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APPLICATION OF STABLE ISOTOPE FORENSICS FOR PREDICTING REGION OF ORIGIN OF HUMAN REMAINS FROM PAST WARS AND CONFLICTS

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The application of stable isotope analysis has provided novel approaches for provenancing unidentified human remains from forensic contexts. Stable isotope ratios measured in human tissues provide a record of the foods consumed during life as well as the geographic location where drinking water or food was obtained. This study begins with an overview of the application of stable isotope analysis for provenancing human remains, followed by three cases that illustrate how chemical signatures in bone reflect a probable region of origin. Using stable carbon and nitrogen isotopes of human bone, we test whether human skeletal remains recovered by the Joint POW/MIA Accounting Command-Central Identification Laboratory (JPAC-CIL, or CIL) reflect a geographic origin within North America or Asia. Stable carbon and nitrogen isotopes of human bone collagen and stable carbon isotopes of bone apatite reflect consumption of food resources that are expected to vary between world regions due to cultural dietary differences. Based on the isotopic differences, a testable hypothesis of geographic origin can be applied, determining if the remains are more likely of a U.S. service person or of an indigenous local. We believe that this approach can provide useful information for narrowing search parameters in unidentified persons cases; can contribute to human rights cases where an unknown individual is thought to originate from a different geographic area; and, in human remains cases of unknown geographic provenience, can determine whether a person is local or nonlocal. [forensic anthropology, stable isotope analysis, provenancing human remains]

INTRODUCTION

The Joint POW/MIA Accounting Command-Central Identification Laboratory (JPAC-CIL, or CIL) is charged with the mission of achieving the fullest possible accounting of all Americans missing as a result of the nation's past conflicts. There are still approximately

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83,000 missing service members from past wars and conflicts (World War II: 74,000; Korean War: 7,900; and Vietnam War: 1,650; Defense Prisoner of War Missing Personnel Office 2014). JPAC operates under the Department of Defense, and is composed of approximately four hundred seventy-five personnel, the majority of whom are uniformed military members. The CIL is a specialized forensic laboratory, employing approximately forty professional anthropologists, odontologists, and historians. JPAC-CIL operates two facilities; its main base of operations is located on the Joint Base Pearl Harbor-Hickam in Honolulu, Hawaii, and a satellite facility is located in Omaha, Nebraska. Frequently, the CIL participates in humanitarian missions around the world when the staff's specialties are requested, particularly in instances of personal identification, recovery, and mass disasters. Further, the CIL trains members of the worldwide community in forensic anthropology, forensic archaeology, and odontology at the Hawaii facility.

The JPAC-CIL is currently the only accredited forensic anthropology laboratory in the world, with accreditation through the American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB). Members of the CIL have also participated in recovery and identifications from 9/11 (Pentagon), Korean Air Lines Flight 801 in 1997, the Thailand Tsunami in 2005, and more recently, Agni Air Flight 101 in Tibet in 2010. Along with these duties, JPAC anthropologists and odontologists are frequently asked to participate in local and national forensic identification cases for police departments, medical examiners, and court officials. Further, the CIL continues to develop and utilize new methods that may assist in the personal identification of missing and unidentified service personnel from past wars and conflict situations.

RESEARCH OBJECTIVES

This study uses stable isotope analysis for provenancing human remains obtained by the JPAC-CIL. Human remains of missing service personnel are recovered worldwide from a variety of wartime and conflict situations by JPAC recovery teams, consisting of approximately twelve to fourteen personnel (civilian and military), with a forensic anthropologist assigned as recovery leader (Holland et al. 2008). These recovery missions frequently target known aircraft crash sites, as well as incidents where intelligence has provided possible burial locations of fallen service members. Aside from these formal missions, human remains are often unilaterally turned over to the JPAC-CIL by foreign governments or through informal channels, often with little or no provenience information. This has created situations where significant investment is undertaken to identify remains that may not be of U.S. service members, including anthropological, odontological, and mitochondrial DNA (mtDNA) analysis. Methodological approaches, such as stable isotope analysis, offer a potential new tool for screening human remains cases that may be of U.S. versus non-U.S. origin; in addition, these methodological approaches could provide more specific information on region of origin. Accurate determination of origin significantly reduces the time, effort, and cost of analyzing remains that are not of U.S. service persons, and thus not within the purview of the JPAC-CIL's mission. Previous research by Regan (2006) has demonstrated significant isotopic differences in tooth

enamel bioapatite between U.S. Americans and East Asians, indicating this method has enormous potential for CIL casework. More recently, stable carbon and oxygen isotopes of enamel bioapatite were used to identify a single tooth fragment as being of U.S. origin from a Vietnam War–era plane crash site in Laos (Holland et al. 2012).

Stable isotope analysis has proven to be a useful tool for provenancing unidentified human remains from a variety of forensic contexts, including cases from local jurisdictions (Chesson et al. 2014; Ehleringer et al. 2010; Kennedy et al. 2011; Meier-Augenstein 2007, 2010; Meier-Augenstein and Fraser 2008; Prince et al., 2014; Rauch et al. 2007), past wars and conflicts (Bartelink et al. 2014a; Beard and Johnson 2000; Holland et al. 2012; Regan 2006), deaths along the U.S.–Mexico border (Juarez 2008), and mass graves from civil wars (Posey et al. 2010). Stable isotope ratios measured in human tissues (e.g., bone, teeth, hair, and nails) provide a record of an individual's diet and migration history, thus providing a means to potentially trace the origins of unidentified individuals (see reviews in Bartelink et al. 2014b and Meier-Augenstein 2007, 2010). Unlike incremental tissues such as teeth, hair, and nails, bone tissue is continuously remodeled during life, and thus represents an average of diet consumed over several years (Hedges et al. 2007; Manolagas 2000). Thus, stable isotopes of bone collagen and bioapatite will be most useful for identifying a traveler or recent arrival who died in a foreign place.

Stable carbon and nitrogen isotopes of collagen and stable carbon isotopes of bioapatite reflect food consumption patterns, which often vary between geographic regions due to cultural dietary differences. Stable oxygen isotopes of bone and tooth bioapatite, in contrast, vary between regions due to environmental factors (e.g., aridity, elevation, and distance from large bodies of water) that influence the isotopic composition of drinking waters. In this study, we focus specifically on stable carbon and nitrogen isotope analysis of human bone from three individuals as a tool to potentially differentiate U.S. Americans from Southeast Asians. More specifically, we predict that stable carbon isotope values of Southeast Asians will be consistent with consumption of a strictly C_3-based diet (e.g., rice), whereas U.S. Americans will be consistent with greater consumption of a mixed C_3/C_4 diet due to higher consumption of foods containing corn and sugar products. Future research on these samples will focus on stable oxygen isotopes of bioapatite to further aid in predicting region of origin.

STABLE ISOTOPE ANALYSIS

Stable isotopes are atoms of an element that have the same number of protons but differ in the number of neutrons and thus have varying masses (Hoefs 2009). As stable forms of atoms, they do not undergo radioactive decay, and thus record in vivo chemical signatures of organisms. In general, the lighter isotope of an element is the common isotope form, whereas the heavier isotope is the rare isotope form. In the case of stable isotopes such as hydrogen (H), carbon (C), nitrogen (N), oxygen (O), and sulfur (S), the lighter form of the isotope comprises between 95.0 and 99.8 percent of natural abundance, and the heavier form comprises between 1 and 5 percent of natural abundance (Fry 2006). Due to the small differences in atomic mass, the stable isotopes of an element will react at slightly different rates in chemical reactions. In cases where starting materials are not completely

consumed in chemical reactions, the different rates of reaction for stable isotopes result in disproportionally more of the light isotope in the product than the reactants; this process is known as isotopic fractionation (Fry 2006).

Stable isotope values are generally expressed as the ratio of the heavy isotope to the light isotope (e.g., $^{13}C/^{12}C$) in relation to a known standard, and are reported in permil (‰), or parts per thousand (Schoeller 1999). The delta symbol (δ) is used to indicate that the measured isotope ratios originally measured for a material have been converted into permil values. The U.S. National Bureau of Standards and the International Atomic Energy Agency in Vienna, Austria, provide international laboratory standards for reporting isotope values. Stable carbon isotope ratios are expressed relative to the PDB standard, a Cretaceous fossil (*Belemnitella americana*) from the Pee Dee formation in South Carolina. PDB is assigned a value of 0 ‰ by definition and is enriched in ^{13}C relative to organic carbon and most terrestrial carbonate materials. Thus, $\delta^{13}C$ values for most living things are negative relative to the standard. Stable nitrogen isotopes are expressed by the ratio of $^{15}N/^{14}N$ relative to the standard of atmospheric N_2 (AIR, "ambient inhalable reservoir"), also arbitrarily set at 0 ‰. Because atmospheric N_2 is more depleted in ^{15}N than most living things, $\delta^{15}N$ values in organisms are usually positive. The δ-values are calculated as follows for carbon and nitrogen, respectively:

$$\delta^{13}C\,(‰) = \frac{^{13}C/^{12}C_{sample} - {}^{13}C/^{12}C_{standard}}{^{13}C/^{12}C_{standard}} \times 1{,}000,$$

$$\delta^{15}N\,(‰) = \frac{^{15}N/^{14}N_{sample} - {}^{15}N/^{14}N_{standard}}{^{15}N/^{14}N_{standard}} \times 1{,}000.$$

Stable Carbon and Nitrogen Isotopes

The carbon isotope ratios of biological materials are ultimately related to plant photosynthesis and the movement of carbon from the atmosphere to the terrestrial environment. Plant photosynthetic pathways differ based on their specific mechanism used to fix atmospheric CO_2 and relate to adaptive mechanisms that maximize efficiency of carbon fixation in different climates (Heaton 1999; O'Leary 1981, 1988). C_3 plants discriminate more against ^{13}C than do C_4 plants during photosynthesis, and therefore have lower $\delta^{13}C$ values than C_4 plants, with an average $\delta^{13}C$ value of -26.7 ± 2.7 permil ($n = 370$, Cerling et al. 1998). C_3 plants comprise the majority of the earth's vegetation, and include trees, shrubs, legumes, and most grasses and tubers found in temperate regions (O'Leary 1981, 1988). C_4 plants, in contrast, discriminate less against ^{13}C during photosynthesis than do C_3 plants, and thus have more elevated $\delta^{13}C$ values that average -12.5 ± 1.1 permil ($n = 455$, Cerling et al. 1998). Examples of C_4 plants include tropical grasses typical of hot and arid climates, such as maize, millet, amaranth, sorghum, and sugarcane.

Carbon isotope ratios of bone reflect the relative consumption of C_3 versus C_4 resources (i.e., plants and the animals that consume them) by an organism (DeNiro and Epstein 1978). Other dietary resources, such as seafood and CAM plants (desert-adapted succulents and cacti) show elevated $\delta^{13}C$ values that overlap with C_4 plant values (Schoeninger et al. 1983; Schwarcz and Schoeninger 1991). Controlled feeding studies on rodents fed on pure C_3, C_4, or a mixed diet containing both C_3 and C_4 plants demonstrate that the

majority of carbon molecules from dietary protein are preferentially routed to collagen (Ambrose and Norr 1993; Tieszen and Fagre 1993). In contrast, carbon molecules in bioapatite are derived from blood bicarbonate and more closely approximate the whole diet of an organism (Ambrose and Norr 1993; Tieszen and Fagre 1993). Thus, the use of carbon isotopes of both collagen and bioapatite permits greater discrimination of the contributions of different macronutrients to the diet than the isotope analysis of either collagen or bioapatite alone (Froehle et al. 2010; Kellner and Schoeninger 2007).

Nitrogen has two stable isotopes, ^{15}N and ^{14}N, which are incorporated into plants from N_2 in the atmosphere. Stable nitrogen isotopes of bone collagen can be used to track consumption of marine and terrestrial resources in an ecosystem because $\delta^{15}N$ values typically increase 2 to 4 permil for each level in the food web (DeNiro and Epstein 1981; Minagawa and Wada 1984; Schoeller 1999; Schoeninger and DeNiro 1984). This makes it possible to reconstruct food webs of primary producers, herbivores, omnivores, and carnivores within a given ecosystem. The nitrogen contained within bone collagen derives from dietary protein; therefore $\delta^{15}N$ animal tissue values reflect the $\delta^{15}N$ value of the whole diet. Since meat is 85 to 90 percent protein, meat consumption will dominate $\delta^{15}N$ values of collagen as opposed to plants consumed as part of the diet (Ambrose et al. 2003).

Geographic Information from Carbon and Nitrogen

Residents of different geographic regions display dietary differences due to cultural traditions and food preferences, with diets often consisting of locally available foods. These characteristics can impact the $\delta^{13}C$ and $\delta^{15}N$ values of bulk (total) diet and the $\delta^{13}C$ and $\delta^{15}N$ values of human tissues in turn. For example, the practices used for growing and raising food items found in the human diet vary in different regions of the world. In the United States, C_4 plants such as corn and sugarcane are the basis of many human and livestock food items. The preponderance of C_4 in the diet is reflected in the higher $\delta^{13}C$ values of human hair from U.S. Americans (Valenzuela et al. 2011) than hair collected from humans that consume more C_3 plants, such as Europeans (McCullagh et al. 2005; Valenzuela et al. 2012) and Asians (Thompson et al. 2010). Global differences in the consumption of meat and animal-derived foods such as eggs can also impact the $\delta^{13}C$ and $\delta^{15}N$ values of human tissues, resulting in significant differences between omnivores, vegetarians, and vegans (O'Connell and Hedges 1999; Petzke et al. 2005a, 2005b).

Global differences in the $\delta^{13}C$ and $\delta^{15}N$ values of diet—and thus human tissues—can be useful for distinguishing the origin of individuals. Recent forensic investigations have focused on the application of carbon and nitrogen stable isotope analysis for unidentified human remains (Ehleringer et al. 2010; Fraser et al. 2006; Mützel (Rauch) et al. 2009). The ultimate goal is the classification of an individual of unknown origin as a local versus a traveler or recent arrival to a particular location to help assign identity (Bol et al. 2007).

MATERIALS AND METHODS

Samples

This study includes human remains recovered from various sites associated with U.S. military losses in Asia and the Pacific. Initial selection focused on 30 samples from

JPAC-CIL active cases, representing approximately 24 individuals (some individuals were represented by more than one sample from the body, e.g. a femur and a humerus). Each sample or case was selected based on either (1) the decedent(s) being identified, thus being of known geographic origin; or (2) the bone sample having mtDNA extracted and amplified, providing haplogroup designations for the remains, thus also providing geographic origin for the remains. Samples were selected to have a relatively even distribution between U.S. Americans and Asians. In other words, the selected cases were either associated with a missing U.S. service person or a native inhabitant of Asia or the Pacific. The second author was the only individual with initial working knowledge of the selected cases. All other analysts were kept in the blind to ensure the scientific integrity of the results.

Most samples were taken from cases associated with aircraft crash sites in which the incident details are known, and the site is correlated to a specific loss (e.g., identity of the remains might be determined). The majority of the Asian sampled were from a mid-1960's incident in which numerous Army of the Republic of Vietnam (ARVN) personnel were on board an aircraft that crashed into a mountain in Vietnam. Other crash sites were from Papua New Guinea (PNG, World War II era), and Cambodia (Vietnam War era). Finally, three samples were from both air and ground losses during the Korean War. The present study focuses on three individuals from two separate incidents. Two individuals, samples 18 and 19, are associated with the ARVN incident and were determined to be of Asian ancestry based on the loss incident and mtDNA. Sample 9 is associated with a World War II loss in PNG, and was determined to be a U.S. American individual based on mtDNA and loss circumstances.

Samples were obtained from the parent bone following JPAC-CIL's standard sampling procedures for mtDNA and isotopic sampling. These sampling procedures were conducted in a clean environment, using standard personal protective equipment, a fume hood, and DNA-free, cleaned rotary tools. Each sample was documented through photography prior to and after sampling. Samples were cut using clean rotary tools under a positive pressure fume hood to minimize contamination. After removal from the parent bone, the samples were tagged with the appropriate sample numbers, placed in envelopes, and sealed with evidence tape for shipment. Between samples, all tools and the fume hood were sanitized with a 10 percent bleach solution and ultraviolet radiation. After the sampling was completed, the envelopes were registered on a chain-of-custody document and sent to the California State University, Chico (CSU, Chico) Human Identification Laboratory for isotope analysis. Chain of custody was maintained throughout the study period.

Sample Preparation

Bone collagen and bioapatite samples were prepared within the Stable Isotope Preparation Laboratory at CSU, Chico. Approximately two to three grams of bone was subsampled from the submitted bone portion for isotope analysis, and each sample was split for separate collection of the collagen and bioapatite fractions. Samples were first cleaned with

a diamond-studded Dremel tool to remove the external surface, followed by ultrasonic baths in distilled–deionized H_2O, 95 percent EtOH, and 100 percent EtOH.

For collagen preparation, bone chunks were soaked in a 0.25 M hydrochloric acid solution until demineralized (Ambrose 1990; Schwarcz and Schoeninger 1991). Collagen pseudomorphs were next soaked for 24 hours in a 0.125 M sodium hydroxide solution to remove humic acids, and then solubilized in pH≈3 water. Collagen samples were frozen and then freeze-dried in glass scintillation vials. The $\delta^{13}C$ and $\delta^{15}N$ values of collagen were measured by continuous-flow isotope ratio mass spectrometry at the Stable Isotope Facility at the University of California, Davis, using an elemental analyzer (PDZ Europa ANCA-GSL) interfaced with an isotope ratio mass spectrometer (PDZ Europa 20–20). For all 30 samples, atomic C/N ratios and percentage collagen yields fell within the range of well-preserved collagen (i.e., C/N: 3.2–3.3; collagen yield: 7–30 percent; DeNiro 1985; van Klinken 1999).

For the bioapatite fraction, bone samples were ground into a powder using a mortar and pestle and sieved through fine-mesh screen (200 μm). A 1.5 percent sodium hypochlorite solution was used to remove the organic fraction over a 48-hour period, with solution replaced once at 24 hours (Koch et al. 1997). Powdered samples were treated with a 1.0 M acetate-buffered (pH≈4.5) acetic acid solution for 24 hours (replaced once at 12 hours) to remove soluble contaminants. The $\delta^{13}C$ values of bioapatite were measured by continuous-flow isotope ratio mass spectrometry at IsoForensics Inc. in Salt Lake City, Utah, using a GasBench (Thermo Scientific) interfaced to an isotope ratio mass spectrometer (ThermoFinnigan MAT 253).

RESULTS AND INTERPRETATION

Stable carbon isotope values of bone collagen measured for the 30 samples varied from -20.5 to -14.7 permil (mean and standard deviation [SD] $= -17.4 \pm 2.1$ permil), and stable nitrogen isotope values varied from 9.2 to 14.2 permil (mean and SD $= 11.9 \pm 1.1$ permil). Bioapatite values for the same samples varied from -17.1 to -8.9 permil (mean and SD $= -13.2 \pm 2.6$ permil) for stable carbon isotopes. As seen in the histograms (Figures 1 and 2), the stable carbon isotope values of both collagen and bioapatite form bimodal distributions. The distributions were interpreted as diets consisting of varying amounts of C_3 and C_4 resources. When origin of the samples was taken into account, minimal overlap in collagen and bioapatite stable carbon isotope values between U.S. Americans and Asians was observed. As expected, U.S. American stable carbon isotope values were significantly higher relative to native Asians, reflecting greater contribution of C_4 resources in the diet of U.S. Americans as compared to Asians.

As the principal goal in this study was to determine sample origin, discrimination between the measured isotope ratios of U.S. American and Asian samples was paramount. An effective tool for this procedure was the linear discriminant function (LDF); FORDISC 3.1 (Jantz and Ousley 2005) allowed for custom functions to be generated with imported datasets. Output from the program included posterior and typicality probabilities, as well as a cross-validation procedure that showed how well the

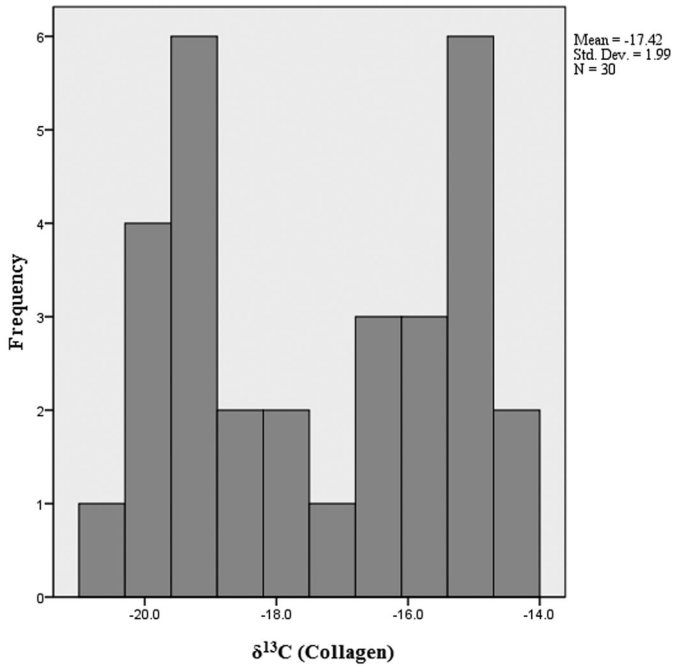

FIGURE 1. Bimodal distribution of collagen stable carbon isotope values. The distribution on the left side comprises individuals with primarily a C_3 diet and distribution on the right side comprises individuals with a greater contribution of C_4 in the diet.

function works for classifying an unknown sample. Linear discriminant function analysis correctly classified 96.7 percent (cross-validated) of all 30 study samples as to origin (U.S. American vs. Asian) based on stable carbon isotope values of collagen and bioapatite. Stable carbon isotopes of collagen yielded posterior probabilities ranging between 1.000 and 0.557, showing overall reasonable model performance (given the small sample size). Carbon isotopes of bioapatite yielded similar results. Only one sample did not fit this pattern; the sample derived from an ARVN individual who consumed a high level of C_4-based plants or possibly low–trophic level marine resources. In contrast, stable nitrogen isotope values of bone collagen among the samples had much lower variation than carbon isotope values, and were not significantly different between the U.S. American and Asian groups.

Individual Sample Performance

Three individuals from the complete dataset were selected as test cases. $\delta^{13}C$ values were the focus here since $\delta^{15}N$ showed poor discrimination of sample origin. Sample 18, an individual associated with the Vietnam War–era ARVN aircraft crash site, had a collagen $\delta^{13}C$ value of −19.7 permil and a bioapatite $\delta^{13}C$ value of −17.1 permil. Similarly, sample 19, a second individual from the same incident had a $\delta^{13}C$ value of −18.7 permil and a bioapatite $\delta^{13}C$ value of −15.6 permil. When selected within the LDF both individuals

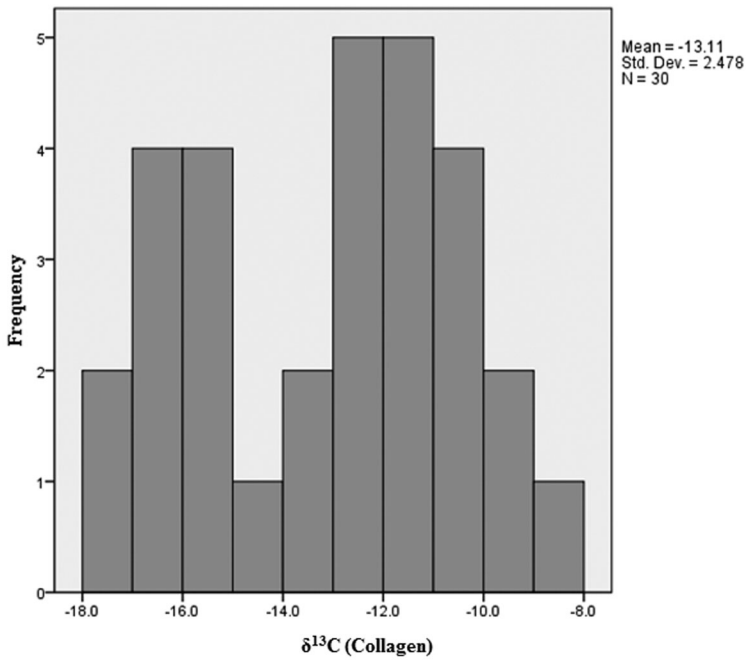

FIGURE 2. Bimodal distribution of bioapatite stable carbon isotope values. The distribution on the left side comprises individuals with primarily a C_3 diet and distribution on the right side comprises individuals with a greater contribution of C_4 in the diet.

were classified as Asians, with posterior probabilities of 0.998 and 1.000, respectively. Typicality probabilities showed one to be highly typical of the Asian profile at 0.957 and the other less so, at 0.216. The low typicality in this instance is because the $\delta^{13}C$ value is much less than the Asian group mean (−15.7 permil), which is not unexpected due to the small sample size. The $\delta^{13}C$ values for both individuals fit our hypothesis that a C_3-based diet that included rice versus a C_4-based diet based on plants such as corn would allow for discrimination between U.S. Americans and Asians; this was confirmed by LDF analysis. Further, the recovery context of the specimens, as well as the mtDNA determined haplogroups (B4c2 and B4b1a, both Asian) reconfirmed the LDF analysis from the isotopic data to be effective at discriminating U.S. Americans from Asians.

Sample 9, an individual recovered from a World War II site location in PNG had a collagen $\delta^{13}C$ value of −16.7 permil and a bioapatite $\delta^{13}C$ value of −12.2 permil. The $\delta^{13}C$ values from this individual were consistent with a mixed C_3/C_4 diet. The same procedure was undertaken as with Samples 18 and 19. A custom LDF was used to predict region of origin from the overall sample database. The LDF returned a posterior probability of 0.984 and a typicality probability of 0.469, classifying the sample as a U.S. American. Again, the accuracy rate was 96.7 percent for this model. The recovery context of the remains (a C-47 cargo aircraft in PNG) and the mtDNA determined haplogroup (J1c,

European) confirmed the isotopic findings. The individual is currently undergoing the final phases of identification for repatriation back to the next of kin.

CONCLUSIONS

Analysis of stable carbon isotope ratios in bone bioapatite and collagen was hypothesized to be able to differentiate between groups consuming different diets. In this study, we analyzed the $\delta^{13}C$ and $\delta^{15}N$ values from 30 samples from two primary groups, U.S. Americans and Asians. Samples were provided from the JPAC-CIL and were associated with war incidents from World War II, the Korean War, and the Vietnam War. The $\delta^{13}C$ values proved to be highly discriminatory between these groups; LDF analysis provided a 96.7 percent accuracy rate for region-of-origin assignment for each sample. In contrast, stable nitrogen isotope ratios were not useful for differentiating between the groups. The accuracy rates of the LDF analyses were confirmed for three test cases through recovery context of the remains as well as haplogroup information derived from mtDNA analysis. Given the powerful ability of $\delta^{13}C$ values to distinguish between U.S. Americans and Asians, we recommend this procedure as an analytical tool during origin investigations to screen human remains cases that may be of U.S. versus Asian origin.

Looking forward, future work will focus on the use of oxygen isotope ratios to further refine geographic origin predictions based on carbon isotopes. This study has demonstrated that stable isotope analysis provides valuable information for estimating the provenance of human remains and can aid in eliminating samples from consideration that are unlikely to be of forensic significance. This approach can further provide useful information for narrowing search parameters in unidentified persons cases, as well as aid in provenancing remains from human rights cases where an unknown individual originated from a different geographic area.

NOTE

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REFERENCES CITED

Ambrose, Stanley H.
1990 Preparation and Characterization of Bone and Tooth Collagen for Isotopic Analysis. Journal of Archaeological Science 17(4):431–451.

Ambrose, Stanley H., and Lynette Norr
1993 Experimental Evidence for the Relationship of the Carbon Isotopes Ratios of Whole Diet and Dietary Protein to Those of Bone Collagen and Carbonate. *In* Prehistoric Human Bone: Archaeology at the Molecular Level. Joseph B. Lambert and Gisela Grupe, eds. Pp. 1–37. Berlin: Springer-Verlag.

Ambrose, Stanley H., Jane Buikstra, and Harold W. Krueger
2003 Status and Gender Differences in Diet at Mound 72, Cahokia, Revealed by Isotopic Analysis of Bone. Journal of Anthropological Archaeology 22(3):217–226.
Bartelink, Eric J., Gregory Berg, Melanie M. Beasley, and Lesley A. Chesson
2014a Application of Stable Isotope Forensics for Provenancing Human Remains Recovered from Southeast Asia and the Pacific. Proceedings of the 66th Annual Meetings of the American Academy of Forensic Sciences, Seattle.
Bartelink, Eric J., Rachel Berry, and Lesley A. Chesson
2014b Stable Isotopes and Human Provenancing. *In* Advances in Forensic Human Identification. Xanthe Mallett, Teri Blythe, and Rachel J. Berry, eds. Pp. 157–184. Bosa Roca: CRC Press.
Beard, Brian L., and Clark M. Johnson
2000 Strontium Isotope Composition of Skeletal Material Can Determine the Birth Place and Geographic Mobility of Humans and Animals. Journal of Forensic Sciences 45(5):1049–1061.
Bol, Roland, Jen Marsh, and Timothy H. E. Heaton
2007 Multiple Stable Isotope (^{18}O, ^{13}C, ^{15}N and ^{34}S) Analysis of Human Hair to Identify the Recent Migrants to a Rural Community in SW England. Rapid Communications in Mass Spectrometry 21(18):2951–2954.
Cerling, Thure E., James R. Ehleringer, and John M. Harris
1998 Carbon Dioxide Starvation, the Development of C_4 Ecosystems, and Mammalian Evolution. Philosophical Transactions of the Royal Society London B 353(1365):159–171.
Chesson, Lesley A., Brett J. Tipple, John D. Howa, Gabriel J. Bowen, Janet E. Barbette, Thure E. Cerling, and James R. Ehleringer
2014 Stable Isotopes in Forensic Applications. *In* Treatise of Geochemistry 12. 2nd edition. Thure E. Cerling, ed. Pp. 285–317. New York: Elsevier.
Defense Prisoner of War Missing Personnel Office
2014 http://www.dtic.mil/dpmo/summary_statistics/, accessed January 3, 2014.
DeNiro, Michael J.
1985 Postmortem Preservation and Alteration of In Vivo Bone Collagen Isotope Ratios in Relation to Palaeodietary Reconstruction. Nature 317:806–809.
DeNiro, Michael J., and Samuel Epstein
1978 Influence of Diet on the Distribution of Carbon Isotopes in Animals. Geochimica et Cosmochimica Acta 42(5):495–506.
1981 Influence of Diet on the Distribution of Nitrogen Isotopes in Animals. Geochimica et Cosmochimica Acta 45(3):341–352.
Ehleringer, James R., Alexandra H. Thompson, David Podlesak, Gabriel J. Bowen, Lesley A. Chesson, Thure E. Cerling, Todd Park, Paul Dostie, and Henry Schwarcz
2010 A Framework for the Incorporation of Isotopes and Isoscapes in Geospatial Forensic Investigations. *In* Isoscapes: Understanding Movement, Pattern, and Process on Earth Through Isotope Mapping. James B. West, Gabriel J. Bowen, Todd E. Dawson, and Kevin P. Tu, eds. Pp. 357–387. Dordrecht: Springer.
Fraser, Isla, Wolfram Meier-Augenstein, and Robert M. Kalin
2006 The Role of Stable Isotopes in Human Identification: A Longitudinal Study into the Variability of Isotopic Signals in Human Hair and Nails. Rapid Communications in Mass Spectrometry 20(7):1109–1116.
Froehle, Andrew W., Corina M. Kellner, and Margaret J. Schoeninger
2010 Effect of Diet and Protein Source on Carbon Stable Isotope Ratios in Collagen: Follow up to Warinner and Tuross (2009). Journal of Archaeological Science 37(10):2662–2670.
Fry, Brian
2006 Stable Isotope Ecology. New York: Springer Science.
Heaton, Timothy H. E.
1999 Spatial, Species, and Temporal Variations in the $^{13}C/^{12}C$ Ratios of C_3 Plants: Implications for Palaeodiet Studies. Journal of Archaeological Science 26(6):637–649.

Hedges, Robert E. M., John G. Clement, C. David L. Thomas, and Tamsin C. O'Connell
2007 Collagen Turnover in the Adult Femoral Mid-shaft: Modeled from Anthropogenic Radiocarbon Tracer Measurements. American Journal of Physical Anthropology 133(2):808–816.
Hoefs, Jochen
2009 Stable Isotope Geochemistry. 6th edition. Berlin: Springer-Verlag.
Holland, Thomas D., Gregory E. Berg, and Laura A. Regan
2012 Identification of a United States Airman Using Stable Isotopes. Proceedings of the 64th Annual Meetings of American Academy of Forensic Sciences, Atlanta. 18:420–421.
Holland, Thomas, John Byrd, and Vincent Sava
2008 Joint POW/MIA Accounting Command's Central Identification Laboratory. *In* The Forensic Anthropology Laboratory. Michael W. Warren, Heather A. Walsh-Haney, and Laurel E. Freas, eds. Pp. 47–63. Boca Raton: CRC Press.
Jantz, Richard L., and Stephen D. Ousley
2005 FORDISC 3: Computerized Forensic Discriminant Functions, Version 3.1. University of Tennessee, Knoxville.
Juarez, Chelsey A.
2008 Strontium and Geolocation, the Pathway to Identification for Deceased Undocumented Mexican Border-Crossers: A Preliminary Report. Journal of Forensic Sciences 53(1):46-49.
Kellner, Corina M., and Margaret J. Schoeninger
2007 A Simple Carbon Isotope Model for Reconstructing Prehistoric Human Diet. American Journal of Physical Anthropology 133(4):1112–1127.
Kennedy, Casey D., Gabriel J. Bowen, and James R. Ehleringer
2011 Temporal Variation of Oxygen Isotope Ratios ($\delta^{18}O$) in Drinking Water: Implications for Specifying Location of Origin with Human Scalp Hair. Forensic Science International 208(1–3):156–166.
Koch, Paul L., Noreen Tuross, and Marilyn L. Fogel
1997 The Effects of Sample Treatment and Diagenesis on the Isotopic Integrity of Carbonate in Biogenic Hydroxylapatite. Journal of Archaeological Science 24(5):417–429.
Manolagas, Stavros C.
2000 Birth and Death of Bone Cells: Basic Regulatory Mechanisms and Implications for the Pathogenesis and Treatment of Osteoporosis. Endocrine Reviews 21(2):115–137.
McCullagh, James S.O., Jennifer A. Tripp, and Robert E. M. Hedges
2005 Carbon Isotope Analysis of Bulk Keratin and Single Amino Acids from British and North American Hair. Rapid Communications in Mass Spectrometry 19(22):3227–3231.
Meier-Augenstein, Wolfram
2007 Stable Isotope Fingerprinting – Chemical Element "DNA." *In* Forensic Human Identification: An Introduction. Tim Thompson and Sue Black, eds. Pp. 29–53. Boca Raton: Taylor and Francis Group.
2010 Stable Isotope Forensics: An Introduction to the Forensic Applications of Stable Isotope Analysis. Wiltshire: Wiley.
Meier-Augenstein, Wolfram, and Isla Fraser
2008 Forensic Isotope Analysis Leads to Identification of a Mutilated Murder Victim. Science and Justice 48(3):153–159.
Minagawa, Masao, and Eitaro Wada
1984 Stepwise Enrichment of ^{15}N Along Food Chains: Further Evidence and the Relation Between $\delta^{15}N$ and Animal Age. Geochimica et Cosmochimica Acta 48(5):1135–1140.
Mützel (Rauch), Elisabeth, Christine Lehn, Oliver Peschel, Stefan Hölzl, and Andreas Roßmann
2009 Assignment of Unknown Persons to Their Geographical Origin by Determination of Stable Isotopes in Hair Samples. International Journal of Legal Medicine 123(1):35–40.
O'Connell, Tamsin C. and Robert E. M. Hedges
1999 Investigations into the Effect of Diet on Modern Human Hair Isotopic Values. American Journal of Physical Anthropology 108(4):409–425.
O'Leary, Marion H.
1981 Carbon Isotope Fractionation in Plants. Phytochemistry 20(4):553–567.
1988 Carbon Isotopes in Photosynthesis. Bioscience 38(5):328–335.

Petzke, Klaus J., Heiner Boeing, Susanne Klaus, and Cornelia C. Metges
2005a Carbon and Nitrogen Stable Isotopic Composition of Hair Protein and Amino Acids Can Be Used as Biomarkers for Animal-Derived Dietary Protein Intake in Humans. Journal of Nutrition 135(6):1515–1520.
Petzke, Klaus J., Heiner Boeing, and Cornelia C. Metges
2005b Choice of Dietary Protein of Vegetarians and Omnivores is Reflected in Their Hair Protein ^{13}C and ^{15}N Abundance. Rapid Communications in Mass Spectrometry 19(11):1392–1400.
Posey, Robert, Jurian Hoogewerff, Henriette Ueckermann, Ermengol G. Ballbé, and Simon Vriend
2010 Development of Isoscapes to Aid the Provenancing of Human Remains from Spanish Civil War. Science and Justice 50(1):36.
Prince, Julia, Stefanie Kline, Amy MacKinnon, and Eric J. Bartelink
2014 Provenancing Human Remains from Forensic Contexts: Application of Stable Isotope Analysis in Forensic Anthropology. Proceedings of the 66th Annual Meetings of the American Academy of Forensic Sciences, Seattle.
Rauch, Elisabeth, Susanne Rummel, Christine Lehn, and Andreas Büttner
2007 Origin Assignment of Unidentified Corpses by Use of Stable Isotope Ratios of Light (Bio-) and Heavy (Geo-) Elements: A Case Report. Forensic Science International 168(2-3):215–218.
Regan, Laura A.
2006 Isotopic Determination of Region of Origin in Modern Peoples: Applications for Identification of U.S. War-Dead from the Vietnam Conflict. Ph.D. dissertation, Department of Anthropology, University of Florida, Gainesville.
Schoeller, Dale A.
1999 Isotope Fractionation: Why Aren't We What We Eat? Journal of Archaeological Science 26(6):667–673.
Schoeninger, Margaret J., and Michael J. DeNiro
1984 Nitrogen and Carbon Isotopic Composition on Bone Collagen from Marine and Terrestrial Animals. Geochimica et Cosmochimica Acta 48(4):624–639.
Schoeninger, Margaret J., Michael J. DeNiro, and Henry Tauber
1983 $^{15}N/^{14}N$ Ratios of Bone-Collagen Reflect Marine and Terrestrial Components of Prehistoric Human Diet. Science 220(4604):1381–1383.
Schwarcz, Henry P., and Margaret J. Schoeninger
1991 Stable Isotope Analyses in Human Nutritional Ecology. Yearbook of Physical Anthropology 34:283–321.
Thompson, Alexandra H., Lesley A. Chesson, David W. Podlesak, Gabriel J. Bowen, Thure E. Cerling, and James R. Ehleringer
2010 Stable Isotope Analysis of Modern Human Hair Collected From Asia (China, India, Mongolia and Pakistan). American Journal of Physical Anthropology 141(3):440–451.
Tieszen, Larry L., and Tim Fagre
1993 Effect of Diet Quality and Composition on the Isotopic Composition of Respiratory CO_2, Bone Collagen, Bioapatite, and Soft Tissues. *In* Prehistoric Human Bone: Archaeology at the Molecular Level. Joseph B. Lambert and Gisela Grupe, eds. Pp. 121–155. Berlin: Springer-Verlag.
Valenzuela, Luciano O., Lesley A. Chesson, Gabriel J. Bowen, Thure E. Cerling, and James R. Ehleringer
2012 Dietary Heterogeneity among Western Industrialized Countries Reflected in the Stable Isotope Ratios of Human Hair. PLoS One 7(3):e34234.
Valenzuela, Luciano O., Lesley A. Chesson, Shannon P. O'Grady, Thure E. Cerling, and James R. Ehleringer
2011 Spatial Distributions of Carbon, Nitrogen and Sulfur Isotope Ratios in Human Hair across the Central United States. Rapid Communications in Mass Spectrometry 25(7):861–868.
Van Klinken, Gert J
1999 Bone Collagen Quality Indicators for Palaeodietary and Radiocarbon Measurements. Journal of Archaeological Science 26(6):687–695.