Carbon and Oxygen Isotope Analysis to Document Childhood Diet and Local vs. Non-Local Status among African Slave Burials from the Grassmere Plantation, Nashville, Tennessee

Molly K. Shea, Lindsey T. Yann, Dr. Larisa DeSantis, Dr. Tiffiny A. Tung

KEYWORDS. slavery, carbon and oxygen isotope analysis, diet reconstruction, migration

BRIEF. Carbon and oxygen isotope analyses of human teeth from a captive slave population from the Grassmere Plantation in Nashville, Tennessee.

ABSTRACT Carbon and oxygen isotope analyses of human teeth from a captive slave population (ca. 1840s) from the Grassmere Plantation in Nashville, Tennessee were examined to reconstruct childhood diet and determine if individuals were local or non-local. Carbon and oxygen isotope ratios were obtained from the hydroxyapatite of 18 tooth enamel samples, representing 7 individuals. The isotopes from dentition reflect certain aspects of juvenile diet because as teeth are forming in childhood, the inorganic components of teeth incorporate carbon and oxygen isotopes from food and water, and they do not change throughout one's lifetime. Agricultural census records from the plantation indicate that corn and wheat were major crops on the plantation, but it is unknown if the slave population had equal access to those foods. The carbon isotope analysis, which can easily detect corn in the diet because it has a C_4 photosynthetic pathway, enables partial reconstruction of the diet of the slave population. Oxygen isotope values were used to determine if some or all of the individuals are from Middle Tennessee. Preliminary data show that the carbon isotope ratios are similar, suggesting that the captive slaves consumed a fairly homogenous diet that included maize (i.e. corn) as a staple food source. The $\delta^{13}C$ values ranged from -9.8 to -1.7‰ with a mean of -4.3‰ (s.d.=2.4‰). The non-converted $\delta^{18}O_{PDB}$ ranged from -5.4 to -3.4‰ with a mean of -4.6‰ (s.d.= 2.1‰). The converted $\delta^{18}O$ values suggest that at least two individuals were likely born and raised on or near the Grassmere Plantation, while the others exhibit values that are not expected for individuals who consumed water from the Grassmere Plantation.

INTRODUCTION

The trans-Atlantic slave trade was the largest long-distance forced migration of humans in history and, prior to the mid-nineteenth century, made up the majority of the re-peopling of the Americas after the collapse of the Amerindian population (Eltis, 2007). The South played a predominant role in this history of the slave trade since the majority of the Africans were being sent to work on plantations in the southeastern region of the United States. Agriculture was an important part of the South, and due to the high labor demands of producing things like cotton and tobacco, workers were needed to harvest them. The plantation owners thus subsequently purchased slaves, typically from West Africa, to labor in those fields. The number of slaves in the South grew as plantations expanded to meet the higher demands for agricultural goods. In 1840, the total slave population in the U.S. was 2,482,556, and Tennessee's slave population constituted about five percent of that total (n=183,059) (Historical Census Browser, University of Virginia Library). The West Africa to United States slave trade was abolished in 1808, but the law was loosely enforced, so some slaves were forcibly brought to U.S. soil until the beginning of the Civil War. However, there was also a significant internal slave trade; slaves were forcibly moved between regions, so it possible that the slaves at the Grassmere Plantation in Nashville, Tennessee were born and spent their childhood in West Africa or some other southern state before being purchased to labor in the fields at the Grassmere Plantation .

This study is a stable isotope analysis of black slaves from the Grassmere Plantation. The analysis is done to reconstruct aspects of childhood diet and examine whether the individuals were native to Middle Tennessee or whether they were recent immigrants from West Africa or from some other southeastern region in the U.S. These issues are examined through analysis of carbon isotope ratios from dental enamel, which aids in reconstructing aspects of childhood diet, and through analysis of oxygen isotope ratios, which aids in determining sources of imbibed water during childhood, and thus serves as a proxy for childhood residence.

The Grassmere Plantation

In Middle Tennessee, the land that came to be known as the Grassmere Plantation (and now the Nashville Zoo) was originally awarded to William Simpson in 1786 as compensation for his service in the revolutionary war against Britain (Riegel 1989). After being passed to his male heirs, one of Simpson's sons sold 272.5 acres to Michael C. Dunn in 1810 (Riegel 1989). Population census data for the Grassmere Plantation is available from 1820 to 1900; for the first 40 years, no names of inhabitants are provided. From 1840-1860 only the names of the white population are listed, and from 1870-1900, the names of the white and black population are listed. In 1840, the census data lists the following: one white female (15-20 years old) and one white male (70-80 years old) and 30 black slaves: 19 males and 11 females. They ranged in age from under 10 years old to mid-fifties, plus one male categorized as between 55-99 years old (Riegel, 1989:7). It is likely that the 20 burials that were archaeologically excavated at the Nashville Zoo in the spring of 2014 are from those individuals listed in the Grassmere Plantation census.

Agricultural census records from the Grassmere Plantation show that corn and oats were the major crops, but the plantation also produced wheat, potatoes, sweet potatoes, peas, and beans. Cotton was not produced there until 1870. It is unknown if the captive slave population consumed the wide variety of crops produced there, or if they had more limited access to those foods. Carbon isotope analysis can aid in addressing part of this question, because the technique can detect whether corn was a significant part of an individual's diet (discussed below).

The Archaeological Excavations

Excavations by the environmental service firm, TRC Inc., uncovered 20 human burials from an unmarked cemetery at the Nashville Zoo (formerly the Grassmere Plantation). Grave goods and coffin style suggest that the burials date to the early to mid-nineteenth century (Guidry 2015). After the burials were excavated, the adult burials were delivered to Shannon Hodges at Middle Tennessee State University (MTSU) for skeletal analysis and the juvenile burials were delivered to Tiffiny Tung at Vanderbilt University. Because the burials were still in their original soil matrix, they underwent detailed excavation and cleaning at the MTSU and Vanderbilt Osteology Labs. Tung collected small dental and bone samples from the adult burials and Tung and Shea collected dental and bone samples from the juveniles for the stable isotope study. After skeletal analysis and sample collection were completed, the burials were returned to the Nashville Zoo, where they were reburied in a new plot near the family cemetery. The same spatial organization was maintained, as it is likely that kin were buried near each other; this was done out of respect for the mourners who had originally buried them.

Previous Bioarchaeological Analysis

Demographic analysis of the skeletons shows that of the 20 burials, 11 were juveniles and 9 were adults, and of those adults 4 were male, 4 were female, and one was of unknown sex (Hodges, 2014; Tung 2014). Cranial morphological analysis of those that were well enough preserved suggests that seven of the individuals exhibit African ancestry (Hodges 2014). Three individuals were examined to obtain ancient mtDNA, and one belongs to haplogroup L, which is found exclusively in populations of African descent, the second was haplogroup H, common in western Eurasia, and the third sample did not yield mtDNA (Fratpietro 2014). These mtDNA data indicate that at least one individual had a mother of African descent, while admixture along the maternal line from non-African populations was also likely. Nonetheless, the suite of biological data strongly suggest that these individuals represent African slaves, an interpretation that is bolstered by the archaeological evidence; the unmarked graves, separation from the white cemetery (Guidry, 2015), and the presence of powder glass bead molds that were found when Tung re-excavated Burial 15 at the Vanderbilt Osteology Lab; this cultural item is evidence of strong ties to Africa, particularly Ghana.

Background on Stable Isotope Analysis

Humans incorporate carbon and oxygen isotopes into their tissues (i.e., bone, blood, teeth, etc.) via the foods they eat and the water they drink. Specifically, isotopes obtained from teeth reflect diet and water consumed in infancy and childhood because the enamel in teeth forms during those juvenile years. Unlike bone, the isotopes in teeth do not change during an individual's lifetime. Thus, carbon isotopes from teeth inform us about childhood diet, and oxygen isotopes from teeth can provide clues to place of childhood residence as geographic regions vary in oxygen isotope values (White, et al. 1998).

The ratios of carbon 12 to 13 ($^{12}C/^{13}C$) and oxygen 16 to 18 ($^{16}O/^{18}O$) fractionate minimally as they move through the food chain (White et al. 1998). Thus, carbon isotope ratios in human tissues can be used to estimate diet, especially differences in plant foods that have distinct photosynthetic pathways (eg. oat and wheat are C_3 plants and maize is a C_4 plant). These different photosynthetic pathways lead to different ratios of ^{12}C to ^{13}C in the plant and the animals and humans that eat those plants; thus a C_3 plant can be distinguished from a C_4 plant in human tissues. If maize was a significant part of their diet, as it was suspected to be, the $\delta^{13}C$ from the dental apatite should fall within the range of -4.5 to -1 ‰.

Oxygen isotope ratios vary depending on precipitation levels, climate, altitude, and distance from the equator, among other factors, and thus, it is a good proxy for estimating geographic origin of an individual. However, if two regions have overlapping oxygen isotope values, the geographic origin of a person may not be able to be distinguished. But if the oxygen isotope ratios of suspected native locations are distinct, a person's childhood location may be estimated. For example, a person who spent their childhood in coastal Ghana in West Africa should have oxygen isotope ratios in early forming teeth that are similar to the ground water there. See Table 1 for the expected oxygen isotope values for parts of West Africa and Middle Tennessee (Jorgensen and Banoeng-Yakubo 2001; Schroeder, et al. 2009). Future strontium isotope analysis will help to distinguish their geographic origins in greater detail.

The carbon and oxygen isotope ratios from a specimen are compared to a standard, known as the Vienna Pee Dee Belemnite (VPDB). Results are reported in per mil (‰) as δ values (Coplen 1994).

MATERIALS AND METHODS.

Enamel Collection

Eighteen dental isotope samples were taken from seven individuals. Teeth were cleaned with ultrapure water to remove dirt and other contaminants. Teeth were drilled with a Dremel drill to collect the enamel powder, which was then placed in a plastic micro-vial. Three teeth per person were sought, so C and O isotope values for different phases of childhood could be evaluated. Molar 1 forms in infancy/early childhood, Molar 2 forms in middle childhood, and Molar 3 forms in early adolescence. Two samples from each tooth were drilled when possible; one from near the occlusal surface (crown), which forms first, and the other near the cemento-enamel junction (CEJ), which forms later. This permits nuanced comparisons of diet within a person's childhood. If enamel chunks chipped off during drilling, they were converted into "bulk" samples and crushed with an agate mortar and pestle.

Table 1. Expected oxygen isotope ratios if an individual is from a particular region.

Region	**Expected $\delta^{18}O$**	**Citation**
Coastal Ghana	-3 to -2‰	Jorgensen &
Inland West Africa	-7 to -6‰	Schroeder et al. 2009
Highland Guinea and Cameroon	-10 to -9‰	Schroeder et al. 2009
Middle TN	-5.7 to -5.0‰	waterisotopes.org

Chemical Preparation

Approximately 1 mg of enamel powder was soaked in 1 ml of 30% hydrogen peroxide (H_2O_2) and periodically agitated for 10 seconds. After reaction was complete, the H_2O_2 was pipetted off and samples were rinsed with ultrapure water. Samples were then soaked in 1ml of acetic acid for exactly 18 hours and then rinsed with ultrapure water. To assist with drying, samples were soaked in 1ml of methanol, agitated, and then the methanol was removed immediately. Samples were then placed in a desiccator to allow the samples to dry. Once samples were dry, they were shipped to the University of Wyoming Isotope Facility for processing in the Thermo Gasbench coupled to a Thermo Delta Plus XL IRMS (Isotope Ratio Mass Spectrometry).

RESULTS.

Carbon and Oxygen Isotope Ratios

The $\delta^{13}C_{VPDB}$ values from the 18 samples range from -9.8 to -1.7‰; mean $\delta^{13}C_{VPDB}$ = -4.2‰ (s.d.=2.4‰). Individuals also exhibit variation in their carbon isotope ratios, which is consistent with changing dietary practices (and the process of weaning) throughout the developmental years (Figure 1, Table 2). Burial 4, an adolescent of unknown sex, exhibits the lowest $\delta^{13}C$ value, suggesting that this person had very little access to C_4 foods such as maize, at least during infancy when the first molar was forming. Burial 12, a middle-aged female (30-50 years old), had the highest carbon isotope ratio, suggesting that she had consistent access to maize as a food source.

Excluding the sample from the cemento-enamel-junction of Molar 2 from Burial 4, which likely represents an instrumentation error, the $\delta^{18}O_{VPDB}$ ratios from enamel carbonate ranged from -5.4 to -3.4‰; mean $\delta^{18}O_{VPDB}$ = -4.2‰ (s.d.= .5‰). These ratios are not yet converted and should not yet be compared to the expected values (see below for those comparisons).

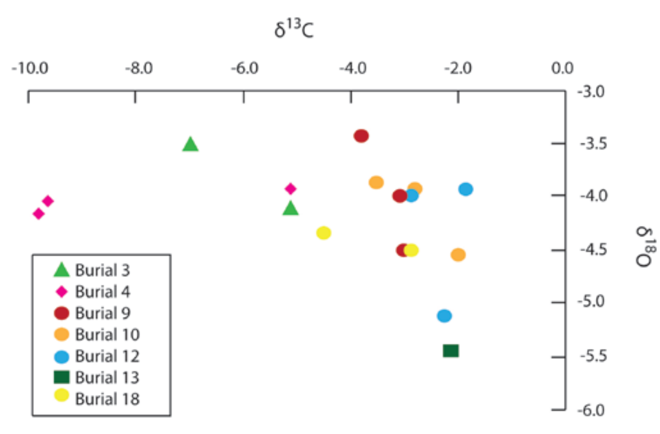


Table 2. The burial number, tooth sample, demographic information and carbon and oxygen isotope results.

Burial	Tooth	Quad	Tooth section	Sex	Age-at-death	$\delta^{13}C_{PDB}$	$\delta^{18}O_{PDB}$
3	RM1	Max	CEJ	?	20-35	-6.9	-3.5
3	RM2	Max	CEJ	?	20-35	-5.2	-4.1
4	RM1	Mand	Occ.	?	11-15	-9.8	-4.0
4	RM1	Mand	CEJ	?	11-15	-9.5	-3.9
4	RM2	Mand	Occ.	?	11-15	-5.2	-3.9
4	RM2	Mand	CEJ	?	11-15	-6.7	-13.0*
9	RM1	Mand	CEJ	F?	20-35	-3.7	-3.4
9	LP3	Mand	CEJ	F?	20-35	-3.1	-4.0
9	LM3	Mand	bulk	F?	20-35	-3.0	-4.5
10	RM1	Mand	CEJ	F	20-35	-3.5	-3.8
10	RM2	Mand	CEJ	F	20-35	-2.9	-3.9
10	LM3	Mand	CEJ	F	20-35	-1.9	-4.5
12	RM1	Max	Occ.	F?	35-50	-2.2	-5.1
12	RM2	Max	CEJ	F?	35-50	-1.7	-3.9
12	RM3	Max	CEJ	F?	35-50	-3.0	-4.0
13	LM3	Max	CEJ	M?	15-25	-2.1	-5.4
18	LM1	Max	Occ.	F	20-35	-4.6	-4.3
18	LM3	Max	CEJ	F	20-35	-2.8	-4.5

Occ. = enamel sample from near the occlusal portion of tooth.
CEJ = enamel sample from near the cemento-enamel-junction of tooth.
* = error in sample, possibly instrumentation error.

DISCUSSION

The carbon isotope ratios show that maize was a significant part of childhood diet for the captive slave population at Grassmere Plantation, though there were dietary contributions from other C_3 foods, likely oats and wheat. One exception is the adolescent known as Burial 4 (molar 1 $\delta^{13}C$ = -9.8 and -9.5‰): these are much lower than other burials. Because enamel in Molar 1 is forming in early infancy, and infants of this era were commonly breastfed, the values also reflect the mother's diet. In short, this individual's mother apparently consumed a diet distinct from the other slaves at the plantation (ie, the mother, and her breastfeeding infant, had little to no maize in their diets). An examination of the carbon isotope ratios from different teeth or parts of teeth indicate that diet may have changed throughout the childhood years. Specifically, slightly more maize and other carbon-enriched foods were consumed as an individual aged. Although marine resources can also lead to higher carbon isotope values (Schwarcz and Schoeninger 1991), it is highly unlikely that seafood was a significant part of the childhood diet among slaves in Middle Tennessee.

The oxygen isotope ratios are presented in PDB, but to compare them more directly to the drinking water values expected for parts of West Africa and Middle Tennessee, they were converted to another standard known as Vienna Standard Mean Ocean Water (VSMOW), using the formula below (Coplen, et al. 1983):

$$\delta^{18}O\ VSMOW_{carb} = (1.03091 \times \delta^{18}O\ VPDB_{carb}) + 30.91$$

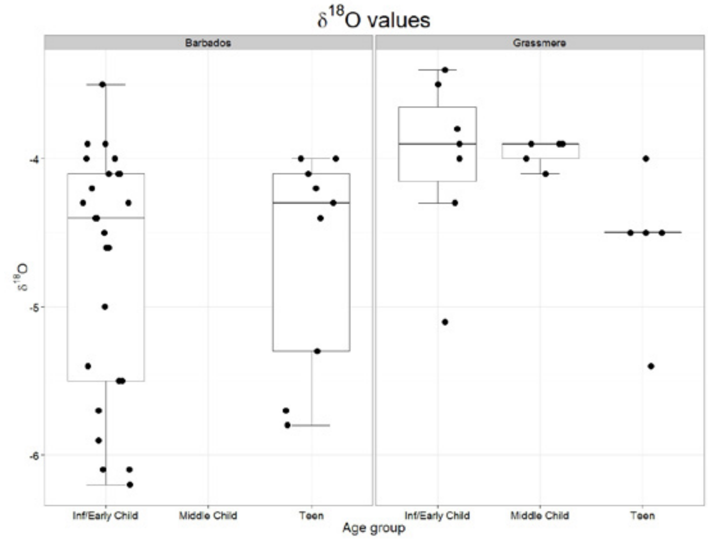
Those VSMOW values were then ultimately converted to drinking water values (Iacumin, et al. 1996; Luz, et al. 1984). Specifically, the δ18O VSMOW, which came from the carbonates (carb) in the hydroxyapatite of the teeth, was converted to VSMOW for phosphates (phos)

$$\delta^{18}O\ VSMOW_{phos} = (0.98 \times \delta^{18}O\ VSMOW_{carb}) - 8.5$$

Those values were then converted to make them comparable to drinking water (dw)

$$\delta^{18}O\ VSMOW_{dw} = (VSMOW_{carb} - \delta^{18}O\ 22.7)/\ 0.78$$

These conversions result in a range of oxygen isotope values of -8.2‰ to -5.5‰, which overlap with Middle Tennessee, Virginia, and parts of inland Africa (as well as more northern regions in the United States). It does not, however, overlap with highland Guinea, highland Cameroon, or coastal Ghana (Table 1), suggesting that none of the Grassmere Plantation slaves were from those regions. Instead, the preliminary data suggest that at least two individuals were native to Middle Tennessee (or other southern states with similar $\delta^{18}O$ values of drinking water (Bowen, et al. 2007), as their oxygen isotope values fall within the range expected for the region (waterisotopes.org). However, the majority of the sample exhibit values that do not match those expected for Middle Tennessee, where the slaves were buried. It is possible that local water sources were indeed used, but the water was boiled, leading to non-local values. It is also possible that the streams and groundwater in the immediate vicinity of the Grassmere Plantation is distinct from the larger river systems in Middle Tennessee. Future research will establish the oxygen isotope ratios of the groundwater and stream on the actual property of the Nashville Zoo (the former Grassmere Plantation).



Although Burial 13 exhibits oxygen isotope values that do not match that expected for Middle Tennesse, he may have suffered from a disease that altered his oxygen isotope ratio. Previous skeletal analysis of Burial 13, a male between the ages of 15-25 years old, reveals that he suffered from a slipped capital femoral epiphysis and possible gout (S. Hodges, Personal Communication 2014), conditions that could have resulted from the genetic disorder, sickle cell disease (SCD), which is relatively common among populations of West African descent. Burial 13 has the lowest $\delta^{18}O$ in the sample, which may further suggest that he suffered from SCD; mice with SCD have significantly lower $\delta^{18}O$ relative to healthy mice (Reitsema and Crews 2011). However, there are other possible factors that could lower enamel $\delta^{18}O$ values (i.e., drinking from a different water source, boiling water, smoking, tuberculosis), so the interpretation of SCD in this person must be further tested with additional enamel and bone samples.

Stable isotope data from a captive slave population at the Newton Plantation in Barbados was compared to the Grassmere Plantation to evaluate differences, if any, in forced migration patterns among slaves in two New World regions. The Newton Plantation in Barbados dates to between the late seventeenth century and early nineteenth century (Schroeder, et al. 2009), so it overlaps with the Grassmere Plantation burials. (These comparisons in the oxygen isotope values are kept in the original VPDB standard, because that is how the authors of the Barbados study presented their results.) The $\delta^{18}O$ from the Grassmere slave population is more homogeneous than the Barbados population, suggesting that the Grassmere captive slaves were from the same area or regions (potentially southeastern states with similar $\delta^{18}O$ values of drinking water (Bowen, et al. 2007), not from diverse regions (i.e., southeastern and southwestern states, or northern and southern states). For example, the teeth that correspond to infancy and early childhood yielded values in the -3.8 to -5.1‰ range, with the exception of two higher values. Among the Grassmere sample, the tooth that forms during adolescence (Molar 3) was also very homogeneous, with a range between -4 and -5.4‰. In Barbados, in contrast, the oxygen isotope ratios suggest that the slave population was drawn from a wider variety of regions. Among the Barbados slave burials, the teeth that formed during infancy and early childhood show much more variability in oxygen isotope ratios, ranging from -3 to -6.5 (Schroeder, et al. 2009) (Figure 2). The authors concluded that perhaps seven young children were taken from West Africa to the Newton Plantation; they were not born on the planation (Schroeder, et al. 2009). The Schroeder et al (2009) study also had strontium isotope data, which enabled them to exclude the coastal regions of West Africa as a place of origin; however, owing to overlapping values of oxygen and strontium isotope ratios in other parts of West Africa, they could not pinpoint the native locations for those seven slaves who had been moved to Barbados after childhood.

CONCLUSIONS

The preliminary carbon isotope data presented here suggest that the Grassmere Plantation slaves consumed maize or other carbon-enriched foods in childhood, and the data from the early forming teeth also suggest that females, who likely breastfed the infants, also consumed maize. The preliminary oxygen isotope data suggest that at least two individuals buried on the Grassmere Plantation were from the local area, while the non-local oxygen isotope values from others suggest that some may have been forcibly relocated to the Grassmere Plantation after childhood. However, because data on the oxygen isotope values of the small streams in and around the Grassmere Plantation are not yet available, these results do not permit a conclusive statement that some Grassmere slaves were from inland West Africa. Future studies will collect rain water and stream water from the Nashville Zoo (the former Grassmere Plantation) to obtain a more precise measurement of oxygen isotope ratios for the immediately local area. Further, strontium isotope studies, which track strontium isotopes from the soil to the food to the humans, will aid in clarifying the geographic origins of the Grassmere slaves.

ACKNOWLEDGMENTS.

We want to express our gratitude to Dr. Kimberly Mulligan and the REHSS program for facilitating this research. We thank Steve Wernke for assistance in obtaining the oxygen isotope values for the Middle Tennessee region from the interactive website, waterisotopes.org and the ArcGrid data created by the University of Utah. We thank Hannah Guidry for providing us with background information on the archaeological excavations, and we thank Tori Mason at the Nashville Zoo for providing other background information on the Grassmere Plantation and for coordinating with us to help the Nashville Zoo make long term plans for protecting the cemetery. Funding for this project was provided by a grant to Tung from the College of Arts and Science at Vanderbilt University and a grant to DeSantis by the College of Arts and Science at Vanderbilt University.

REFERENCES.

1. Bowen, G. J., J. R. Ehleringer, L. A. Chesson, E. Stange and T. E. Cerling
2007 Stable isotope ratios of tap water in the contiguous United States. Water Resources Research 43(3).
2. Coplen, T., K. C and H. J
1983 Comparison of stable isotope reference samples. Nature 302:236-238.
3. Coplen, T. B. 1994 Reporting of stable hydrogen, carbon, and oxygen isotopic abundances. Pure and Applied Chemistry 66:273-276.
4. Fratpietro, S. 2014 mtDNA Analysis of 3 Tooth Samples from the Nashville Zoo Burials. Lakehead University, Canada.
5. Guidry, H. 2015 Archaeological Investigation and Relocation of a Slave Cemetery at the Nashville Zoo, Davidson County, Tennessee. Paper presented at the Society for American Archaeology, San Francisco.
6. Hodges, S. 2014 Bioarchaeological Analysis of the Adults Burials from the Grassmere Property, Nashville Zoo, Tennessee. Middle Tennessee State University.
7. Iacumin, P., H. Bocherens, A. Mariotti and A. Longinelli
1996 Oxygen isotope analyses of co-existing carbonate and phosphate in biogenic apatite: a way to monitor diagenetic alteration of bone phosphate? Earth and Planetary Science Letters 142(1):1-6.
8. Jorgensen, N. O. and B. K. Banoeng-Yakubo 2001 Environmental isotopes (18O, 2H, and 87Sr/86Sr) as a tool in groundwater investigations in the Keta Basin, Ghana. Hydrogeology Journal 9:190-201.
9. Luz, B., Y. Kolodny and M. Horowitz
1984 Fractionation of Oxygen Isotopes between Mammalian Bone-Phosphate and Environmental Drinking Water. Geochimica et Cosmochimica Acta 48:1689-1693.
10. Reitsema, L. J. and D. E. Crews 2011 Brief communication: Oxygen isotopes as a biomarker for sickle-cell disease? Results from transgenic mice expressing human hemoglobin S genes. American Journal of Physical Anthropology 145(3):495-498.
11. Riegel, V. A. 1989 A historical, architectural, and archaeological assessment of the Grassmere Property, Nashville, Tennessee. Tennessee Department of Conservation.
12. Schroeder, H., T. C. O'Connell, J. A. Evans, K. A. Shuler and R. E. M. Hedges
2009 Trans-Atlantic slavery: Isotopic evidence for forced migration to Barbados. American Journal of Physical Anthropology 139(4):547-557.
13. Schwarcz, H. P. and M. J. Schoeninger 1991 Stable isotope analyses in human nutritional ecology. American Journal of Physical Anthropology 34(S13):283-321.
14. White, C., M. W. Spence, H. L. Q. Stuart-Williams and H. P. Schwarcz
1998 Oxygen Isotopes and the Identification of Geographical Origins: The Valley of Oaxaca versus the Valley of Mexico. Journal of Archaeological Science 25:643-655.

Molly K. Shea is a student at Hillsboro High School in Nashville, Tennessee. She participated in the Research Experience for High School Students (REHSS) program at Vanderbilt University.