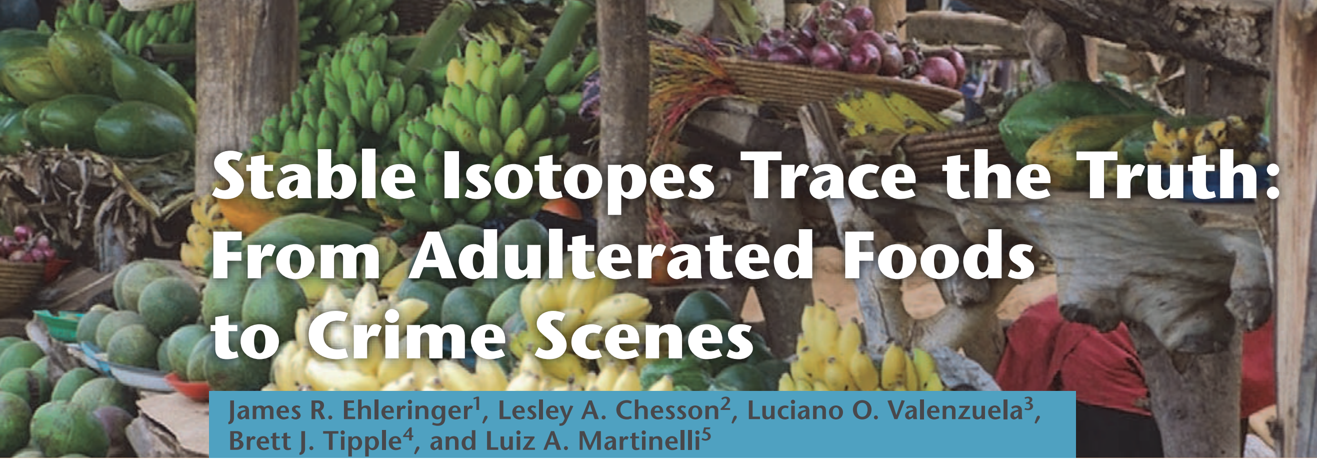

Stable Isotopes Trace the Truth: From Adulterated Foods to Crime Scenes

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Stable isotopes are valuable biogeochemical markers for solving problems faced by society today, such as distinguishing authentic from adulterated foods and beverages or tracing the origins of illicit drugs. Hydrogen and oxygen isotopes in water exhibit distinct continental patterns (isoscapes), which provide useful region-of-origin information. We ourselves reflect the stable isotope ratios of the water we drink and the food we eat: our hair records any isotopic changes to our diets, which can often be related to location. This latter aspect can be of interest to law enforcement in determining the origins and travel histories of unidentified murder victims.

Keywords: biogeochemistry, stable isotope, food, law enforcement, humans

INTRODUCTION

Changes in stable isotope abundances in materials are nature's recorder of many aspects of climatology, geology, hydrology, biology, and anthropology. Whether through successive layers in lake sediments, individual segments along a human hair, or annual layers in an ice core, a sequence of isotope ratios, once deciphered, leads to a quantitative understanding of patterns, processes, and history (Bowen 2010). The results of stable isotope fractionation appear as differences in the relative abundances of heavy-to-light isotopes of an element within the constituent molecules. For society today, the analysis of stable isotopes serves as a valuable, and diagnostic, biogeochemical tool when applied to water, food, human diets, travel movement patterns, and manufacturing methods (Fig. 1). Stable isotope analyses have been applied in medicine (O'Brien 2015) and as a tool to trace pollution (Elsner et al. 2012).

The subtle, but significant, changes in the hydrogen, carbon, nitrogen, oxygen, and sulfur stable isotope compositions of the water we drink and the foods we eat become reflected in our hair (Valenzuela et al. 2012; Nechlich 2015), which can thereby record geographic and dietary information (Ehleringer et al. 2008; Thompson et al. 2014; Chesson et al. 2014). Human hair becomes a linear recorder of our diets and geographic origins; this leads to applications that include the investigation of historical practices, modern dietary patterns, and even forensic cases (Ehleringer et al. 2008; Thompson et al. 2014). Furthermore, even slight differences in how a chemical is manufactured or the substitution of ingredients from one source of raw materials to another can be recorded in the isotope ratios of organic molecules of the product itself. And this can lead to useful insights into the origins, authenticity, and verification of a food product (Chesson et al. 2014).

HOW AUTHENTIC IS YOUR FOOD?

"Adulteration" is the illegal substitution of one ingredient or product for another, often driven by a desire for increased profits. In many aspects of food security, stable isotope biogeochemistry has become a critical tool in determining food and beverage authenticity (Simon et al. 2005). Here, the large differences in carbon isotope ratios between the two major types of photosynthesis in plants (C3 and C4: plant groups named for either the 3-carbon or the 4-carbon molecule present when they initially convert atmospheric CO_2 to organic carbon) become a diagnostic tool (Ehleringer and Sandquist 2014). A common adulteration approach is adding sugars from corn or sugar cane (both C4 plants) into either honey or maple syrup (both from C3 plants) to cheaply increase product volume (Guler et al. 2014). But because honey and maple syrup come from C3 plants and have carbon isotope ratios of –28‰ to –23‰, the addition of corn or sugar cane, with carbon isotope ratios of –14‰ to –10‰, results in a significant and detectable change in the expected carbon isotope ratio of the honey or syrup. The attempted importation of adulterated foreign honey to the United States is pervasive, which has led to federal guidelines that describe the acceptable ranges of carbon isotope values and allow authorities to differentiate between authentic and adulterated honeys.

Carbon isotopes can also be used to distinguish between other types of authentic and adulterated foods and beverages, such as beer (Brooks et al. 2002) and the sources of caffeine (Zhang et al. 2012). It seems that when there is an economic opportunity, producers will augment beverages with corn and cane sugars to extend their products to make them sweeter than they would be naturally or else to substitute cheaper synthetic ingredients for the more expensive naturally occurring compounds.

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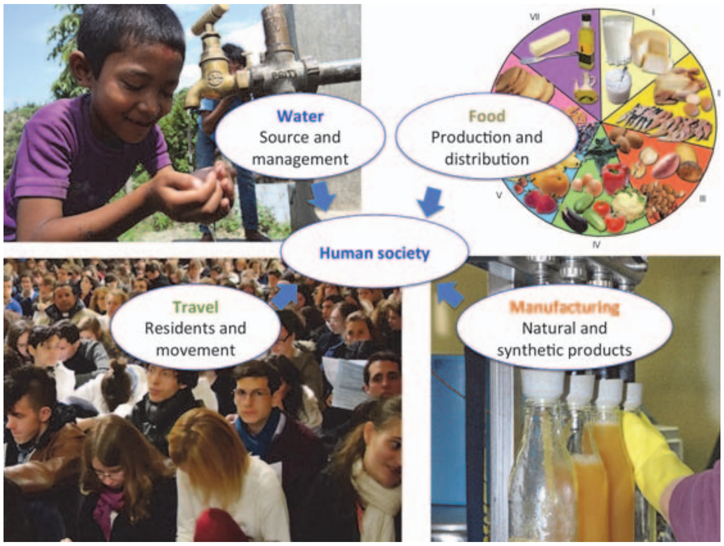

FIGURE 1 Stable isotope analyses are applied to many aspects of human society. These range from tracing the food and water we drink, verifying the claimed growing regions where our food is produced, recording via our hair where we have been, and checking the authenticity of the products we use. PHOTO CREDIT: COMMONS.WIKIMEDIA.ORG

While carbon isotopes have been used to detect food adulteration, nitrogen isotopes are commonly analyzed to distinguish organic from nonorganic fertilizers used in the production of vegetable crops (Chesson et al. 2014). Vegetables raised with synthetic fertilizers often have nitrogen isotope ratios of between +3‰ and +5‰; plants raised on organic fertilizers are expected to have higher nitrogen isotope ratios between +10‰ and +20‰. A popular undergraduate student exercise is to visit stores and local farmer's markets to see which of the vegetables advertised as organically grown really have nitrogen isotope ratios consistent with those expected from plants grown with organic fertilizers.

For each of the food items mentioned above, the carbon and nitrogen isotope ratios are faithful recorders of food history and serve as a more quantitative means of verifying authenticity than simply using bar codes or package labels. Protecting consumers from fraud has always been a challenge. However, stable isotopes provide government and industry with a means by which to identify fraud.

WAS TONIGHT'S DINNER GROWN LOCALLY?

Eating locally grown foods is an increasing trend in Western societies, and consumers are often willing to pay more for local produce. But how can one be sure that a vegetable, fruit, or animal meat was produced locally, as opposed to having been transported in from afar?

Geographic Origins Revealed through Isoscapes

Think of a continental landscape where the different geographical regions are characterized by the stable isotope ratio of the geology, hydrology, or biology of the region. This is the isotopic landscape, or "isoscape" context where the hydrogen and oxygen isotope ratios of local water act as a tool to distinguish between different geographical regions (Bowen 2010). How does this work?

Heavier isotopes of water (^{2}H, ^{18}O) are less stable in the vapor form and therefore more likely to precipitate from clouds than the lighter isotopes (^{1}H, ^{16}O). This leads to a spatial gradient in water isotope ratios. As most drinking waters are largely derived from precipitation or recently recharged groundwaters, we find that there are pronounced spatial patterns in the stable isotopes of tap waters across a geographic region based on this rain-out effect. For example, cold, winter precipitation in the United States largely originates as storms from the eastern Pacific Ocean while warm, summer precipitation also draws in moisture from the Gulf of Mexico. As a result, we see distinct tap water isotope-ratio gradients defining geographic bands or zones (FIG. 2A), allowing us to isotopically distinguish between various regions of the United States. The isotope fractionation during precipitation affects both hydrogen and oxygen isotopes similarly, so either could be used to generate water isoscapes.

Our Foods Record Regional Isotope Biogeochemistry

The hydrogen and oxygen isotope ratios of local waters enter our bodies in many ways: tap water, bottled water, milk, fruit or vegetable juice, bottled or canned alcoholic and nonalcoholic drinks, and brewed beverages (Chesson et al. 2010a,b). The spatial gradients in water isotope values provide a quantitative tool and a type of geographic mapping system for determining if a beverage is of local origin or whether it was imported from a separate and isotopically distinct region. For instance, is the wine at dinner really from the Napa Valley of California or did it originate from another region or country? Is the bottled water served at lunch local, regional, or imported?

The hydrogen and oxygen isotopes of water serve as the raw material used to build proteins within our hair, nails, muscles, bones, and teeth and also within the "flesh foods" we eat daily. As a consequence, the hydrogen and oxygen isotope ratios of beef, chicken, eggs, freshwater fish, and pork reflect the local isotope ratios of water for the region in which these protein sources were grown (FIG. 2B). A survey of milk and meat sources in restaurants across the United States revealed a tendency of local restaurants to serve regionally produced foods. In contrast, the national chain restaurants tended to provide foods not of local origin and in some cases foods were transported large distances or even imported (Chesson et al. 2010a,b; Martinelli et al 2011).

Stable isotope analyses provide consumers with a tool that allows them to make a choice: foods of local origin or foods transported in from another part of the country (Chesson et al. 2009). Consider, for example, hamburgers that were purchased at different restaurants in Salt Lake City, Utah (location shown as black dot in FIG. 3). When these hamburgers were analyzed, the protein's hydrogen and oxygen isotope ratios fell into two distinct groupings. FIGURE 3 shows possible cattle-raising regions for hamburgers purchased from local restaurants as well as national chain restaurants. Local restaurants tended to have protein consistent with being regionally raised, whereas hamburgers from national chain restaurants tended to have isotope ratios expected for cows that had been raised farther away. FIGURE 3 shows all of those regions that were isotopically consistent with the observations. Because beef cattle are actually raised in only a subset of these possible locations, then overlaying those locations would further reduce the scope of possible geographic origins of beef sold in the different restaurants.

Stable isotope analyses also serve as valuable diagnostic tools for characterizing foods that are from protected domains of origin, which include many of the more desirable wines, cheeses, hams, and olive oils.

YOU ARE WHAT YOU EAT AND DRINK

Hair serves as a wonderful linear recorder of human diet (Thompson et al. 2014). Growing at a rate of about 1 cm per month, hair provides a continual record of diet and drinking water behavior even if we want to deny specific eating habits or movements. So, your mother was right, "You are what you eat and drink."

The carbon and nitrogen isotope ratios found in all animals reflect their food resources. Because foods are consumed as the macromolecules of protein, fat, and carbohydrate, there is little carbon isotope fractionation between the food resource and the tissues produced by animals consuming those foods. This holds true even when we consider animals along the entire length of a food chain—including humans, at the top of the food chain.

However, for nitrogen isotopes there is approximately a 3‰ fractionation between animals and their food resources at each trophic level (i.e. their position in the food chain), reflecting fractionation events that occur when animals excrete nitrogen (as urine). Thus, there are increasing differences in the nitrogen isotope ratios of animals along a food chain, with animals at the top of the chain having the highest nitrogen isotope ratios (Post 2002). This insight allows us to study ourselves (humans) and our diets. Within humans, we see similar patterns: vegans, vegetarians, and omnivores can be distinguished through characterization of nitrogen isotope ratios of their hair (Thompson et al. 2014). When we use isotope analyses to study broad patterns between Europeans and North Americans, we find absolutely no differences in the population-level distributions of nitrogen isotope ratios (Valenzuela et al. 2012). Most individuals appear to be omnivores, and there is no difference in the occurrence of vegans or vegetarians in the populations of the two continents. On average, Europeans and North Americans eat at the same trophic level.

Dietary Differences Between Continents

What has been remarkable to discover is that even with the emergence of global supermarket diets, individuals from different continents often show very distinct and different carbon and sulfur isotope ratios (Martinelli et al. 2011; Valenzuela et al. 2012). Sulfur isotopes appear to reflect the relative contributions of marine versus nonmarine proteins (Nechlich 2015). The carbon isotope ratios of proteins from terrestrial sources reflect animals that were raised on either C3 or C4 plants. Because it is common in North America for beef, eggs, and pork to come from concentrated animal feeding operations where corn is the major food source, it is not surprising that the carbon isotopes of North American protein foods reflect a strong C4 carbon isotope ratio (Jahren and Kraft 2008). In contrast, European animals are more often field raised where the predominant forages are C3 grasses.

The difference in carbon isotope ratios of dietary protein is so large that there is virtually no overlap in the carbon isotope ratios of hair from individuals living in Europe versus those in North America (Valenzuela et al. 2012). Thus, we can use this isotopic separation to distinguish an international visitor from a local resident.

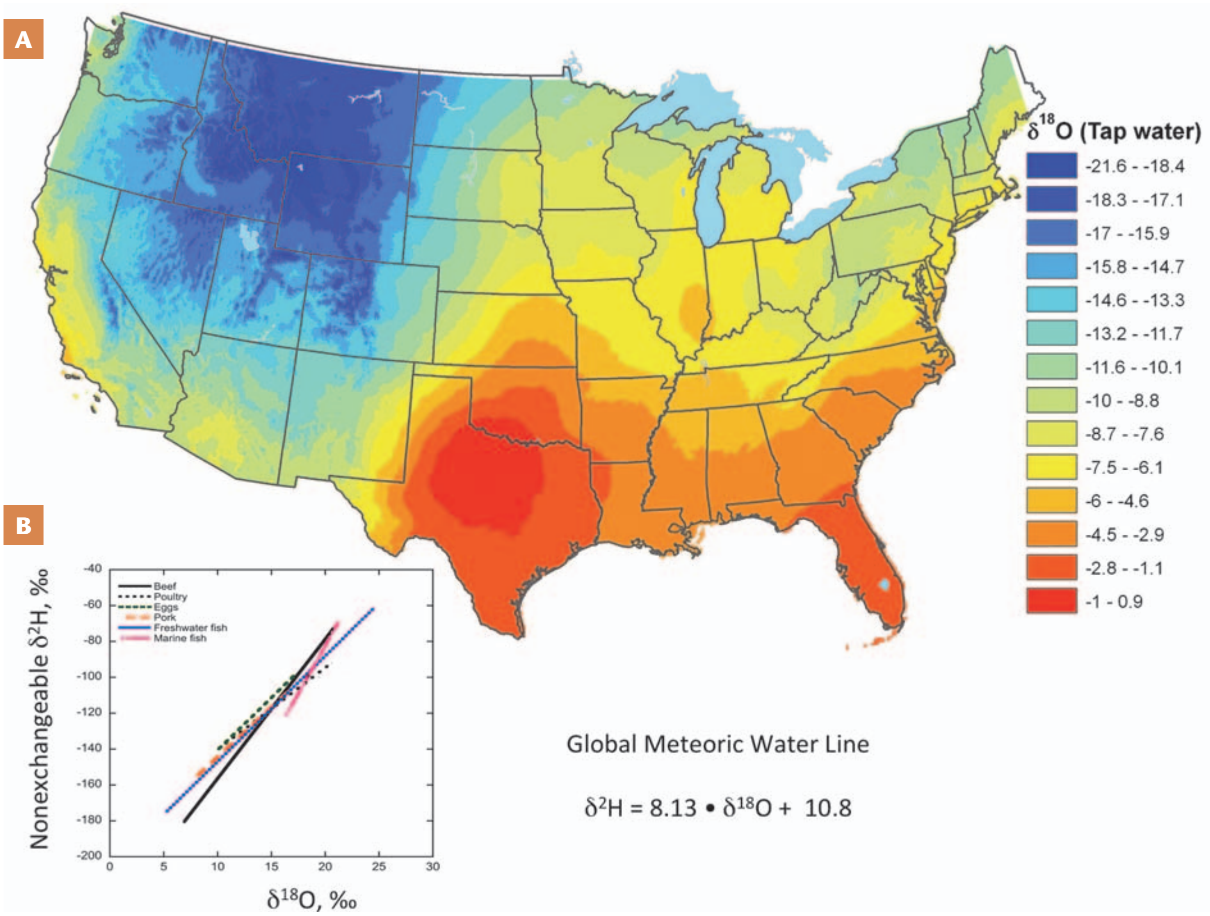

Figure 2 (**A**) An "isoscape," or geographical depiction, of oxygen isotope ratios in the drinking waters across the United States (Adapted from Bowen et al. 2007). The equation for the Global Meteoric Water Line describes the relationship between hydrogen and oxygen isotope ratios in precipitation. (**B**) A plot of the stable isotopes of nonexchangeable H and O atoms in various protein sources common in our diets (Adapted from Chesson et al. 2011)

CASES SOLVED WITH ISOTOPES

Stable isotope analysis is now making its way into the US judicial system as an investigative tool. As such, it will receive the same level of scrutiny as any other analytical approach applied to legal matters (Ehleringer and Matheson 2010). We next explore some recent cases along these lines.

Graves Speak of the Past

The dead can speak from the grave using the language of stable isotopes. Corpses and skeletons from the past can provide key information on their once-living dietary practices and travel histories. A well-known example is that of Ötzi, the "Iceman" discovered by hikers in the Ötzal Alps (on the border between Austria and Italy) in 1991. Ötzi is one of the best-preserved natural mummies in the world, and many of his remains (hair, teeth, bones) have undergone stable isotope analysis.

The carbon and nitrogen isotope ratios of Ötzi's hair suggest he ate a vegetarian diet of primarily C3 grains in the weeks before death (Macko et al. 1999). The oxygen isotopes of Ötzi's teeth, which were incorporated into the enamel during initial formation, suggest that the Iceman spent his childhood approximately 60 km south of the site of his discovery (Müller et al. 2003). Isotopes in adult bones are incorporated at a later stage of life than in teeth. Differences between the isotopic composition of his teeth and bones indicate Ötzi was migratory during adulthood. The analysis of strontium was also useful in constraining his origins because strontium isotopes vary with local geology. Strontium is taken up passively and without fractionation as part of drinking water and in the food eaten, so strontium isotopes in biological tissues reflect the local bedrock. Thus, strontium isotopes become a second marker for geographic location.

Another example of stable isotopes as a forensic tool involves a man well-known before death: King Richard III of England. Killed in battle in 1485, his body was hastily buried without ceremony, the burial site remaining lost for more than 500 years. It was not until 2012 that his remains were recovered during an excavation to construct a car park in Leicester (UK). Analysis of residual DNA confirmed the identity of Richard III, but it was stable isotope analyses that revealed details of his lifestyle (Lamb et al. 2014). Enriched nitrogen isotopes ratios of his bones and enriched oxygen isotope ratios of his teeth have been interpreted as indicating that his diet was richer later in life than in childhood, reflecting consumption of more meat and wine as an adult.

Bones and teeth also provide dietary information through carbon isotopes. As described previously, a North American diet is heavily reliant on C4 plants (corn, sugar cane); in contrast, a Southeast Asian diet typically includes more reliance on C3 plants. As war-zone human remains are occasionally recovered in Vietnam, the bone tissues are first analyzed isotopically to determine their carbon isotope ratios. In these cases, differences in carbon isotope ratios in bone tissues of remains from these regions are applied to determine if a set of remains are more likely to be from a US serviceperson or an indigenous local (Bartelink et al. 2014).

Sequential stable isotope analysis of hair can provide more information than the "snapshots" in time provided by measurements of teeth and bones. Because hair acts like a linear tape recorder as it grows, it is possible to construct chronological histories for individuals that span weeks to months, depending on lengths of the hair strands. For instance, Incan civilization children from Peru, who were to be sacrificed for religious purposes, were first fed rich diets in an effort to provide an offering that would please

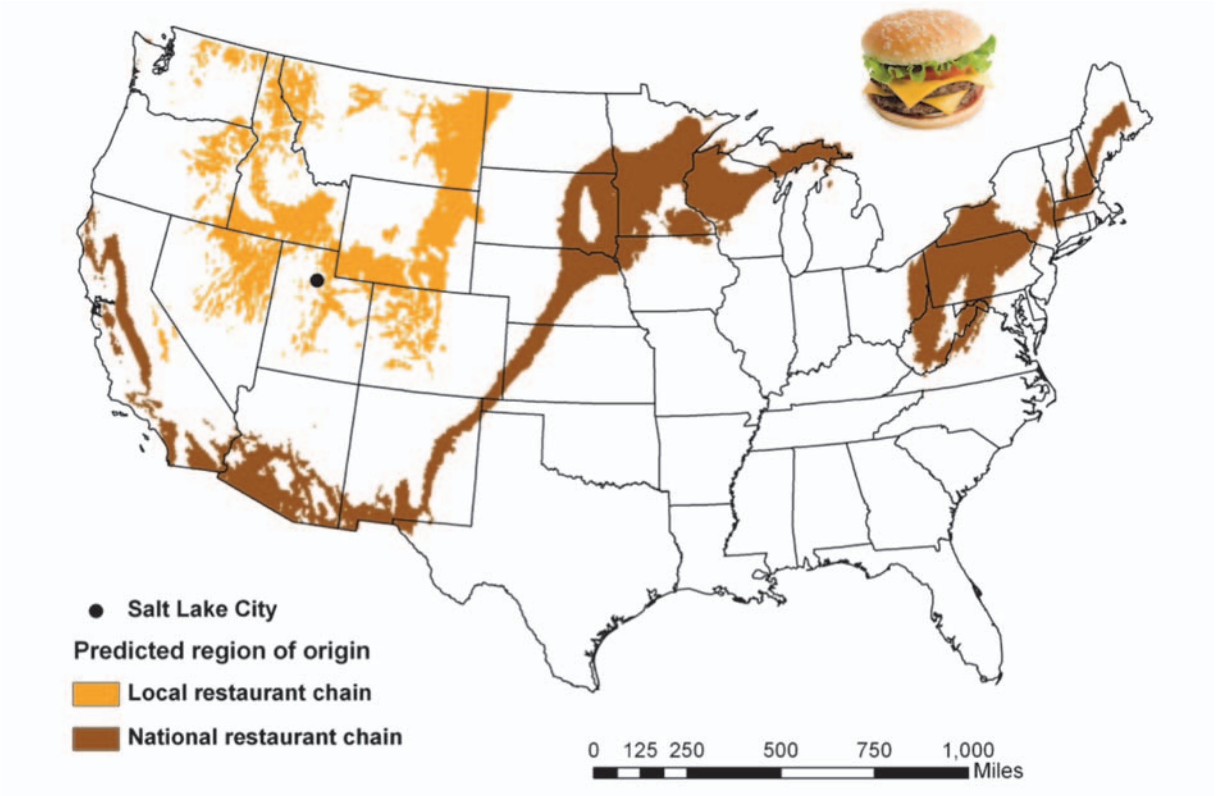

FIGURE 3 Possible geographic regions in which the beef in hamburgers from a local restaurant and from a national-chain restaurant in Salt Lake City (Utah) could have been raised. The geographic origin of the meat is determined from the isoscape shown in FIGURE 2 using the relationship between the oxygen isotope ratios of water and beef. LA Chesson (unpublished data).

the gods. Carbon isotope analyses of hair from sacrificed and mummified children show that in the year preceding their sacrifice, at the highest peaks of the south Central Andes, these children went from diets with limited corn to a diet that was rich in corn (Wilson et al. 2007).

Unraveling Other Mysteries and Crimes

Applying stable isotope analyses to assist law enforcement on cases with unidentified murder victims represents a contribution that has gained wider attention from biogeochemical sciences in recent years. The American crime drama *CSI: Crime Scene Investigation* is a popular television series, and millions of viewers are fascinated by the power of the show's analytical methods to solve mysteries. Yet few consider that the acronym CSI could just as easily be "**C**ases **S**olved with **I**sotopes."

Hair is a common piece of crime-scene evidence, and stable isotope analytical methods have applications in identifying murder victims and combating human trafficking and illegal immigration because travel histories for individuals can be established (Ehleringer et al. 2010). As noted earlier, the oxygen isotope ratios of hair act as a linear recorder of drinking waters and so becomes a recorder of an individual's geographical history or travel movements.

Consider the case of "Saltair Sally", initially an unidentified female buried near the freeway in the desert west of Salt Lake City, Utah (Fig. 4). The oxygen isotope ratios of her hair suggested that Saltair Sally was a traveler. Yet this same isotope record showed isotope values that were consistent with her being a resident of Salt Lake City (Region 1 and blue dot in Fig. 4). Despite her apparent residency in the Salt Lake City region, no one came forward to identify her as a missing person. She was simply unidentified for many years. Where to go next in this investigation? New clues were needed. When stable isotope analyses were applied, two other isoscape regions were identified as possible locations where Saltair Sally lived or had visited prior to her death (Regions 2 and 3 of Fig. 4). Perhaps Saltair Sally had traveled to these regions to visit family members. Working on this hunch, Detective Todd Park (Salt Lake Unified Police Department) sent inquires to the major cities within these isoscape regions. In August 2012, he received a positive response[1]. An individual last seen by her family in the Seattle area a year before Saltair Sally's discovery matched the timeline reconstruction shown in Figure 4 (Seattle is represented by the yellow dot). Subsequent genetic analyses confirmed that Saltair Sally was no longer an unidentified decedent, but was actually Nikole Bakoles. Stable isotope analyses confirmed that she visited her mother about a year before her death (Region 2 in Fig. 4). Thus, stable isotopes had proved useful in assisting with what is now an active homicide investigation. The literature is filling with additional examples of how stable isotopes analyses are being applied to investigations of other unidentified persons' cases.

A Tool Applied to Sourcing Drugs

The source of illicit drugs, such as heroin and cocaine, can also be determined, or at least ruled out, using stable isotope ratio analyses. Unique combinations of carbon and nitrogen isotope ratios provide regional information of benefit to federal agents, allowing the agents to determine from which region illicit drugs originated (Ehleringer et al. 2000). Within the US Drug Enforcement Administration, stable isotope analyses are now used to identify cocaine that has originated from various regions in Central and South America. One particularly striking and unusual example for this application of stable isotopes is not of cocaine but instead involved heroin.

1 www.sltrib.com/sltrib/news/54649101-78/bakoles-saltair-detectives-police.html

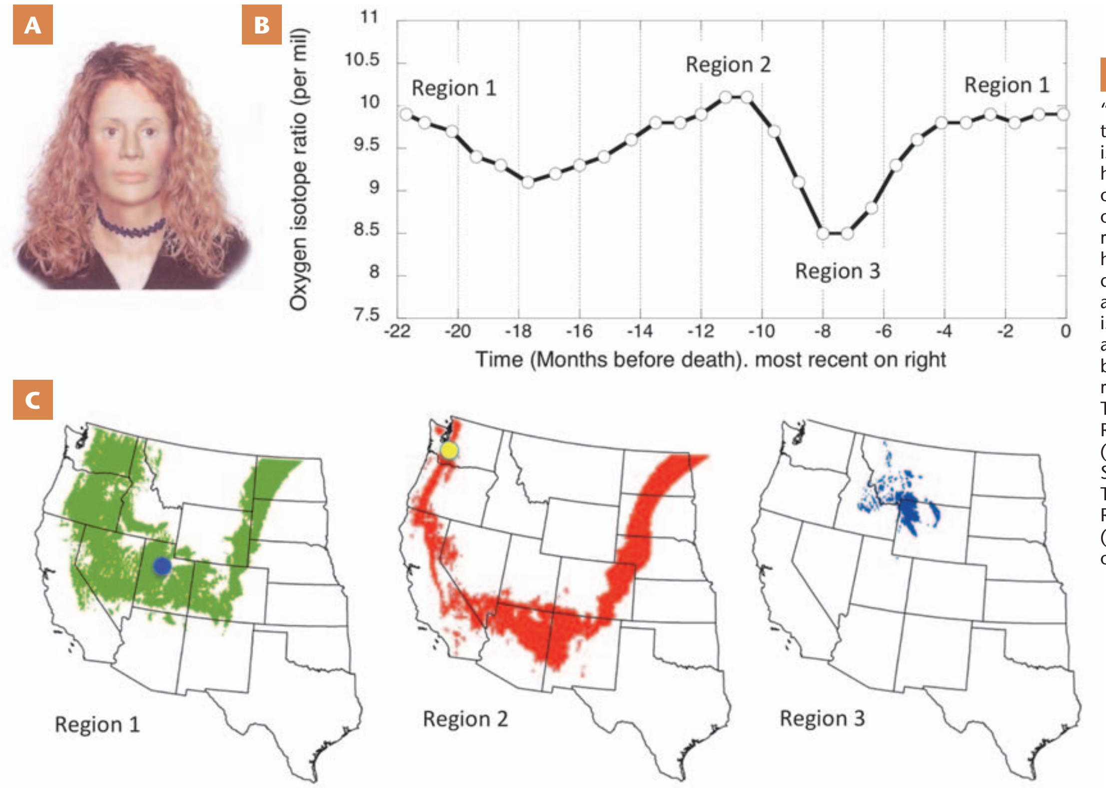

Figure 4 (**A**) An artist's sketch of "Saltair Sally". (**B**) A plot of the changes in the oxygen isotope ratio of Saltair Sally's hair over the last 22 months of her life. (**C**) The isoscapes of three geographical regions where Saltair Sally had traveled prior to her death (Remien et al. 2014) are based on the tap water isoscape shown in FIGURE 2 and the relationship between the oxygen isotope ratios of tap water and hair. The blue spot on isoscape Region 1 is Salt Lake City (Utah) from where Saltair Sally's body was recovered. The yellow spot on isoscape Region 2 is Seattle (Washington) where she was originally from.

In 2003, a large amount of heroin was seized from the North Korean cargo ship, the *Pong Su* (Casale et al. 2006). Australian officials confiscated heroin from the cargo ship as well as from off-load sites on Australian soil. When these heroin samples were isotopically characterized, they were all found to have stable isotope ratios significantly different from any of the known poppy growing regions used in heroin production (i.e. Mexico, South America, Southeast Asia, Southwest Asia). Both the combination of stable isotopes and alkaloid compositions of the heroin were unlike any samples seized before or of any authentic samples previously analyzed by Australian or United States investigators. The origin(s) of the *Pong Su* heroin remains a mystery to this day, but several regions could be ruled out on the basis of the isotopic analyses. Authorities are currently awaiting heroin samples collected from a poppy-growing region in Asia where the isotope ratios and alkaloid profiles are similar to the *Pong Su* samples.

ON THE FUTURE BENEFITS OF STABLE ISOTOPE BIOGEOCHEMISTRY TO SOCIETY

As we move into an era of increased capacities to analyze specific compounds, human metabolism, and spatial patterns across the globe, the possibilities for stable isotope analyses to benefit society seem boundless. We are already seeing these contributions in relation to health, understanding our environment, and clarifying matters on the food we eat. As stable isotope analyses are applied to more situations, society will continue to benefit from this valuable biogeochemical tool.

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