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PAPER

ANTHROPOLOGY; CRIMINALISTICS

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Resident and Nonresident Fingernail Isotopes Reveal Diet and Travel Patterns*,†,‡

ABSTRACT: Global travel has increased, and having a diagnostic tool to distinguish residents from visitors would be valuable. This study examined stable isotope biomarkers of fingernail tissues of resident (n = 26) and nonresident (n = 22) participants in Salt Lake City (SLC), UT, from 2015 to 2016. The purpose of this research was to determine whether fingernail isotopes could be used for reconstructing geolocation movements and to examine the convergence in nonresident fingernail isotopes to that of the resident signal following their arrival to SLC. Resident isotope values defined a baseline to make comparisons to. Initial nonresident hydrogen and oxygen isotope values were correlated with precipitation isotopes of their prior location. Fingernail isotope turnover rates were rapid and nonresident isotopes were indistinguishable from residents after ~71–90 days. The results of our study highlight the utility of stable isotope measurements of fingernail clippings to examine travel history reconstruction that could aid in identification of human remains.

KEYWORDS: forensic science, forensic anthropology, stable isotope analysis, travel history reconstruction, keratin biomarker, fingernails

Human travel and relocation have dramatically increased over the past 20 years; the world is getting smaller. Increased travel will continue, and it is estimated that within a decade, ~1.8 billion people will travel internationally (1). From a forensic science perspective, we can imagine the value of a diagnostic tool to determine whether unidentified human remains were from a resident or a visitor. Recently, stable isotope analyses have aided forensic investigations in determining the origin of living humans and also of unidentified remains (2–4). With the increase in global movements of human populations for a variety of reasons, distinguishing recent visitors from residents of a particular region could be of great importance and determining how long it takes for a nonresident to adopt the same stable isotope signal of a resident could be equally valuable. Additionally, questions related to the similarity between stable isotope profiles of individuals who travelled and lived together could be used for reconstructing travel patterns and associating individuals to each other.

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Stable isotope biomarkers based on diet and drinking water inputs have been commonly used to assess a person's dietary patterns and to distinguish individuals from different geographic regions using bones, teeth, muscles, or keratin tissues (5–9). Carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$) stable isotopes found in human tissues broadly reflect the isotope profile of the foods that an individual has consumed directly and indirectly. $\delta^{13}C$ values differentiate diets based on the consumption of C_3 versus C_4 photosynthetic plants (9–12). $\delta^{15}N$ values indicate the trophic level of the consumer (13–15) and may be used to differentiate a person's dietary eating preference (omnivores, ovo-lacto vegetarians, and vegans) (12) and reveal agricultural farming practices (16–18). $\delta^{34}S$ isotope values can shed further light on $\delta^{13}C$ and $\delta^{15}N$ by distinguishing terrestrial and marine food sources (11,19–21). Lastly, oxygen ($\delta^{18}O$) and hydrogen ($\delta^{2}H$) relate to a person's direct and indirect water consumption through drinking and food water that are tied with geographic location (22–25).

We have focused our investigation on the use of fingernail keratin tissues to assess questions related to an individual's region of origin and travel history. Fingernail tissues are ubiquitous to all humans and are comprised of α-keratin which represent an integrated dietary signal of protein intake (26). The abundances of different stable isotopes in keratin do not change once formed and therefore serve as linear "tape recorders" of isotope information of a particular time period (27). The isotope records for fingernails are primarily limited in utility by the length of the tissue, which could represent a period of several months to as long as half a year.

Stable isotope biomarkers are incorporated into the nail plate of the finger through an underlying structure called the nail matrix (28,29). This tissue matrix is where cell proliferation occurs and approximately 80% of the nail plate is formed from the proximal half of the matrix and the remaining 20% comes from the distal half of the matrix (30). The nail matrix extends to the distal edge of the lunula (Figure S1), where maturation is

complete (29,30) and the isotope signature of that time remains unchanged. From there the nail plate must grow to the free edge of the nail, where it can be clipped and this growth may take ~3–3.5 months (30,31). Estimates of fingernail growth in healthy adult populations have ranged from ~3 to 4 mm/month (32–35), suggesting that an individual thumb nail could provide up to 4.5–6 months of travel and residency history from human remains based on these growth rates.

The benefits of using human fingernails to assess travel history and residency in unidentified human remains are numerous, and it is therefore surprising that there have been so few long-term studies examining their forensic potential (27,36). Studies using fingernails have predominantly looked at singular time points (8,36,37); few have examined populations over longer timescales (38). With the potential for fingernail isotope biomarkers to aid in questions related to travel history and region of origin, we sought to further examine a longitudinal study of two participant groups (resident and nonresident) in Salt Lake City, UT. Our objective was to characterize stable isotope biomarkers that related to dietary foods ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) and culinary or tap waters ($\delta^{2}H$ and $\delta^{18}O$) to assess potential seasonal patterns and establish an isotope baseline in a resident population. Second, we wanted to examine how fingernail stable isotopes of a nonresident group moving into Salt Lake City would change and converge on the resident isotope signal over time. Lastly, we examined pairs of volunteers that exclusively traveled and lived together, to examine whether isotope signals of these couples with similar dietary and travel patterns would also be similar.

Materials and Methods

Volunteer Recruitment

Forty-eight volunteers were recruited from the Salt Lake City region, UT, in 2015. Volunteers were primarily recruited from the University of Utah (Salt Lake City, UT) and included undergraduate and graduate students, faculty, and staff. These individuals were separated into two groups: resident ($n = 26$) and nonresident ($n = 22$). The resident group was defined as individuals who, at the start of the study period (2015), had resided consistently in the Salt Lake City region for a minimum of 9 months; most volunteers had been residents for 2+ years. The nonresident group was defined as individuals who had recently moved to the Salt Lake City region (within 1 month of the start of this study).

Fingernail clippings were collected from both groups for a period of 9–12 months. Additionally, demographic surveys (sex, age, travel history, and dietary questions) were obtained from volunteers throughout the study period to monitor changes in diet or travel. Nonresident volunteers had moved to Salt Lake City from different locations throughout the United States and from China, India, and South America (Table 1). The University of Utah's Institutional Review Board approved this study (IRB protocol 00035524). All volunteers provided written consent to participate in the study and were free to choose which sample(s) and demographic information to provide. These choice decisions account for the variation in sample sizes for analyses presented in this study.

Fingernail Samples

Sample Collection—Fingernail clippings were collected from all volunteers over a 9-month period in 2015–2016. A subset of the resident group volunteers were asked to provide fingernail clippings for an additional 3 months in the fall of 2016 in order to fully explore seasonal changes. Volunteers were provided with labeled coin envelopes and were asked to record the date when they clipped their fingernails and to separate clippings from right versus left hands. This distinction was requested because there is evidence that fingernails on an individual's dominant hand grow faster (30,31). To maintain consistency among volunteers, we used fingernail clippings from the dominant hand for all analyses whenever possible. Volunteers were instructed to clip their fingernails as they normally would (dates between clippings averaged at 16 ± 7 days for all volunteers) and to collect all samples over the study period. Sample processing and isotope analyses were conducted at the Stable Isotope Ratio Facility for Environmental Research (SIRFER) at the University of Utah, Salt Lake City, UT.

TABLE 1—*Nonresident volunteers' region of origin.*

Country	City	State
United States of America	Oakland	CA
	Washington	DC
	Lewiston	ID
	Pocatello	ID
	Dekalb	IL
	Baton Rouge	LA
	Bethesda	MD
	Silverspring	MD
	Geneseo	NY
	Oklahoma City	OK
	Nashville	TN
	Lynchburg	VA
Argentina	San Luis	
China	Xianyang	Shaanxi Province
Colombia	Medellin	
India	N/A	

Sampling Plan

At the completion of the study, we had collected between 8 and 27 sets of fingernail clippings from different individuals in our resident group and between 6 and 29 sets from different individuals in our nonresident group. The number of volunteer samples varied, as they collected samples in accordance with their normal grooming routine and volunteers could choose to not provide sample(s) during the study. All resident fingernail clippings were analyzed for light stable isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $\delta^{18}O$, and $\delta^{2}H$) and 4–5 measurements were made per volunteer throughout the study period. Similarly, all nonresident fingernail clippings were also analyzed for the same light stable isotopes, specifically 4–5 measurements of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ and 6–10 measurements of $\delta^{18}O$ and $\delta^{2}H$.

Stable Isotope Analyses

All stable isotope ratio measurements were analyzed at SIRFER (http://sirfer.utah.edu). Fingernail clippings were cleaned using methods similar to those described by O'Connell et al. (37). Briefly, fingernail clippings were cleaned of any visible surface dirt using deionized (DI) water. Afterward, samples were washed twice (for 60 and 30 min) in a 2:1 chloroform:methanol mixture to remove lipids and other surface contaminants. Lastly, they were rinsed with DI water for 20–40 min in an ultrasonic bath. Cleaned samples were dried for 24–48 h at 65°C in a convection oven.

Carbon, Nitrogen, and Sulfur Isotope Analyses

For measurements of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values, fingernail samples were cut into 500-μg (±10%) pieces and placed into tin capsules for analyses. Fingernail samples and laboratory reference materials were analyzed using an elemental analyzer attached to an isotope ratio mass spectrometer (Delta V, Thermo Scientific, Bremen, Germany) operated in continuous flow mode. Data were analyzed using IsoDat version 3.0.94.12. The laboratory reference materials for $\delta^{13}C$ and $\delta^{15}N$ consisted of two glutamic acids and ground bovine muscle. $\delta^{34}S$ laboratory references included two sulfides and ground eider down. All stable isotope ratios are reported using the standard δ-notation relative to an international standard ($\delta^{13}C$: Vienna Pee Dee Belemnite (VPDB); $\delta^{15}N$: AIR; and $\delta^{34}S$: Vienna Canyon Diablo Troilite (VCDT)) in units per mil (‰) using the following: $\delta X = (R_{sample}/R_{standard} - 1) * 1000$, where X is the isotope of interest and R_{sample} and $R_{standard}$ are the molar ratios of the heavy to the light isotopes (e.g., $^{13}C/^{12}C$) of the sample and international standard, respectively. The analytical precision (1σ), based on long-term measurements of internal laboratory reference materials for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$, was 0.1‰, 0.1‰, and 0.2‰, respectively.

Oxygen and Hydrogen Isotope Analyses

Measurements of $\delta^{18}O$ and nonexchangeable $\delta^{2}H$ values in fingernails required only 150 μg (±10%) samples and were loaded into silver capsules that had been treated to remove oxides. Fingernail samples were analyzed in duplicate. Samples for $\delta^{18}O$ and $\delta^{2}H$ analyses were equilibrated following established methods (39) and stored under vacuum for a period of 5–7 days prior to analysis to remove adsorbed water. Three laboratory keratin reference materials included powered DS, ORX, and POW, for which the $\delta^{2}H$ ratios of the nonexchangeable hydrogen fraction and total $\delta^{18}O$ ratios were known, and were also loaded in a similar manner following the principle of identical treatment. The $\delta^{18}O$ and $\delta^{2}H$ values of the fingernail clippings were measured using a temperature conversion elemental analyzer (TC/EA; Thermo Scientific, Bremen, Germany) attached to an isotope ratio mass spectrometer (IRMS, Delta Plus XL; Thermo Scientific, Bremen, Germany). All results for $\delta^{2}H$ data are expressed on a nonexchangeable hydrogen basis, and both $\delta^{2}H$ and $\delta^{18}O$ are expressed in per mil (‰) units relative to the international standard Vienna Standard Mean Ocean Water (VSMOW). The analytical precision (1σ), based on long-term measurements of internal laboratory reference materials for $\delta^{18}O$ and $\delta^{2}H$, was 0.2‰ and 1.1‰, respectively.

Equilibration and Reaction Progress Modeling of Hydrogen and Oxygen Isotope Values

To examine the equilibration of $\delta^{2}H$ and $\delta^{18}O$ isotope ratios in the fingernail clippings of our nonresident population, we used the two-pool reaction progress variable model (short term, $t_{1/2}$ = 2 days; and long term, $t_{1/2}$ = 30 days) as described by Cerling et al. (26). The initial starting value (d_i) for the model was derived from the mean initial fingernail sampling from the nonresident population ($\delta^{2}H$: −85.2 ± 11.8‰; and $\delta^{18}O$: 12.5 ± 2‰). The equilibration value (d_e) was based on the resident population mean $\delta^{2}H$ (−104.1 ± 5‰) and $\delta^{18}O$ (8.9 ± 1.3‰) isotope values.

As fingernails develop from the proximal and distal portions of the nail matrix, regional isotope signals are incorporated into the keratin proteins (30), and once they are formed, they do not change with time. It may take several months for the nail plate to grow from the proximal fold (where the nail emerges from the cuticle) to the free edge, where it can be clipped as part of a normal grooming routine (Figure S1). This time period reflects a "lag" in the isotope incorporation of a new location. We hypothesized that the initial and subsequent fingernail clippings (for ~3–4 months) collected from our nonresident volunteers would reflect the isotope signal of their previous location and that following this period, we would begin to see the incorporation of the Salt Lake City isotope signal in their nails (time of arrival or t = 0 was defined as the period when the fingernail emerged from the proximal fold). Therefore, during this "lag" period in the reaction progress variable model, we hypothesized that the isotope ratio would remain constant until the time of an individual's arrival.

Keratin proteins are incorporated into the nail plate through the distal end of the lunula (where the nail matrix ends; Figure S1) (29,30). When our nonresident volunteers arrived in Salt Lake City (t = 0), they began incorporating the local isotope signal in their fingernails. It is estimated that the nail matrix ranges from 4 to 6 mm in length, with the majority of the tissue located beneath the proximal fold (40). Based on this range and the average nail growth rates (~3.4 mm/month), the nail matrix could reflect a time of 35–53 days. Taking the middle value of the two extremes, we estimated that the earliest time we could see the initial stable isotope changes in our nonresident volunteers would be ~44 days before t = 0, as the new isotope signal would be incorporated through the distal end of the lunula (which is distal to the proximal fold). We used this estimated length of time as an offset for when we anticipated seeing changes in stable isotope ratios of nonresident fingernail samples. Modeled values (with population variation) were plotted along with the observed isotope ratios from the nonresident volunteers. Nonresident volunteer isotope data were pooled together (~30-day increments) based on the number of days since volunteer arrival.

Estimates of fingernail growth (mm/day) were calculated from the length of fingernail clippings and time in between clippings from our volunteers. We calculated that average daily fingernail growth was approximately 0.112 ± 0.04 mm/day (3.4 mm/month) in our population and that the average length of the nail plate (from the proximal fold to the free edge of the nail) averaged at 13.8 ± 2.4 mm. Using the average growth rate and length of the nail plate, we estimated that the length of time it could take to go from the proximal fold to the free edge of the nail plate could vary from 101 to 180 days (from mean ± SD). Using this as a starting value, we took the difference of the nonresident day of arrival means for each time point and plotted this with the modeled data. We examined the fit for the mean ± SD and found that the lower range of the nail plate length of 11.4 mm (~101 days) fit the observed data the best and was used for analysis.

Statistics

Statistical significance and graphic output were generated using Prism v 5.0 (GraphPad Software). Data were summarized as mean ± SD. Normality of distributions was assessed using the Shapiro–Wilk test. Comparisons between the resident and nonresident groups were calculated using either an unpaired t-test or a Mann–Whitney test (based on normality of data). Comparisons within a group were made using either a paired t-test or Wilcoxon matched-pairs test. Finally, assessment of $\delta^{18}O$ and $\delta^{2}H$ values over time for the nonresident group was determined using a Kruskal–Wallis test with a Dunn's post-test

or a one-way ANOVA with a Bonferroni multiple comparison post-test to look for significant differences between specific times. Differences were statistically significant at the $p < 0.05$ level.

Results

Resident Stable Isotope Ratios Establish a Baseline for the Salt Lake City Region

Fingernail clippings from resident volunteers ($n = 27$) were used to establish a baseline isotope signal for Salt Lake City looking at both dietary ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) and tap water ($\delta^{18}O$ and $\delta^{2}H$) inputs. The mean and variance of these isotopes (Table 2) were used to make subsequent comparisons with the nonresident population as they converged on the resident signal.

Fingernail $\delta^{18}O$ and $\delta^{2}H$ were measured throughout the study period to see whether there were seasonal variations (Table S1). Between August 2015 and April 2016, resident fingernail $\delta^{18}O$ and $\delta^{2}H$ values gradually increased, with fingernail clippings collected in April having the most positive values. To determine whether this trend could be related to seasonality in water isotopes, samples were collected from a subset of the original volunteers ($n = 14$–16) from September to November 2016. By the end of this sampling period, isotope ratios were indistinguishable from previous April 2016 observations ($\delta^{18}O$: $p = 0.231$; $\delta^{2}H$: $p = 0.105$). Isotope ratios of individuals within the resident population were similar to themselves over time, with some volunteers having consistently higher or lower values relative to the population mean ($\delta^{18}O$: 8.9 ± 1.4‰; and $\delta^{2}H$: −104 ± 5.2‰). These were consistent with previously reported longitudinal resident studies (38,41).

The $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values of fingernails (dietary isotope ratios) generally remained constant throughout the study (Table S2). Specifically, $\delta^{13}C$ and $\delta^{15}N$ ratios in our resident population did not show significant differences over the course of the study ($p = 0.363$ and $p = 0.083$, respectively); however, significant changes did occur in fingernail $\delta^{34}S$ values ($p = 0.039$). Average dietary stable isotope ratios for each individual volunteer over the course of the study were calculated and compared to values for the overall resident population (mean ± 1 or 2 SD) ($\delta^{13}C$ −18.2 ± 0.7‰, $\delta^{15}N$ 9.5 ± 0.4‰, $\delta^{34}S$ 4.4 ± 0.7‰; Fig. 1). Throughout the study, volunteers maintained similar diets; that is, the average fingernail stable isotope ratios did not fall outside 2 SD of the population mean. However, fingernails of a few individuals ($n = 3$) exhibited $\delta^{13}C$ or $\delta^{34}S$ variation, which may have related to seasonal changes in diet (e.g., fish versus red-meat proteins).

Initial Nonresident Oxygen and Hydrogen Isotope Ratios of Fingernail Samples are Correlated with Calculated Precipitation of Their Previous Location

Our study included volunteers who moved to Salt Lake City in the summer of 2015 (August and September, $n = 21$–22) from locations within the United States as well as from foreign locations (China, India, and South America; Table 1). Based on geospatial models, some of these volunteers moved to Salt Lake City from locations where the tap water $\delta^{18}O$ and $\delta^{2}H$ values should have been different (25,42–45). Given previously published literature, we knew that scalp hair (22) and local tap waters were strongly related to geographic locations and we anticipated that fingernails would exhibit similar relationships.

TABLE 2—*Average resident (*n *= 27) isotope values from fingernail clippings collected throughout the study period. Values listed as mean ± SD.*

$5^{13}C$ (‰) (VPDB)	$5^{15}N$ (‰) (AIR)	$5^{34}S$ (‰) (VCDT)	$5^{18}O$ (‰) (VSMOW)	$5^{2}H$ (‰) (VSMOW)
−18.2 ± 0.7	9.5 ± 0.4	4.4 ± 0.7	8.9 ± 1.4	−104 ± 5.2

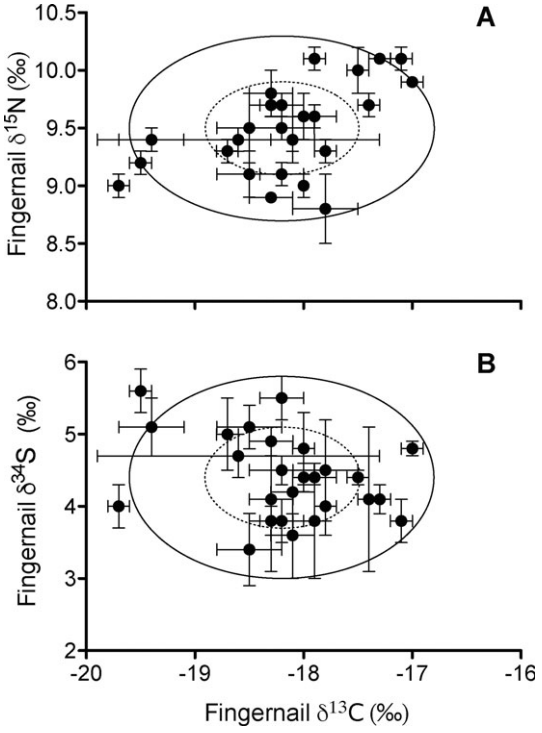

FIG. 1—*Resident (A) $\delta^{13}C$ and $\delta^{15}N$ and (B) $\delta^{13}C$ and $\delta^{34}S$ of fingernail clippings; individual points reflect the mean ± SD for each volunteer during the study period, and the ovals represent the resident group mean ± 1 (dotted oval) and 2 (solid oval) SD.*

To examine the tap water inputs that our nonresidents would have drunk prior to their arrival in Salt Lake City, precipitation stable isotope data from the Online Isotopes in Precipitation Calculator (version OIPC3.1, http://wateriso.utah.edu/waterisotopes/pages/data_access/oipc.html) were used to determine the mean annual precipitation for that volunteer's location prior to arriving in Salt Lake City (43–45). Regression analysis of the predicted precipitation stable isotope values ($\delta^{2}H = 8.3 \times \delta^{18}O + 11.7$, $r^2 = 0.99$, $p < 0.0001$) were indistinguishable from the Global Meteoric Water Line (GMWL) (46) (Fig. 2*A*). We then used the calculated annual precipitation stable isotope value as a proxy for the stable isotope values of local drinking water, as locations use local precipitation for municipal tap waters (47–49). Additionally, precipitation and tap water isoscapes across the U.S.A. have shown similar patterns (25,43).

Initial fingernail samples of the nonresident population displayed a wide range of $\delta^{18}O$ and $\delta^{2}H$ values, with the highest values from volunteers who had moved from India, and the lowest values from volunteers from Lewiston, ID. The initial fingernail samples ranged from 9.3 to 18.1‰ and −56.8 to −101.9‰ for of $\delta^{18}O$ and $\delta^{2}H$, respectively. Fingernail $\delta^{18}O$ and $\delta^{2}H$ values were related to each other; a linear regression of the data yielded the relationship: $\delta^{2}H = 4.84 \times \delta^{18}O - 145.4$‰ ($r^2 = 0.657$, $p < 0.0001$; Fig. 2*A*). The observed variations in the initial fingernail stable isotope values suggested a geographic pattern when we regressed fingernail $\delta^{18}O$ and $\delta^{2}H$ values to

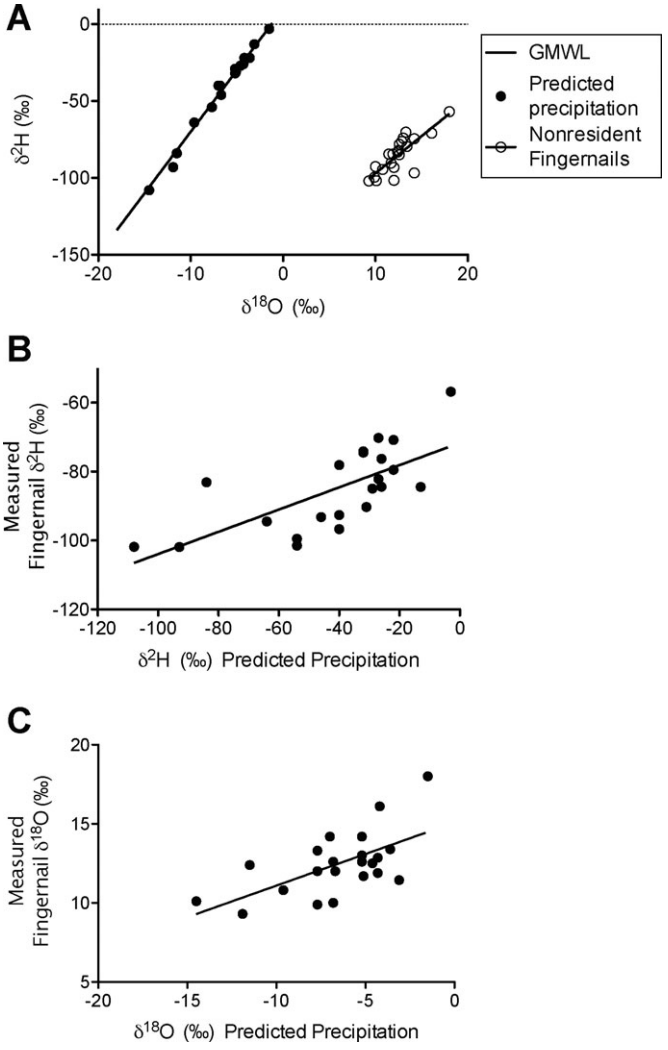

FIG. 2—*Nonresident initial $\delta^{18}O$ and $\delta^{2}H$ sampling compared to calculated precipitation isotope values for their original location. (A) $\delta^{18}O$ and $\delta^{2}H$ values measured from calculated mean annual precipitation for nonresident original locations (left, $\delta^{2}H = 8.28 \times \delta^{18}O + 11.7‰$, $r^2 = 0.99$, $p < 0.001$) and the initial fingernail sampling from nonresident volunteers (right, $\delta^{2}H = 4.84 \times \delta^{18}O - 145.4‰$, $r^2 = 0.99$, $p < 0.001$), (B) $\delta^{2}H$ measured from paired initial fingernail sampling ($\delta^{2}H_f$) and calculated mean annual precipitation ($\delta^{2}H_p$) for nonresident original location ($\delta^{2}H_f = 0.32 \times \delta^{2}H_p - 71.7‰$, $r^2 = 0.4838$, $p = 0.0003$), and (C) $\delta^{18}O$ measured from paired initial fingernail sampling ($\delta^{18}O_f$) and calculated mean annual precipitation ($\delta^{18}O_p$) for nonresident original location ($\delta^{18}O_f = 0.40 \times \delta^{18}O_p - 15.1‰$, $r^2 = 0.3796$, $p = 0.0023$).*

calculated precipitation isotope values. Similarly, we observed that regressions of both fingernail and calculated precipitation stable isotope values (Fig. 2*B*: $\delta^{2}H_f = 0.32 \times \delta^{2}H_p - 71.7‰$, $r^2 = 0.484$, $p = 0.0003$; Fig. 2*C*: $\delta^{18}O_f = 0.40 \times \delta^{18}O_p + 15.1$, $r^2 = 0.380$, $p = 0.002$; where $\delta^{2}H_f$ or $\delta^{18}O_f$ reflects the isotope value of the fingernail clipping and $\delta^{2}H_p$ or $\delta^{18}O_p$ reflects the calculated precipitation isotope value) suggested that approximately 32% and 40% of their $\delta^{2}H$ and $\delta^{18}O$ were derived directly from local drinking waters.

Nonresident Isotope Ratios of Fingernail Samples Vary Initially and Converge on the Resident Signal Over Time

Once the mean resident isotope values for both dietary and culinary water inputs had been established, significant trends were detected within the stable isotope values of the nonresident population. Over the course of the study period, we detected significant stable isotope differences ($\delta^{18}O$: $p < 0.0001$; and $\delta^{2}H$: $p < 0.0001$, paired *t*-test) between initial ($\delta^{18}O$: $12.5 \pm 2.0‰$; and $\delta^{2}H$: $-85.1 \pm 12.3‰$) and final ($\delta^{18}O$: $9.8 \pm 1.4‰$; and $\delta^{2}H$: $-98.5 \pm 5.8‰$) values for nonresident volunteer fingernails (Table 3). To determine whether these shifts were moving toward the resident group value, we compared the initial and final samples with those of the resident fingernail stable isotope values (mean ± 1 and 2 SD; Fig. 3). Stable isotope ratios of the initial nonresident fingernail samples were significantly different from those of the mean resident stable isotope values ($p < 0.0001$, unpaired *t*-test). Most nonresident fingernail stable isotope values did not fall within 1 or 2 SD of the resident group means (Fig. 3*A*). Initial observations of fingernail stable isotopes from some nonresidents fell within the range of values expected for the resident group; those individuals had moved to Salt Lake City from nearby Idaho where water stable isotope values were similar to those in Salt Lake City. By the time of the final sampling of the nonresident group, their fingernail stable isotope values had converged to within the range of values observed for the resident group (Fig. 3*B*). However, for some nonresident individuals, significant differences still persisted when compared to the resident mean ($\delta^{18}O$: $p = 0.008$; and $\delta^{2}H$: $p < 0.0001$). Based on travel history surveys, it is likely that travel to conferences and vacations in locations where tap water inputs would be more positive compared to Salt Lake City could be driving this observation.

Knowing that the nonresident group was significantly different from the resident group at the start of the study, we were interested in explaining the time course of changes in nonresident fingernail stable isotope values. Nonresident stable isotope data were analyzed based on the calculated days since their arrival (date of arrival, $t = 0$). To determine how nonresident fingernail stable isotope values changed over time, we calculated the average difference of the nonresident fingernail stable isotope values from that of the resident group mean stable isotope value (Fig. 4). Data were clustered into ~30-day periods; however, because not all volunteers provided samples on the same dates, the final sample period extended 30–60 days. For both $\delta^{2}H$ and $\delta^{18}O$ fingernail values, there were significant changes over the course of the study as nonresident fingernail stable isotope values converged on the resident group mean ($\delta^{2}H$: $p < 0.0001$; and $\delta^{18}O$: $p < 0.0001$). Stable isotope values of nonresident fingernails became indistinguishable from those of the residents within 161 days ($p = 0.106$) and 181 days ($p = 0.056$) from arrival for $\delta^{18}O$ and $\delta^{2}H$, respectively. However, the first ~90 days of fingernail clippings likely represented the nonresidents' prior location due to the nail plate growth rate. Therefore, the actual time that was required before the stable isotope values of nonresident fingernails became indistinguishable from those of the resident group was only 71–91 days.

With an estimated time for fingernail clippings from nonresidents to reach the resident fingernail stable isotope range, we were interested to see how the trajectories of stable isotope changes in fingernail observations would partition into pools, reflecting current water versus previous water inputs into keratin formation. For these analyses, we used the two-pool reaction progress model described by Cerling et al. (26). When fingernail $\delta^{18}O$ values were fitted to the model, we calculated a rapid response with a rapid half-time response of 43 days followed by a 95% turnover to the resident fingernail stable isotope mean value within 116 days (~3.8 months). The modeled and

TABLE 3—*Nonresident initial and final sampling for $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $\delta^{18}O$, and $\delta^{2}H$ ratios.*

$5^{13}C$ (‰) (VPDB)		$5^{15}N$ (‰) (AIR)		$5^{34}S$ (‰) (VCDT)		$5^{18}O$ (‰) (VSMOW)		$5^{2}H$ (‰) (VSMOW)	
Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
−18.1 ± 1.4	−18.0 ± 1.1	9.2 ± 0.9	9.5 ± 0.7	5.2 ± 2.0	4.2 ± 0.8	12.3 ± 2.2	9.7 ± 1.3	−81.7 ± 21.8	−98.9 ± 5.6
$p = 0.8948$		$p = 0.8086$		$p = 0.0800$		$p < 0.0001$		$p < 0.0001$	

Values listed as mean ± SD; statistical differences were determined using a paired *t*-test or Wilcoxon matched-pairs test depending on normality of data. Statistical significance was determined at $p < 0.05$.

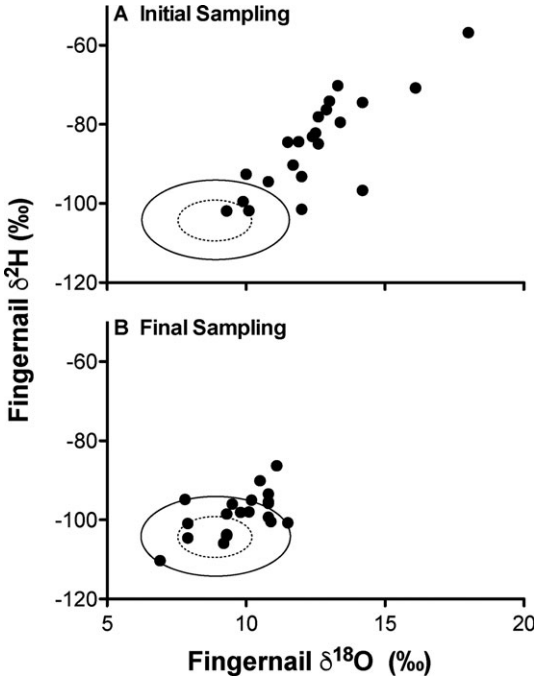

FIG. 3—*Fingernail $\delta^{18}O$ and $\delta^{2}H$ ratios of (A) initial and (B) final nonresident sampling. Individual points represent nonvolunteer initial and final fingernail sampling, and ovals represent 1 (dotted oval) and 2 (solid oval) SD of the resident group mean.*

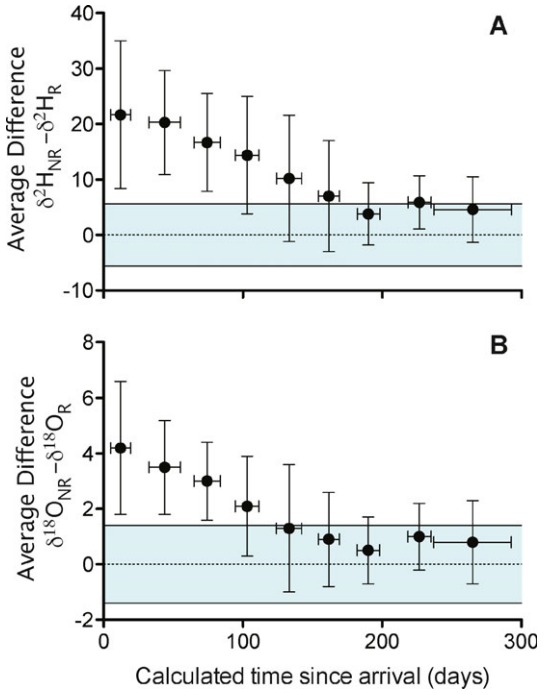

FIG. 4—*The average difference of (A) $\delta^{2}H$ and (B) $\delta^{18}O$ ratios of the nonresident group to the resident group mean. By day 181 for $\delta^{2}H$, nonresidents are indistinguishable from residents* (p = *0.1058, Mann–Whitney), and 161 for $\delta^{18}O$* (p = *0.0564, unpaired* t*-test). Light blue rectangle represents the resident group, where 0 represents the resident group mean ± SD. [Color figure can be viewed at wileyonlinelibrary.com]*

observed changes in nonresident fingernail $\delta^{18}O$ values are plotted in Fig. 5.

Within each observed time period, there were significant variations among nonresident isotope values, because they were not starting from the same initial oxygen isotope ratio values (Fig. 3). Observed fingernail $\delta^{18}O$ values of the entire nonresident group displayed variation at each time point compared to the modeled data; however, the individual fingernail $\delta^{18}O$ values from some volunteers fit the model well, while other deviated from predicted values (Figure S2). Even with the wide range of $\delta^{18}O$ values at each time point, the nonresident group still fell within the extremes of the modeled data (Fig. 5). We subsequently examined how the observed fingernail $\delta^{18}O$ values from nonresidents compared with the predicted fingernail $\delta^{18}O$ values to determine whether there were particular time periods that were deviating from the model (Figure S3). No clear patterns emerged, as the spread of predicted versus observed fingernail $\delta^{18}O$ values was nearly constant over the range of observations.

Volunteers did not apparently change their dietary inputs during the course of this study, even if they had moved to Salt Lake City from another region (Table 3). There were no significant changes in stable isotopes ratios associated with dietary markers from initial to final sampling periods ($\delta^{13}C$: $p = 0.895$; $\delta^{15}N$: $p = 0.809$; $\delta^{34}S$: $p = 0.080$). Initial fingernail $\delta^{13}C$ and $\delta^{15}N$ values from nonresidents were positively correlated, while fingernail $\delta^{13}C$ and $\delta^{34}S$ values were negatively correlated. By the time of the final sampling, nonresident fingernail $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values had converged to the range of values observed in the resident population (Fig. 6). Although fingernail stable isotope ratios from nonresident volunteers had converged to the ranges characterizing the resident population, we did not find significant differences between the initial or final sampling for $\delta^{13}C$ ($p = 0.771$ and 0.859) and $\delta^{15}N$ ($p = 0.755$ and 0.195). However, initial nonresident fingernail $\delta^{34}S$ values were significantly different from those of residents ($p = 0.002$), although final values were not ($p = 0.415$).

Individuals that Reside and Travel Together are Isotopically Similar

Lastly, several volunteers in both groups consisted of paired couples that both lived and traveled together. We compared fingernail dietary isotope values of these pairs and detected strong affiliation for fingernail $\delta^{13}C$ and $\delta^{15}N$ ratios among each pair

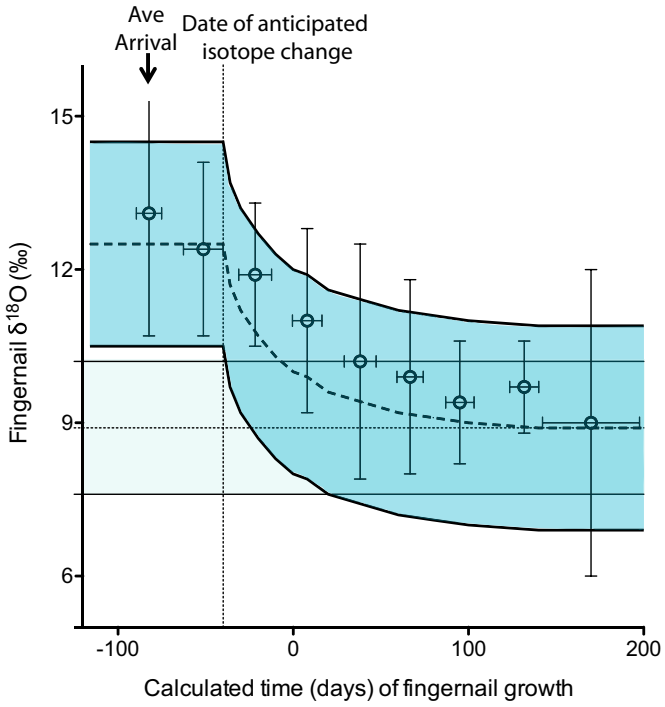

FIG. 5—*Reaction progress variable modeled data (solid line) and observed nonresident $\delta^{18}O$ ratios from fingernail clippings. Individual points display nonresident mean ± SD at ~30-day intervals. Blue rectangle reflects the resident group mean ± SD for $\delta^{18}O$ ratios. [Color figure can be viewed at wileyonlinelibrary.com]*

(Figs 7 and S4). Temporal variations in fingernail $\delta^{34}S$ values also showed similar patterns within each pair for most couples, although some differences were observed. While fingernail dietary isotopes were almost identical among pairs, there were greater differences for paired fingernail $\delta^{18}O$ and $\delta^{2}H$ values. The overall isotope patterns were similar among pairs. Yet there were near-constant offsets in absolute stable isotope ratio values, with one individual tending to be more enriched in the heavy isotope compared to the other (Figure S5).

Discussion

Longitudinal Study—Resident Group

The isotopic characterization of keratin samples from a resident population has typically sampled individuals at a single time point (22,37,50). While this snapshot of a location can provide general information of a particular population, longitudinal studies allow us to examine any natural variability in isotope signatures within a population. Such long-term stable isotope studies of resident populations have been limited (Belfast, Ireland, and Cambridge, MA) (38,41). The Salt Lake City data in this study have shown similar patterns and consistency in the variability of isotope ratios of human fingernail clippings in previous studies.

Resident volunteers apparently had isotopically similar diets, which were reflected in the similarity of fingernail dietary isotope values. The magnitude of stable isotope variability within the Salt Lake City population was consistent with values previously reported in fingernail and scalp hair studies (8,41). The greatest seasonal changes in fingernail stable isotope values occurred in $\delta^{13}C$ and $\delta^{34}S$ values and suggest dietary changes

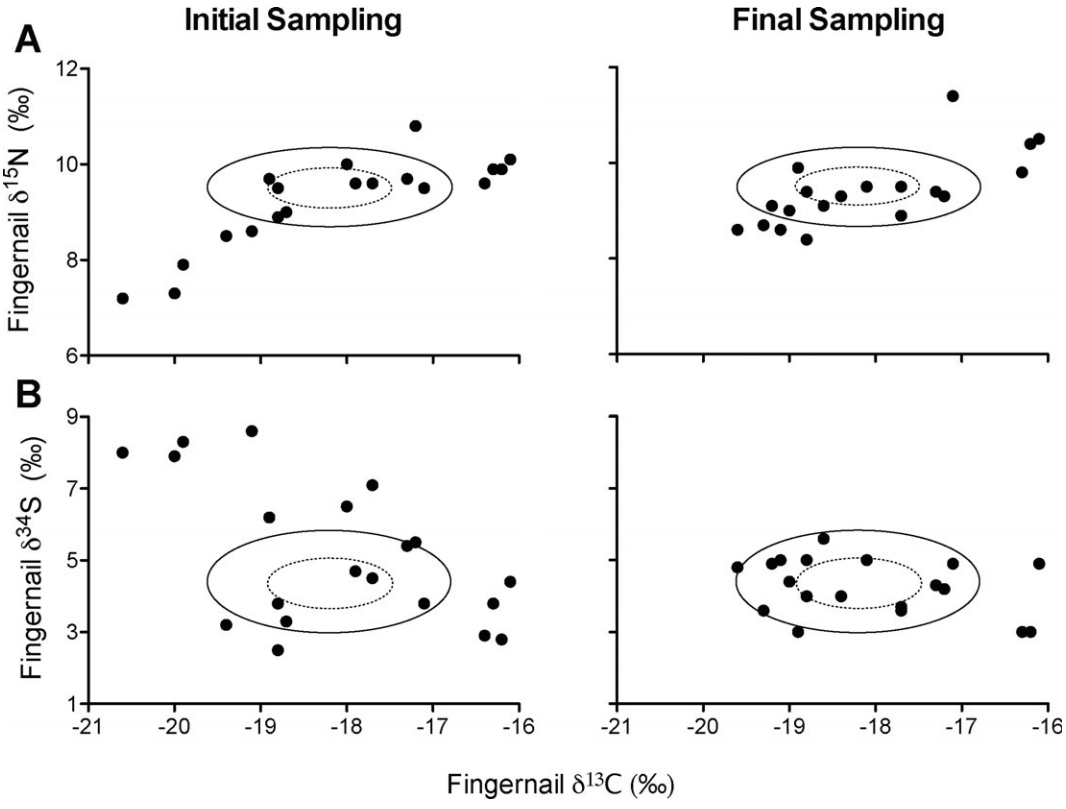

FIG. 6—*Initial and final sampling of fingernail (A) $\delta^{13}C$ and $\delta^{15}N$ and (B) $\delta^{13}C$ and $\delta^{34}S$ from nonresident volunteers. Individual points reflect nonvolunteer samples, and ovals reflect the isotope ratios of the resident group with 1 (dotted oval) and 2 (solid oval) SD.*

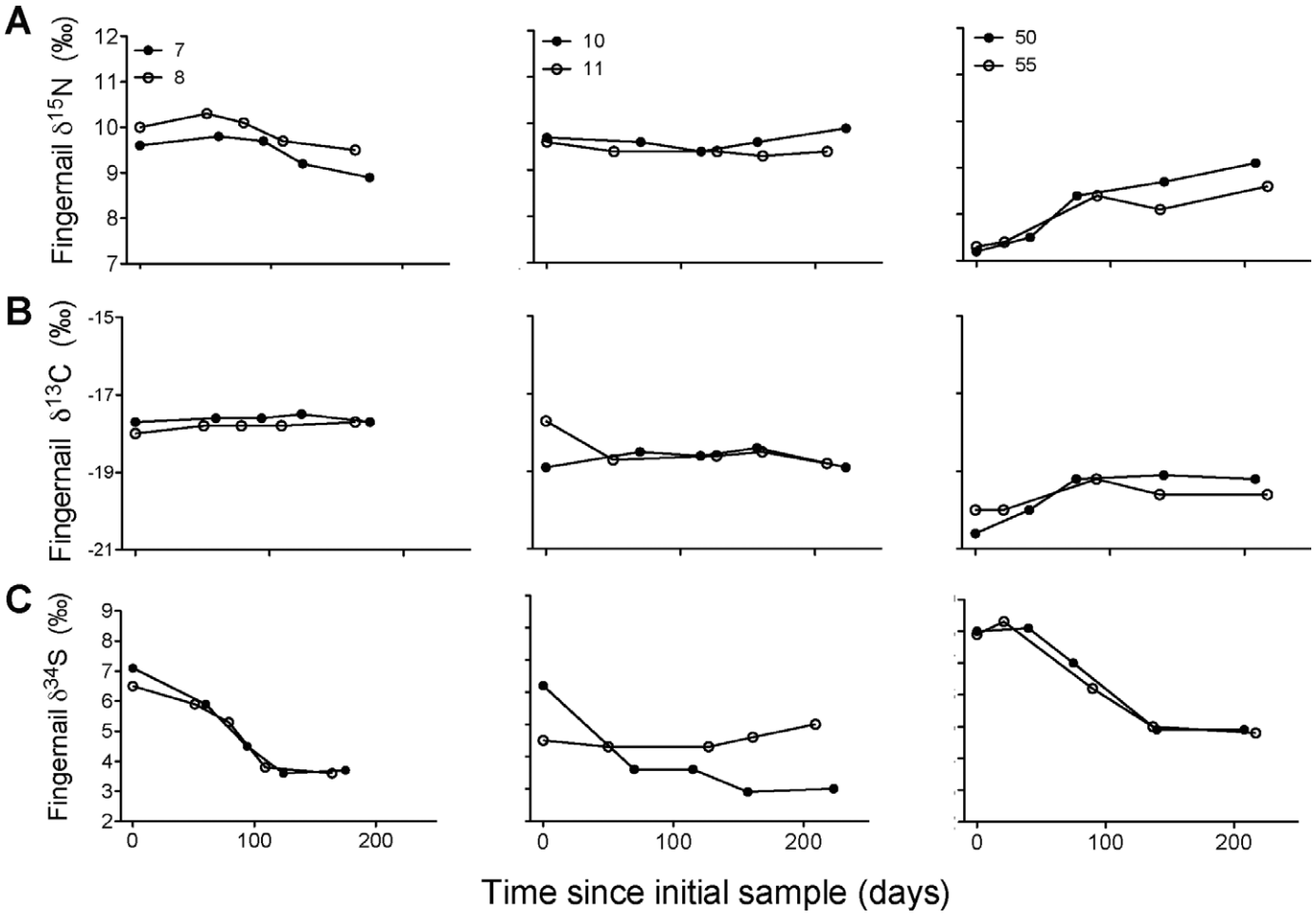

FIG. 7—*$\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ ratios of paired volunteers that traveled and lived together over the study period.*

that might be associated with seasonal eating patterns (e.g., fish versus beef protein sources; Fig. 1). This was not surprising as most of our volunteers self-identified as "omnivore" eaters. However, different proportions of fast food meals, animal proteins, and marine food sources were reported in their diets (9,18,51,52). Proportional changes in dietary inputs could account for the observed ranges of fingernail $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values.

Fingernail $\delta^{18}O$ and $\delta^{2}H$ values fluctuated among resident volunteers over the course of the study (Table S1) with values becoming more positive by the end of the original study period. Each of the resident volunteers resided in the Salt Lake City municipality and should therefore drink isotopically similar water sources throughout the study. We speculate that the increase in fingernail $\delta^{18}O$ and $\delta^{2}H$ isotope ratios over time could be related to management-based changes in municipal water sources (e.g., snowmelt, reservoir, or groundwater sources). Local tap waters are primarily of montane origin and therefore originate from cold-season, high-elevation precipitation (48,53). Most drinking waters are stored in open reservoirs (54) until they are needed, and it is known that reservoir waters become isotopically heavier during the summer as a result of evaporation. In addition to montane water sources, municipal waters for Salt Lake City also utilize groundwater depending on the season (54), which could also be driving the small temporal trends in resident fingernail $\delta^{18}O$ and $\delta^{2}H$ values.

Findings from Jameel et al. (2016) support the hypothesis that the increase in fingernail $\delta^{18}O$ and $\delta^{2}H$ as a result of tap water inputs. This study measured $\delta^{18}O$ and $\delta^{2}H$ tap water samples in Salt Lake City during the spring and fall of 2015, which overlapped with our study. Tap waters that were collected in the spring were isotopically lighter compared to those collected in the fall (53). Tap waters from these time periods would be incorporated into the fingernail tissues of our resident group and clipped in the beginning (August and September 2015) and end of our study (February and April 2016), where we saw the lowest and highest $\delta^{18}O$ and $\delta^{2}H$ values, respectively. Culinary practices (boiling, brewing, and stewing) may also influence the $\delta^{18}O$ value of the fingernail clippings over time, as previous work by Brettell et al. (2012) has shown that these cooking methods increase the $\delta^{18}O$ value of waters (55).

Nonresident Population and Comparisons to Human Scalp Hair

Our study provides the first longitudinal study of keratin stable isotope changes for a nonresident population residing in a new location. While there have been several studies that have examined stable isotope changes within individuals over time, these studies have focused on hair keratin (4,22,24,56). Here, we have shown that nonresidents moving from multiple locations outside of Salt Lake City change over time and could mimic the realistic influx of migrants as they assimilate into a new region. Additionally, we

highlight the similarities of fingernail and scalp hair keratin isotope values as they relate to drinking water inputs.

As human scalp hair has traditionally been used for travel history and region of origin investigations, we were interested to see how well fingernail $\delta^{18}O$ and $\delta^{2}H$ relationships would show similar patterns. Previously reported relationships of $\delta^{18}O$ and $\delta^{2}H$ values of modern scalp hair (the U.S.A.: $\delta^{2}H = 5.73 \times \delta^{18}O - 166‰$ (22); and Asia: $\delta^{2}H = 5.64 \times \delta^{18}O - 177‰$ [5]) were similar to the initial fingernail sampling in our study ($\delta^{2}H = 4.84 \times \delta^{18}O - 145.4‰$). The relationships between the initial fingernail isotope ratios and the calculated precipitation isotope values for those regions further explain the similarities between the two tissues. We found that 32% and 40% of the hydrogen and oxygen in human fingernails were directly related to local drinking waters and were similar to modern scalp hair studies (~27–42% hydrogen and 35–40% oxygen contributions [5,22,57]). The consistency of the relationships between drinking waters and keratin tissues suggests that similar dietary and physiologic factors influence the incorporation of $\delta^{18}O$ and $\delta^{2}H$ in keratin tissues, further validating the use of fingernail samples for region of origin determination.

Examination of the temporal changes in the $\delta^{18}O$ and $\delta^{2}H$ ratios in our nonresident group's fingernails over time is critical to their function to designate resident versus nonresident groups. Nonresident fingernail $\delta^{18}O$ and $\delta^{2}H$ values were indistinguishable from the mean resident population by ~71 and 90 days, respectively, and had similar equilibration and turnover times as scalp hair (22). Differences in the turnover times of the $\delta^{18}O$ and $\delta^{2}H$ ratios could relate to the exchange processes of proteins with gut water and the *in vivo* synthesis of nonessential amino acids. As ingested proteins are broken down, they undergo isotopic exchange with the gut body water, which is related to the drinking water source. Oxygen atoms from these proteins are free to fully exchange with that of the gut water, which could contribute to the faster turnover rate. The same free exchange happens with the hydrogen atoms at the c-terminus of amino acids during protein degradation; however, hydrogen atoms associated with the R-group of the amino acids will retain their original isotope value and therefore do not fully exchange with gut body water (56). Hydrogen and oxygen atoms incorporated into nonessential amino acids produced *in vivo* are derived from the gut body water and therefore reflect drinking water inputs (5). Keratin proteins in the nail plate also have structural elements with high levels of cysteine, glycine, and tyrosine (nonessential amino acids) (30), so it is possible that if these amino acids are synthesized *in vivo*, they should reflect body waters. The slower equilibration of $\delta^{2}H$ values could be influenced by the partial exchange with ingested proteins and the *in vivo* synthesis of nonessential amino acids. Understanding the temporal changes and the factors influencing the $\delta^{18}O$ and $\delta^{2}H$ values in fingernail tissues is critical for their use as determinants of geographic residency.

Similarities of Isotope Profiles of Paired Individuals

Our examination of paired individuals in both our resident and nonresident groups revealed remarkable similarities in their isotope profiles over the study period. Dietary inputs ($\delta^{13}C$ and $\delta^{15}N$) were most similar among paired individuals, with $\delta^{34}S$ ratios differences between pairs reflecting the reported amounts of marine and terrestrial sourced proteins consumed (11,21). Any deviations in the dietary isotope profiles of our paired individuals were consistent with individual reported dietary differences. Isotope shifts related to geographic relocation and changes in diet were also similar among paired individuals (e.g., participants 50 and 55) and were consistent with shifts in C_3 to C_4 plant sources (5) and increased animal protein intake (9,11,52) Lastly, inputs from drinking water were also similar between our paired individuals displaying similar changes related to geographic relocation (nonresidents) or minor fluctuations due to holiday travels (residents). In both cases, the offset in the $\delta^{18}O$ and $\delta^{2}H$ values could relate to differences in individual metabolism as the result of differences among sex, water intake, and activity levels (22,38,58); however, further studies are needed to determine the specific driver.

The similarities in the isotope profiles of our paired volunteers have highlighted the influence communal or shared meals within a household and differences between paired individuals are related to differences in known dietary inputs. Changes in food choices related to geographic movement and incorporation of new foods into keratin tissues were also similar. While these observations may not be surprising, couples that eat shared meals and travel together look isotopically similar, and they may provide insight on group living dynamics and travel histories.

While there has been minimal research looking at long-term isotope profiles of paired individuals, studies examining similarities in diets over time in elephant family units (hair samples) (59), medieval royal households in Spain (60), and possible subunit pairings in Anglo-Saxon households (61) have been conducted. The human studies have primarily measured isotope ratios in skeletal remains that are reflective of a longer integrated time period and do not reflect changes within a household unit over time (60,61). It is likely for these archeological and anthropological studies that analysis of individual or paired diets using keratin tissues over time is not possible and that applications of this type are better suited to modern human studies. To our knowledge, the human fingernail keratin isotope ratios have not been used to examine paired individuals or household dynamics related to dietary or tap water inputs. To understand their potential to address these questions, further research is needed to determine the isotope impact of residing with other individuals and to what extent they can be used to determine when new individuals join or leave a household group.

Conclusion

Human fingernails provide a linear tape recorder of travel and residency history that can be used in place of human scalp hair for forensic and homeland security studies. Our study has demonstrated that fingernail samples can capture the natural variation in isotope values of a resident group over time. Our findings have also shown that fingernail and scalp hair incorporate $\delta^{18}O$ and $\delta^{2}H$ from drinking water in similar manners. We observed that fingernail samples have similar turnover rates as human scalp hair and that modeled and observed data show similar patterns. Lastly, we have shown that individuals living and traveling together look isotopically identical for dietary isotopes and display similar patterns for drinking water inputs. $\delta^{18}O$ and $\delta^{2}H$ values of fingernail or hair tissues are able to provide a regional signal that corresponds to local precipitation or tap water inputs; however, precipitation and tap water isotope ratios can be similar in different geographic regions around the world. While $\delta^{18}O$ and $\delta^{2}H$ isotope ratios can narrow down geographic regions where an individual could have originated or traveled from, the use of additional isotope markers (strontium or lead) could further constrain potential geographic origins. Our findings have validated and support the use of a multi-isotope ratio analyses of human fingernails for forensic and

homeland security studies to aid law enforcement agencies in region of origin and travel history reconstruction for unidentified human remains and detainees.

Acknowledgments

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article:

Figure S1. Diagram of a fingernail with anatomical structures.

Figure S2. Predicted versus observed fingernail $\delta^{18}O$ for nonresident volunteers. Solid line reflects 1:1 line.

Figure S3. Reaction progress variable model compared to individual nonresident observed data. Blue box reflects the resident group $\delta^{18}O$ (mean ± SD).

Figure S4. $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ ratios of additional paired volunteers that traveled and lived together over the study period.

Figure S5. $\delta^{18}O$ and $\delta^{2}H$ ratios of paired volunteers that traveled and lived together over the study period.

Table S1. Average resident $\delta^{18}O$ and $\delta^{2}H$ ratios from August 2015 to November 2016. Values listed as Mean ± SD.

Table S2. Average resident $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ ratios from August 2015 to November 2016. Values listed as Mean ± SD.