes were different from the slope of a regression line describing the relationship between hydrogen and oxygen isotope ratios of human hair collected throughout the USA [$m = 5.7$; (Ehleringer et al. 2008)].

The congruence between the slopes of the OLS regression lines fitted to measured δ^2H and δ^{18}O values of beef protein and human hair keratin suggests that the incorporation of H and O atoms from the cow's diet, drinking water, and O_2 inputs is similar to the incorporation of H and O from various dietary and fluid inputs to human hair. A semi-mechanistic model developed to describe the relationship between the stable isotope ratios of inputs to human hair δ^2H and δ^{18}O values (Ehleringer et al. 2008) may therefore also be useful – after some modification – to understand the incorporation of hydrogen and oxygen atoms to beef cattle muscle tissue because both hair and muscle are proteins. Using the Ehleringer et al. (2008) publication for reference, we attempted to adapt the model as follows to predict the measured oxygen stable isotope ratios of beef protein.

We assumed that the O atoms of amino acids are exposed to isotopic exchange within the animal's body water pool when proteins from the diet are cleaved in the stomach and small intestine during digestion at low pH. After absorption across the gut wall, the oxygen isotope ratio of an amino acid is, in essence, fixed. Thus, the oxygen isotopic composition of protein in cow muscle is related to that of water in the cow's gut ($\delta^{18}\mathrm{O_{wg}}$) during digestion such that

$$\delta^{18}O_{muscle} = \alpha_o \bullet (1{,}000 + \delta^{18}O_{wg}) - 1{,}000, \tag{33.1}$$

where α_o is the fractionation in oxygen isotope ratio associated with protein synthesis and is assigned a value of 1.0164 (Kreuzer-Martin et al. 2003). Note that (33.1) presented here is the same as Equation (1) in Ehleringer et al. (2008) and Equation (19) in Podlesak et al. (2008). For cows that eat dry feed, gut water is a mixture of gastric fluids from the body water pool ($\delta^{18}\mathrm{O_{wb}}$) and possibly drinking water ($\delta^{18}\mathrm{O_{we}}$):

$$\delta^{18}O_{wg} = g_1 \cdot \delta^{18}O_{wb} + g_2 \cdot \delta^{18}O_{we}, \quad (33.2)$$

where g_1 and g_2 represent the fraction of water in the gut from body water and drinking water, respectively. Unlike the human hair keratin model, we do not consider water contained within food as a contributor to gut water. We initially assume that the contribution of drinking water to gut water is insignificant ($g_2 = 0$) and thus the oxygen isotopic composition of water in the gut is the same as that of body water ($g_1 = 1$ and $\delta^{18}O_{wg} = \delta^{18}O_{wb}$).

The body water pool does not have the same isotope ratio as drinking water because of the contribution of metabolic water (Gretebeck et al. 1997). The oxygen isotopic composition of body water is described as

$$\delta^{18}O_{wb} = \frac{a \cdot \delta^{18}O_{we} + b \cdot \delta^{18}O_d + c \cdot [\alpha_{O2}(1{,}000 + \delta^{18}O_{O2}) - 1{,}000]}{h \cdot \alpha_{fwlo} + j \cdot \alpha_{CO2} + k}, \quad (33.3)$$

where $\delta^{18}O_d$ and $\delta^{18}O_{O2}$ are the stable isotope ratios of food and inhaled oxygen, respectively; a, b, and c are the fractional contributions of drinking water, food, and atmospheric oxygen, respectively, to body water; h, j, and k are the proportions of O lost as fractionated water, exhaled CO_2, and unfractionated water, respectively, from the body water pool; and α_{O2}, α_{fwlo}, and α_{CO2} are the isotopic fractionation factors for the absorption of O_2, loss of oxygen through breathing and transcutaneous evaporation, and loss of oxygen in exhaled CO_2. Equation (33.3) is unchanged from the Ehleringer et al. (2008) model, presented by the authors as Equation (6). This is also essentially the same model for body water oxygen isotope ratios as Equation (24) in Podlesak et al. (2008), though the authors used experimentally derived values for the proportion of drinking water, food, and O_2 contributing to wood rat body water $\delta^{18}O$ values while we have used values from Gretebeck et al. (1997), which were originally derived for humans.

We previously collected and analyzed a sample of grass-fed beef collected in Soda Springs, ID. Using the predicted tap water oxygen isotope ratio ($\delta^{18}O_{we}$) of the location (Bowen et al. 2007), we attempted to use the modified model described above to calculate a predicted beef meat $\delta^{18}O_{muscle}$ value. We used a value of 21.0‰ for $\delta^{18}O_d$, which was measured for locally grown hay in the Salt Lake City, UT area (unpublished data). Using the predicted $\delta^{18}O_{we}$ value of −17.0‰, the modified protein model predicted a $\delta^{18}O_{muscle}$ value of 12.0‰ for beef cattle raised in Soda Springs. The predicted $\delta^{18}O_{muscle}$ value was greater than the measured oxygen isotope ratio 9.2‰, a difference of 2.8‰. However, the variation (calculated as 1σ) in measured $\delta^{18}O$ values of several samples of beef collected from different individual animals within the same herd of cattle in ID ($n = 8$) was 1.2‰. Indeed, one sample of meat collected from the ID herd had a measured $\delta^{18}O$ value of 12.3‰, very near the theoretical predicted value of 12.0‰.

Tracing the geographical origin of beef has recently been recognized as a food-safety concern in Europe and Asia. Preliminary data suggest the stable isotope analysis of beef may be useful to answer questions of region-of-origin (Nakashita et al. 2008; Bong et al. 2010; Horacek and Min 2010). A recent survey of beef raised in Japan published by Nakashita et al. (2008) observed a relationship between the oxygen isotope ratio of water available to beef cattle and the measured $\delta^{18}O$ value of delipified beef, described by:

$$\delta^{18}O_{muscle} = 0.59 * \delta^{18}O_{we} + 15.56‰. \quad (33.4)$$

We also tested the Nakashita et al. (2008) model using the predicted tap water $\delta^{18}O$ value of −17.0‰ for Soda Springs, ID. Equation (33.4) predicted a muscle tissue oxygen isotope ratio of beef raised in ID of 5.5‰, much lower than the measured $\delta^{18}O_{muscle}$ value of 9.2 and 3‰ lower than any value measured for cattle in the ID herd (minimum = 8.6‰).

33.3.4 Bread Hydrogen and Oxygen Isotope Ratios Are Positively Correlated

Following a similar water-based dependence, the hydrogen and oxygen isotope ratio of both beef and bread should be positively correlated. We expected to see a correlation between the measured δ^2H and $\delta^{18}O$ values of breads collected from McDonald's restaurants as hamburger buns and from supermarkets as loaves because the source of H and O atoms in wheat

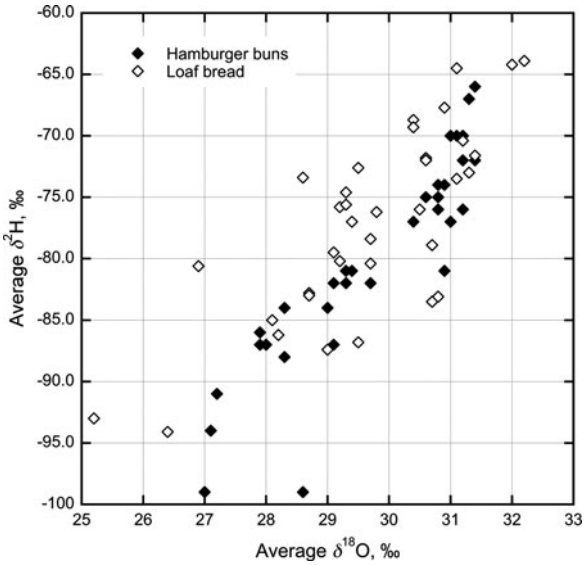

Fig. 33.5 A bi-plot of the measured hydrogen and oxygen isotope ratios of fast food hamburger buns (*closed diamonds*) and supermarket loaf bread (*open diamonds*). The measured δ^2H and δ^{18}O values for each data set were correlated ($r = 0.90$ for hamburger buns and $r = 0.77$ for loaf bread)

are derived from meteoric water. The ranges in δ^2H and δ^{18}O values of hamburger buns spanned from −99 to −66‰ and from 27.0 to 31.4‰, respectively (Fig. 33.5). The ranges in hydrogen and oxygen isotope ratios of bread samples spanned from −94 to −64‰ and from 25.2 to 32.2‰, respectively (Fig. 33.5). Hydrogen and oxygen isotope ratios of the two bread datasets were significantly correlated at the $\alpha = 0.05$ level (two-tailed Pearson correlation; $r = 0.90$ for buns and $r = 0.77$ for loaf bread).

A model of the hydrogen and oxygen isotope ratios of plants carbohydrate (cellulose) has been developed and tested in the past decade (Roden et al. 2000). Recently the hydrogen and oxygen isotope ratios of materials of forensics interest, plant-based poisons and counterfeit money (West et al. 2010a), have also been examined and modeled. Based on the extent of variation in bread δ^2H and δ^{18}O values – and the correlation between the two isotopes – we propose that similar tracing and geolocating approaches could be used for food items like the hamburger buns and loaves of bread collected in the survey.

Models exist to provide a theoretical foundation for predicting the isotopic composition of leaf water (West et al. 2008) based on the distribution of water isotope globally. Other models that use the Craig-Gordon evaporation model as a foundation can be used to interpret the transformation of leaf water δ^2H and δ^{18}O values to plant cellulose δ^2H and δ^{18}O values (Roden and Ehleringer 1999; Roden et al. 2000). With some work, these models may be adapted to model the hydrogen and oxygen isotope ratios of wheat-based carbohydrates like hamburger buns and bread.

In addition to hydrogen and oxygen isotope ratios, we also compared the measured δ^{13}C values of hamburger buns and loaf breads. We found the mean measured δ^{13}C values of the datasets were statistically different (unpaired t test; $t_{69} = 3.8$, $P < 0.001$); the mean carbon isotope ratio for hamburger buns (−23.6‰) was higher than that of loaf bread samples (−24.1‰). This difference is likely due to the ingredients used to make hamburger buns, which include high fructose corn syrup, a C_4-based sweetener. These measured carbon isotope ratios highlight the need for caution when adapting models to trace wheat-based food like bread. Developed models must recognize the potential confounding impact of other ingredients (with other origins) in the bulk bread sample submitted for stable isotope analysis.

33.3.5 Interpreting Potato Hydrogen and Oxygen Isotopes is Challenging at the Moment

We expected the measured hydrogen and oxygen isotope ratios of potatoes and potato products (i.e., French fries) would be significantly correlated because the δ^2H and δ^{18}O values of meteoric water are related (Gat 1996). The ranges in measured δ^2H and δ^{18}O values for delipified French fries and baking potatoes are shown in Fig. 33.6. We found that the hydrogen and oxygen stable isotope values of French fries and baking potatoes collected in this American food survey were significantly and positively correlated at the $\alpha = 0.05$ level, when tested using the two-tailed Pearson correlation. However, the amount of variation in H explained by O was very low for both fries and potatoes ($r^2 = 0.15$ and 0.26, respectively).

There may be multiple explanations why δ^2H and δ^{18}O values in potatoes are not as tightly correlated as they are in the GMWL. One possibility is that starch, the main storage molecule of the potato tuber and

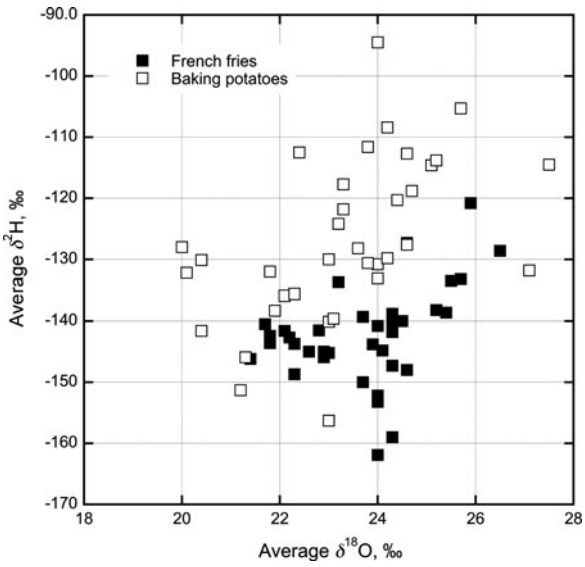

Fig. 33.6 A bi-plot of the measured hydrogen and oxygen isotope ratios of fast food French fries (*closed squares*) and baking potatoes purchased from supermarkets (*open squares*). The measured δ^2H and δ^{18}O values for each data set were correlated though the amount of variance in measured δ^2H values explained by δ^{18}O values was low ($r^2 = 0.15$ for French fries and $r^2 = 0.26$ for potatoes)

containing exchangeable hydroxyl groups, has opportunities for H atoms to interact with different water sources between the time a potato is harvested in the field and sold to the consumer. Despite the protective effects of the potato skin, tubers gain or lose weight according to the relative humidity of the surrounding environment (DeNiro and Cooper 1989). Hydrogen atoms may also have the opportunity for exchange during potato processing post harvest. For example, potatoes for French fries are commonly steam cleaned to remove the skin and any attached debris (Lisińska and Gołubowska 2005). During this process, hydrogen atoms in the hydroxyl groups are subject to exchange with ambient water surrounding the potato tuber. Lisińska and Gołubowska (2005) have shown change in both carbohydrate characteristics and water content during the production of pre-frozen French fries. Thus, the isotopic composition of the water vapor at the storage location and the method of processing post-harvest potatoes could contribute to the decoupling of French fry and potato δ^2H and δ^{18}O values.

In addition, a French fry is not simply a sliced potato. A second possibility is the conversion of carbohydrates during French fry production and/or cooking that were composed of different δ^2H and/or δ^{18}O values, because the local water environment is different than that of the original tuber. For instance, while the delipification step should have removed any fats introduced during the production-cooking processes, conversion of starches to sugars may have altered the overall δ^2H and δ^{18}O values from that originally characterizing the potato. Supporting this argument, a comparison of the mean measured hydrogen and oxygen isotope ratios of French fries and baking potatoes found a significant difference between δ^2H values (unpaired t test; $t_{69} = 6.0$, $P < 0.0001$) but no difference between δ^{18}O values.

33.3.6 Milk Water Isotope Ratios Show Promise for Tracing the Origin of Dairy Products

Chesson et al. (2010a) recently presented a model to predict the isotope ratios of drinking water for cows based on the δ^2H and δ^{18}O values of water extracted from milk. The authors used the OLS regression line describing the relationship between the oxygen isotope ratios of paired cow drinking water and milk water samples as an approximation of a semi-mechanistic model accounting for the proportional contributions of drinking water and meteoric water to body water (i.e., milk water). The relationship is

$$\delta^{18}O_{milk} = 0.86 * \delta^{18}O_{we} + 1.1‰, \qquad (33.5)$$

where $\delta^{18}O_{milk}$ and $\delta^{18}O_{we}$ are the oxygen isotope ratios of water extracted from milk and water in the environment, respectively (Chesson et al. 2010a). The study then examined the predicted drinking water δ^{18}O values for a number of McDonald's milk samples purchased from different cities, which grouped into three distinct data clusters. Predicted water for the three groups of restaurant samples overlapped with known regions of high dairy cow concentration in the US. That study could not go further given the lack of information on the original source of the purchased restaurant samples.

The authors noted that the generally high total water flux (TWF) in dairy cows should drive body water stable isotope ratio values closer to those of

drinking water. This is because a high water input rate has the potential to reduce the impacts of metabolic water on body water isotope values. Thus milk water $\delta^{18}O$ values (which are equivalent to body water $\delta^{18}O$ values) should be closer to drinking water oxygen isotope ratios in dairy cows than non-lactating cows (e.g., beef cattle). With that assumption in mind, we modified (33.3) to predict the oxygen isotope ratio of milk water ($\delta^{18}O_{milk} = \delta^{18}O_{wb}$). Constrained by the lack of information in the literature, we made estimates of how lactating and non-lactating cows might differ. We increased the relative contribution of drinking water to body water from 0.62 to 0.90 to reflect a lactating condition and decreased the proportional contributions of O atoms in food and inhaled O_2 from 0.14 and 0.24, respectively, to 0.05 and 0.05, respectively.

Using cow drinking water ($\delta^{18}O_{we}$) values from the dairy farm collection of Chesson et al. (2010a), $\delta^{18}O_{wb}$ values were predicted for the dairy cows and then compared to the original $\delta^{18}O_{milk}$ values. The slope of the OLS regression line describing the relationship between drinking water and $\delta^{18}O_{milk}$ values ($m = 0.86$; data not shown) was not significantly different from the slope of the line describing the relationship between drinking water and predicted $\delta^{18}O_{wb}$ values ($m = 0.89$; data not shown). On the other hand, the *y*-intercept of the measured milk water line (1.1) was significantly different from the predicted body water line *y*-intercept (1.8; $F_{1,13} = 12.6$, $P < 0.01$).

It appears the regression-based model presented as (33.5) and the process-based model modified from (33.3) may represent equally valid methods for predicting cow drinking water from the oxygen stable isotope analysis of water extracted from milk. Using both model approaches, we predicted the $\delta^{18}O_{we}$ value for a sample of milk collected in Salt Lake City, UT and distributed by local dairy, Winder Farms (www.winderfarms.com). The $\delta^{18}O_{milk}$ value measured for the water extracted from the milk sample was −13.8‰. The predicted value for $\delta^{18}O_{we}$ using modified (33.3) was −17.5‰ and using (33.5) was −17.4‰. Because the two models predicted nearly identical cow drinking water values, we used only the value predicted using (33.5) to then generate a predicted region-of-origin for the cow(s) that produced the milk (Fig. 33.7), using a tap water isoscape for the continental USA (Bowen et al. 2007) and an error estimate of $3\sigma = 1.2$‰ (Chesson et al. 2010a).

The predicted regions-of-origin included the purported site of the dairy farm operation, West Valley City, UT. The predicted regions also included portions of several states north of Utah (Idaho, Montana, and Wyoming). While not normally considered centers of dairy milk production, northern Utah and states to the north are home to several regional milk production facilities. Those regions are characterized by relatively low measured tap water $\delta^{2}H$ and $\delta^{18}O$ values (Bowen et al. 2007) and thus all predicted regions shown in Fig. 33.7 would be consistent with predicted sources of the milk sold under the local Winder Farms label. Despite the fact stable isotope analysis alone is not enough to predict regions with a smaller margin of the error the possible source of the milk sample, the predicted regions are relatively local to the purchase location in Salt Lake City, UT.

33.3.7 Stable Isotope Analyses Reveal Several Patterns in the Modern American Diet

From this case study on the stable isotope analysis of fast food meals for questions of food tracking we have revealed several patterns in the modern American's diet. First, the carbon stable isotope analysis of beef and breads from McDonald's restaurants and supermarkets has shown the impact of a consumer's food choice. The $\delta^{13}C$ value of beef from a restaurant can be markedly different from that of beef purchased from a supermarket in the same town, with consequences for the carbon isotope ratio of dietary inputs incorporated by a consumer. Similarly, the prevalence of C_4-based sweeteners in breads available to fast food diners means the $\delta^{13}C$ value of the hamburger bun input is generally higher than the loaf bread input of a consumer eating bread from the store. Thus, "where you eat matters" when calculating the carbon isotope ratios of inputs in your diet.

Next, the hydrogen and oxygen stable isotope analysis of all components of the fast food meal – and the supermarket correlates – demonstrated the existence of significant isotopic, and thus geographic, variation in the foods available to consumers eating out or dining in at mealtimes. However, our current ability to interpret these isotope ratios varies. For example,

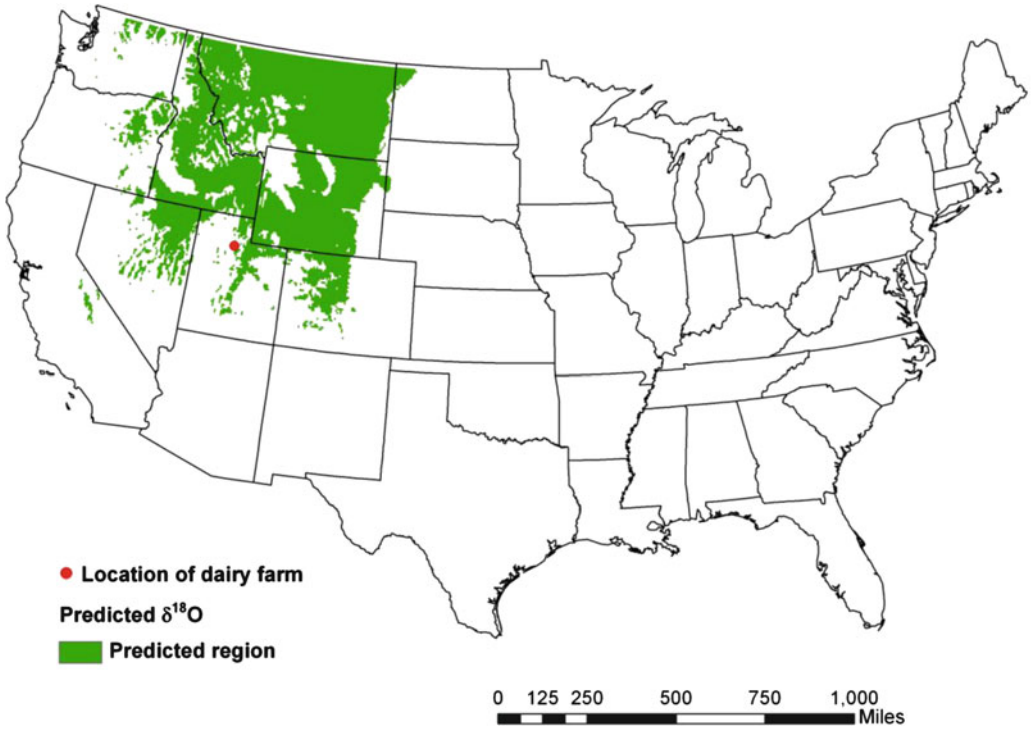

Fig. 33.7 The predicted regions-of-origin for cows that could have produced the Winder Farms milk collected in Salt Lake City, UT. Predictions were generated using the Chesson et al. (2010b) model, (33.5) in the text. The location of the purported dairy farm source is shown by the red point. Predicted regions were generated using a tap water oxygen isoscape for the USA (Bowen et al. 2007) and include an error estimate of 1.2‰ around the predicted $\delta^{18}O$ dairy cow drinking water value

the analysis of beef and milk water for δ^2H and $\delta^{18}O$ values may be very useful for food tracing applications and possible origin assignment of individual samples, as demonstrated for samples of beef and milk collected local to Salt Lake City, UT. The interpretation of bread and potato δ^2H and $\delta^{18}O$ values was not as straightforward but could prove useful in the future as we understand more about the impacts between origin and hydrogen and oxygen isotope ratios of those foods.

Finally, the stable isotope analysis of four elements (H, C, N, and O) provides investigators complimentary information on both the source and production of food. Consider the beef samples collected in this survey. Carbon isotope ratios revealed the different diets of cattle used by fast food restaurants and supermarkets. Despite the dietary differences, the trophic levels of all cattle were similar, as revealed by nitrogen isotope analysis. Contrary to trends in agricultural consolidation, the measured δ^2H and $\delta^{18}O$ values of beef revealed that cattle come from many isotopic regions. These individual pieces of information together show that an aggregated analysis of stable isotope ratio food values can be useful for tracing both the production and origin of food as it travels to a consumer's plate.

33.4 Future Directions

In the second volume of the *Handbook of Environmental Isotope Geochemistry* Hillare-Marcel predicted that stable isotope chemistry had a promising future in the field of food tracing (Hillaire-Marcel 1986). Here two decades later in the *Handbook*'s fourth volume we have highlighted an application of stable isotope analysis for tracing the origins of and production methods for a select group of food items in arguably the most consumed meal in the US today: the fast food restaurant meal. Isotope applications in foods are now approached as including both process and region-of-origin information. This is an active area of international research and one likely to significantly advance in the next two decades as biochemistry,

geography, economics, and stable isotopes find more common ground.

The stable isotope ratios of food items are ultimately linked to two processes: natural environmental variation in stable isotope ratios and processes associated with animal and plant physiology. Pioneering stable isotope research focused on the environmental patterns associated with meteoric water hydrogen and oxygen stable isotopes (Craig 1961) and atmospheric $^{13}CO_2$ (Keeling 1961) laid the foundation for today's advances. Theoretical advances in understanding carbon isotopes (Ehleringer et al. 1993), hydrogen and oxygen in carbohydrates (Roden et al. 2000), and in animal proteins (Ehleringer et al. 2008), to name a few, have laid the foundation for interpreting isotope ratio variations among foods. The exciting area of *isoscapes* has now emerged, advancing our understanding of geospatial isotope patterns (West et al. 2010b).

In order to scale environmental patterns in atmospheric, plant, and water isotopes to animal-derived food items, we believe more fundamental mechanistic research is needed before we can fully understand the isotope physiology of common domesticated animals. Key among the unknowns are relationships related to turnover of protein, carbohydrates, and fats, especially in growing animals and animals shifting diets in the last phases of animal production (e.g., feedlots). This need includes controlled diet studies for food-providing animals like that of Bahar et al. (2005) with beef cattle. It also includes an understanding of between-tissue variation in animals (Bahar et al. 2009) as well as seasonal variation caused by variable environmental conditions or feed changes (Boner and Förstel 2004). For example, consider the prediction of dairy cow drinking water from the measured $\delta^{18}O$ values of milk. Predictions would likely be much improved if we understood more fully the physiology of the cow and how respiration rates, water flux, and the isotopic composition of diet affected final milk isotope ratios.

Second, food traceability should be critically tested to evaluate its potential relative to other technologies. While discussions of food authenticity and potential regulations are discussed globally today verification approaches, such as stable isotope analyses, have not yet been approached with the depth and rigor needed to apply to a global food network. Examples such as the European TRACE Project (www.trace.eu.org) provide a framework for future studies.

Finally, stable isotope analyses are part of a larger, integrated analytical approach to food safety, food authenticity, and food traceability. Stable isotope analyses can complement other approaches, including trace element analysis (Heaton et al. 2008), trace metal analysis (Anderson et al. 1999), radioactive element analysis (Pillonel et al. 2003), and compound characterization [e.g., gas chromatography-mass spectrometry or high pressure liquid chromatography (Földházi 1994; Aichholz and Lorbeer 1999; Cotte et al. 2004)].

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