American Journal of Primatology 74:651–660 (2012)

RESEARCH ARTICLE

Hydrogen and Oxygen Isotope Ratios in Body Water and Hair: Modeling Isotope Dynamics in Nonhuman Primates

SHANNON P. O'GRADY[1], LUCIANO O. VALENZUELA[1*], CHRISTOPHER H. REMIEN[2], LINDSEY E. ENRIGHT[1], MATTHEW J. JORGENSEN[3], JAY R. KAPLAN[3], JANICE D. WAGNER[3], THURE E. CERLING[1,4,5], AND JAMES R. EHLERINGER[1,5]
[1]*Department of Biology, University of Utah, Salt Lake City, Utah*
[2]*Department of Mathematics, University of Utah, Salt Lake City, Utah*
[3]*Department of Pathology, Section on Comparative Medicine, Wake Forest School of Medicine, Wake Forest Primate Center, Winston-Salem, North Carolina*
[4]*Department of Geology and Geophysics, University of Utah, Salt Lake City, Utah*
[5]*IsoForensics, Inc., Salt Lake City, Utah*

The stable isotopic composition of drinking water, diet, and atmospheric oxygen influence the isotopic composition of body water ($^2H/^1H$, $^{18}O/^{16}O$ expressed as δ^2H and $\delta^{18}O$). In turn, body water influences the isotopic composition of organic matter in tissues, such as hair and teeth, which are often used to reconstruct historical dietary and movement patterns of animals and humans. Here, we used a nonhuman primate system (*Macaca fascicularis*) to test the robustness of two different mechanistic stable isotope models: a model to predict the δ^2H and $\delta^{18}O$ values of body water and a second model to predict the δ^2H and $\delta^{18}O$ values of hair. In contrast to previous human-based studies, use of nonhuman primates fed controlled diets allowed us to further constrain model parameter values and evaluate model predictions. Both models reliably predicted the δ^2H and $\delta^{18}O$ values of body water and of hair. Moreover, the isotope data allowed us to better quantify values for two critical variables in the models: the δ^2H and $\delta^{18}O$ values of gut water and the ^{18}O isotope fractionation associated with a carbonyl oxygen–water interaction in the gut (α_{ow}). Our modeling efforts indicated that better predictions for body water and hair isotope values were achieved by making the isotopic composition of gut water approached that of body water. Additionally, the value of α_{ow} was 1.0164, in close agreement with the only other previously measured observation (microbial spore cell walls), suggesting robustness of this fractionation factor across different biological systems. Am. J. Primatol. 74:651–660, 2012.

Key words: mechanistic model; *Macaca fascicularis*; stable isotopes; cynomolgus monkey

INTRODUCTION

The hydrogen and oxygen isotope ratio ($^2H/^1H$, $^{18}O/^{16}O$ expressed as δ^2H and $\delta^{18}O$) analyses of hair and other keratin-based tissues have been used in studies of movement and resource use in the fields of anthropology [Bowen et al., 2009; Ehleringer et al., 2008; Nardoto et al., 2006; O'Connell & Hedges, 1999; Sharp et al., 2003; Thompson et al., 2010], ecology [Brattström et al., 2008; Cerling et al., 2006; Chamberlain et al., 1997; Hobson & Wassenaar, 1997; Hobson et al., 2004; Ramos et al., 2009; Rubenstein & Hobson, 2004; Wassenaar & Hobson, 1998], and more recently in human forensic investigations [Bol et al., 2007; Fraser et al., 2006; Meier-Augenstein & Fraser, 2008; Mützel Rauch et al., 2009]. The link between isotopic composition and geographic location exists because the hydrogen and oxygen isotope composition of environmental water varies predictably across the landscape [Bowen et al., 2007; Craig, 1961; Dansgaard, 1954; Kendall & Coplen, 2001]. As water is first incorporated into plants (as water or in organic matter) and later into primary and secondary consumers in the ecosystem, the isotopic composition of water is integrated into the food web, resulting in an isotopic "signature" of geography [Bowen, 2010; Bowen et al., 2005b; West et al., 2006, 2010]. Animal

Contract grant sponsor: Intelligence Community (IC) Postdoctoral Fellowship; Contract grant sponsor: National Center for Research Resources, contract grant numbers: P40 RR021380; contract grant sponsor: National Science Foundation - Research Training Group.

The first two authors contributed equally to this paper.

*Correspondence to: L.O. Valenzuela, Department of Biology, University of Utah, 257 S. 1400 E., Salt Lake City, UT 84112, USA. E-mail: valenzuela@biology.utah.edu

Received 11 November 2011; revised 24 January 2012; revision accepted 1 February 2012

DOI 10.1002/ajp.22019
Published online in Wiley Online Library (wileyonlinelibrary.com).

tissues commonly used in geolocation studies, such as hair, teeth, feathers, and nails, incorporate the isotopic signature of the water and food consumed at the time of synthesis—thus these tissues provide geographical information about location during these intense periods of growth.

Most ecological studies of primates have focused on the use of carbon and nitrogen isotopes to learn about diet and infer habitat preferences, while oxygen (O) isotopes have only recently been used to address ecological questions [Bedaso et al., 2010; Cerling et al., 2003, 2004, 2011; Chenery et al., 2011; Fourie et al., 2008; Levin et al., 2006; Quade et al., 1995; Smith et al., 2010; Sponheimer & Lee-Thorp, 2001; White et al., 2009; Yeakel et al., 2007]. Furthermore, the use of stable isotopes of hydrogen (H), at natural abundances, in primate research is mostly limited to geolocation of modern humans [Bowen et al., 2009; Ehleringer et al., 2008; Fraser et al., 2006; Meier-Augenstein & Fraser, 2008; Mützel Rauch et al., 2009; Sharp et al., 2003; Thompson et al., 2010]. The analysis of H and O isotope ratios has the potential to clarify several key ecological questions important for primatologists such as niche partitioning and dietary specialization, dispersal, residence patterns, and social organization. Given the many environmental, nutritional, and metabolic variables that influence the isotopic composition of animal tissues, it is important to develop a thorough understanding of hydrogen and oxygen isotope dynamics.

An offset between the isotopic composition of source water and tissue occurs due to inputs of H and O atoms from other sources (e.g., atmospheric oxygen, diet) as well as because of the impact of physiological processes associated with water metabolism (e.g., water flux, metabolic rate). Predictive models that incorporate fluxes and fractionation events have been created for the H and O isotopic composition of phosphate in bones and teeth [Ayliffe & Chivas, 1990; Bryant & Froelich, 1995; Daux et al., 2008; Luz & Kolodny, 1985; Podlesak et al., 2008] and more recently, keratin in hair [Bowen et al., 2009; Ehleringer et al., 2008; Podlesak et al., 2008]. While these are endpoint models for the tissue of interest (e.g., hair keratin), calculations necessarily incorporate the body water pool (i.e., free water in the body) [Gretebeck et al., 1997] and thus produce an intermediate prediction for the isotopic composition of body water.

The complexity of models describing the H and O stable isotopes in body water and proteinaceous tissues [Bryant & Froelich, 1995; Gretebeck et al., 1997; Kohn, 1996] can vary greatly. While some models only require the isotopic values of diet, water, and atmospheric O_2 to generate predictions [Ehleringer et al., 2008], others incorporate physiological processes relative to the organism of interest (i.e., mass, metabolic rate, water economy index), as well as climactic and dietary variables. Few modeling studies [Podlesak et al., 2008] have collected both body water and tissue samples from individual animals in a controlled setting to test the accuracy of the modeled values for not only the endpoint tissue of interest, but also the intermediate body water prediction. Here, we used a nonhuman primate system to test the robustness of mechanistic models of both the δ^2H and $\delta^{18}O$ values of body water [Bryant & Froelich, 1995; Kohn, 1996] and hair [Bowen et al., 2009; Ehleringer et al., 2008; Podlesak et al., 2008]. The goal of this research was to first, test the accuracy of both modeling approaches using a controlled system and second, use data from this system to improve estimates of model parameters.

METHODS

Ethical use of Animals

All procedures involving animals were conducted in compliance with state and federal laws of the US Department of Health and Human Services and guidelines established by the Wake Forest University. All animal procedures were approved by the Wake Forest Institutional Animal Care and Use Committee (IACUC). The research and procedures adhered to the American Society of Primatologists Principles for the Ethical Treatment of Nonhuman Primates.

Animal System

Samples of serum and hair were obtained from nonhuman primates (cynomolgus monkey, *Macaca fascicularis*) housed at the Wake Forest Primate Center (Wake Forest, NC, USA). Serum and hair samples were obtained from ten adult females and ten adult males. As part of an ongoing study at the Wake Forest Primate Center, animals were divided into two treatment cohorts (five females and five males each) and fed a Typical American Diet (TAD) with 35% of calories from fat, a moderate amount of dietary cholesterol, and less carbohydrate than standard monkey chow. One cohort consumed a diet with the protein derived from soy (TAD soy; LabDiet 5L0R) and the other group consumed a diet with the protein derived from casein (TAD casein; LabDiet 5L0P). Both TAD diets satisfy National Research Council recommendations for nonhuman primates and were custom formulated by Purina LabDiet® (Richmond, IN, USA). Dietary macronutrient content of the diets has been published previously [Wagner et al., 2009].

Isotope Analysis

We determined the stable hydrogen (δ^2H) and oxygen ($\delta^{18}O$) isotope ratios of water cryogenically

extracted from serum (hereafter referred to as body water) and hair of TAD soy ($n = 10$) and TAD casein ($n = 10$) monkeys. We also determined the δ^2H and $\delta^{18}O$ values of drinking water ($n = 12$) and of the casein ($n = 2$) and soy ($n = 2$) diets. Following extraction, aliquots of 0.4 ml of body water were transferred to 1.8-ml crimp-top gas chromatography (GC) vials and sealed until analysis. Body water and drinking water samples were prepared and analyzed alongside a set of three laboratory water reference materials previously calibrated to the Vienna Standard Mean Ocean Water (VSMOW) scale. The hydrogen and oxygen isotope ratios of two primary laboratory reference waters (DI and ZE), were −123 and −16.5 ‰ for DI and −0.1 and −0.2 ‰ for ZE, when calibrated against international standards (GISP, SLAP, and VSMOW). As a Quality Control, a third laboratory water reference was used (values −72.5 ‰ for δ^2H and −10.2 for $\delta^{18}O$). Both hair samples and food samples were ground into a homogenous powder. There is a partial isotopic exchange of H atoms in keratin with environmental water (e.g., the atmosphere); thus, all samples were analyzed together with hair reference materials for which the δ^2H of nonexchangeable H had been determined using the principle of identical treatment [Bowen et al., 2005a; Chesson et al., 2009]. Both reference material and samples were equilibrated with water vapor in the laboratory atmosphere and then desiccated under vacuum for 5 days. Samples of 150 μg of ground hair and food were weighed and analyzed.

The δ^2H and $\delta^{18}O$ values of body water, drinking water, hair samples, and food samples were analyzed at the University of Utah Stable Isotope Ratio Facility for Environmental Research (SIRFER; http://sirfer.utah.edu) on a ThermoFinnigan-MAT Delta Plus XL isotope ratio mass spectrometer (Bremen, Germany) with an attached high temperature conversion elemental analyzer (TC/EA). Samples were pyrolyzed at 1400°C to produce H_2 and CO. Resultant gases were separated on a 1-m, 0.25 in (outer diameter) molecular sieve 5Å gas chromatography column (Costech Analytical, Valencia, CA, USA). Water samples were introduced to the pyrolysis column using a PAL autosampler (LEAP Technologies, Carrboro, NC, USA). For hair and food, samples were stored on a zero-blank autosampler (Costech Analytical, Valencia, CA, USA), to ensure that water vapor did not exchange with the samples during analysis. Raw δ^2H and $\delta^{18}O$ measurement values for unknown samples were corrected to the VSMOW scale using a two-point correction with laboratory reference materials. The hydrogen and oxygen isotope ratios of our laboratory reference materials, ground horse hair (FH and UH), were −76.3 and 1.49 ‰ for FH and −141.6 and 5.7 ‰ for UH when calibrated in 2005 [Bowen et al., 2005a]. The analytical process included analyses of QC keratin specimens of known isotopic composition, analyzed also as unknown (its isotope values were −114 ‰ for δ^2H and 12.8 ‰ for $\delta^{18}O$). All QC analyses met the required specifications. The overall analytical precision for water analysis was ±1.55 ‰ and ±0.17 ‰ for H and O, respectively. Analytical precision for hair analysis, calculated from the measured hydrogen and oxygen isotope ratios of powdered keratin used as an internal secondary reference material was ±1.1 ‰ for H and ±0.23 ‰ for O ($n = 18$).

Stable isotope notation

Stable isotope abundances are reported in δ-notation as parts per thousands (‰), where

$$\delta_{\text{sample}} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000 \quad (1)$$

and R_{sample} and R_{standard} are the molar ratios of the rare-to-abundant isotope (e.g., $^2H/^1H$) in the sample and standard, respectively [Coplen, 2011]. The international standard for both hydrogen and oxygen stable isotope analysis is VSMOW.

Modeling

Body water modeling

We applied a mechanistic model describing the isotopic composition of body water to determine if we could accurately predict the H and O isotope ratios of body water of *M. fascicularis* (hereafter referred to as the Primate Body Water Model). Similar models have been constructed and then applied to reconstruct body water in both modern and paleoclimate conditions [Bryant & Froelich, 1995; Kohn, 1996; Luz & Kolodny, 1985] and to determine energy expenditure via the doubly labeled water technique [Gretebeck et al., 1997; Schoeller et al., 1986]. We constructed a body water model by modifying the parameters of Bryant & Froelich [1995] and Kohn [1996] to most closely approximate the physiological traits of nonhuman primates.

In brief, given the assumption that an animal is in isotopic equilibrium, the general mass balance equation of influxes and effluxes can be written as

$$\begin{aligned} &R^O_{dw}F^O_{dw} + R^O_{fw}F^O_{fw} + R^O_{food}F^O_{food} + R^O_{wvg}F^O_{wvg} + R^O_{air}F^O_{air} \\ &= \alpha^O_{co2}R^O_{bw}F^O_{co2} + \alpha^O_{bwv}R^O_{bw}F^O_{bwv} + \alpha^O_{twv}R^O_{bw}F^O_{twv} + R^O_{bw}F^O_{rw} \end{aligned} \quad (2)$$

for ^{18}O, and similarly

$$\begin{aligned} &R^H_{dw}F^H_{dw} + R^H_{fw}F^H_{fw} + R^H_{food}F^H_{food} + R^H_{wvg}F^H_{wvg} \\ &= \alpha^H_{bwv}R^H_{bw}F^H_{bwv} + \alpha^H_{twv}R^H_{bw}F^H_{twv} + R^H_{bw}F^H_{rw} \end{aligned} \quad (3)$$

for ^2H. Isotope abundances are expressed as ratios (R) of heavy-to-light isotopes, fluxes (F) are in units mole/day, and isotopic fractionation relative to body water is expressed as α. Oxygen and hydrogen influxes and effluxes considered include drinking water (*dw*), free water in food (*fw*),

TABLE I. Values Used in Body Water Modeling. Flux Value Ranges Represent Those Calculated for the Actual Mass Range in the Sample Set (2 kg–13 kg)

Influx (moles/day)	Oxygen flux	Hydrogen flux	Source
dw–drinking water	8.3–36.7	16.6–73.3	[Bryant & Froelich, 1995]
fw–food water	0	0	Measured
food–bound O and H	0.4–1.6	1.2–5.0	[Bryant & Froelich, 1995]
wvg–water vapor gain	0.2–0.8	0.4–1.5	[Bryant & Froelich, 1995]
air–atmospheric O	1.4–5.7	n/a	[Bryant & Froelich, 1995]
Efflux (moles/day)	Oxygen flux	Hydrogen flux	Source
CO_2–carbon dioxide	1.2–4.9	n/a	[Bryant & Froelich, 1995]
bwv–breath water vapor	1.3–5.1	2.5–10.2	[Kohn, 1996]
Twv–transcutaneous water vapor	0.8–2.9	1.6–5.8	[Schoeller et al., 1986]
rw–remaining water	7.0–32.0	14.0–64.0	Calculated
Fractionation factors	Oxygen	Hydrogen	Source
a_{co2}	1.038	n/a	[Chamberlain et al., 1997; Pflug et al., 1979]
a_{bwv}	0.991	0.946	[Hobson & Wassenaar, 1997; Horita & Wesolowski, 1994]
a_{twv}	0.981	0.935	[Hobson & Wassenaar, 1997; Horita & Wesolowski, 1994]
Isotope ratio (‰)	Oxygen casein, soy	Hydrogen casein, soy	Source
dw–drinking water	−5.8	−32.9	Measured
fw–food water	n/a	n/a	n/a
food–bound O and H	27.4–26.8	−116.1–119.8	Measured
wvg–water vapor gain	−15.3	−80.5	[Kohn, 1996]
air–atmospheric O	15.1	n/a	[Kohn, 1996]

oxygen and hydrogen bound in food (*food*), water vapor gain (*wvg*), atmospheric oxygen (*air*), carbon dioxide (CO_2), breath water vapor (*bwv*), transcutaneous water vapor (*twv*), and unfractionated remaining water loss (*rw*) which includes urine, fecal water, and sweat.

Solving for the isotope ratio of body water yields

$$R^O_{bw} = \frac{R^O_{dw}F^O_{dw}+R^O_{fw}F^O_{fw}+R^O_{food}F^O_{food}+R^O_{wvg}F^O_{wvg}+R^O_{air}F^O_{air}}{\alpha^O_{co2}F^O_{co2}+\alpha^O_{bwv}F^O_{bwv}+\alpha^O_{twv}F^O_{twv}+F^O_{rw}} \quad (4)$$

for oxygen, and

$$R^H_{bw} = \frac{R^H_{dw}F^H_{dw}+R^H_{fw}F^H_{fw}+R^H_{food}F^H_{food}+R^H_{wvg}F^H_{wvg}}{\alpha^H_{bwv}F^H_{bwv}+\alpha^H_{twv}F^H_{twv}+F^H_{rw}} \quad (5)$$

for hydrogen.

Influxes and effluxes of oxygen and hydrogen fall into two categories, those associated with free water (*dw*, *fw*, *wvg*, *bwv, twv*, *rw*) and those derived from the metabolism of carbohydrates, fats, and protein (*food, air, CO_2*). Oxidation reactions for glucose, palmitic acid, and alanine are considered first order representatives of carbohydrate, fat, and protein substrates [Bryant & Froelich, 1995]. The flux terms associated with metabolism are therefore functions of diet composition, specifically the fraction of energy derived from carbohydrates, fats, and protein, as well as total energy expenditure (TEE).

All parameters were either measured directly or estimated from directly measurable parameters (Table I). We used the equations described by Bryant & Froelich [1995] and Kohn [1996] to estimate the molar influxes and effluxes H_2O. Where it was possible, we used calculations specific to nonhuman primates to estimate physiological variables. For example, TEE was estimated using the following equation, formulated using the TEE for 19 species of primates [Key & Ross, 1999; Pontzer & Wrangham, 2004]:

$$\mathrm{TEE} = 93.3\ \mathrm{M}^{0.75} \quad (6)$$

where TEE is kCal/day and M is body mass in kilograms. Five of the females considered in this study were nursing during sample collection and thus TEE during lactation was calculated as 1.39 × TEE [Key & Ross, 1999]. Animals were housed in an indoor–outdoor facility with temperature-controlled indoor housing areas. Facility-recorded averages were used as a best guess estimate for temperature (21°C) and humidity (40%) inputs in the model. These estimates for temperature and humidity are not perfect, as animals are given access to a large outdoor enclosure when weather is good and outdoor measures of these variables change seasonally.

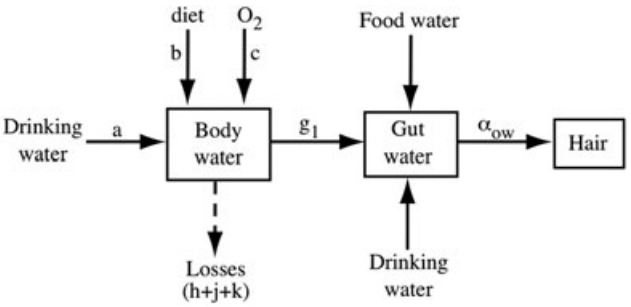

Fig. 1. Schematic of the flow of oxygen atoms into hair keratin as described by the Ehleringer et al. [2008] hair model. Model parameters are: a = Proportion of body water O derived from drinking water; b = Proportion of body water O derived from diet; c = Proportion of body water O derived from O_2; g_1 = Proportion of gut water derived from body water; α_{ow} = Fractionation of O between carbonyl oxygen and water; h = Proportion of O in body water that is lost as fractionated water associated with breathing and evaporation; j = Proportion of O in body water lost as CO_2; k = Proportion of O in body water that is lost as unfractionated water.

Hair modeling

The semimechanistic model for the hydrogen (H) and oxygen (O) isotopic composition of human hair was developed by Ehleringer et al. [2008] and later modified slightly by Bowen et al. [2009] to incorporate additional dietary considerations (hereafter referred to as "Hair Model"). These models estimate δ^2H and $\delta^{18}O$ of H and O atoms fixed in vivo by calculating an isotopic mass balance of H and O influxes (i.e., a, b, and c in Fig. 1) and effluxes (i.e., h, j, and k in Fig. 1). The models assume that the nonexchangeable H atoms in hair amino acids are a mixture of H atoms from dietary amino acids and amino acids synthesized internally which would have H isotopes reflecting body water. Oxygen atoms in hair are assumed to be the result of isotopic exchange with gut water during hydrolysis of dietary protein.

The controlled diet and environmental study system allowed us to constrain the ranges of possible values for two critical variables presented in the hair model for the estimation of the oxygen isotope ratios of hair ($\delta^{18}O_h$): the fractional contribution of body water to gut water (g_1) and the oxygen isotope fractionation associated with a carbonyl oxygen–water exchange in the gut (α_{ow}) (Fig. 1). Such calculations are possible for the flow of O atoms into hair keratin because of the (assumed) primary contribution of body water to the system. Similar calculations for hydrogen are currently not possible because the primary contribution of H atoms to keratin come from both essential (diet derived) and nonessential (synthesized in vivo) amino acids. The hair model assumes that oxygen atoms flow from the body water pool, to gut water (where isotopic exchange can occur), and into hair follicles where amino acids are condensed to produce hair keratin (Fig. 1). Body water is calculated as an essential intermediate in the hair model and is estimated based on data collected for modern humans [Gretebeck et al., 1997]. The model calculates the isotopic composition of gut water as a mixture of body water and water in food. The estimated contribution of body water to gut water (g_1) is poorly constrained by existing data, but a previous study [Malagelada et al., 1976] suggested a value of $g_1 = 0.57$. Using that value in the hair model developed by Ehleringer et al. [2008] provided reasonable results. Using the oxygen isotopic composition of body water and hair of nonhuman primates, we explore how variable settings of g_1 affect estimated values relative to measured hair stable isotope values.

If amino acids remain in a neutral pH environment following protein hydration in the gut, the O atoms in the carbonyl O should reflect the isotopic composition of gut water during digestion. This relationship is illustrated in the following equation from Ehleringer et al. [2008]

$$\delta^{18}O_h = \alpha^*_{ow}\left(1000 + \delta^{18}O_{wg}\right) - 1000 \quad (7)$$

where α_{ow} is the oxygen isotope fractionation associated with a carbonyl oxygen–water interaction in the gut. Based on experimental observations, [Kreuzer-Martin et al., 2003] calculated αow as 1.0164 for microbial spore cell walls during nonlog phase growth.

Assuming a rapid water exchange across intestinal walls and making g_1 equal to one, (therefore the isotopic composition of body water ($\delta^{18}O_{wb}$) is equal to gut water ($\delta^{18}O_{wg}$)), we are able to use the data collected for nonhuman primates to calculate the value of α_{ow} in this system.

$$\alpha_{ow} = (\delta^{18}O_h + 1000)/(\delta^{18}O_{wg(wb)} + 1000) \quad (8)$$

Statistical analysis

Data are presented as means ± 1 standard deviation unless stated otherwise. Welch two sample *t*-test was used to compare stable isotope values of samples from groups [Welch, 1947]. Data analysis and statistical calculations were conducted using *R* [R Development Core Team, 2008]. Statistical significance is reported as exact *P* values; significance level was set at $\alpha = 0.05$.

RESULTS

Measured Parameter Values

The δ^2H and $\delta^{18}O$ values of treatment diets were similar. The δ^2H values of the casein and soy diets were -116.1 ± 0.7 ‰ and -119.8 ± 0.5 ‰, respectively, and the $\delta^{18}O$ values of the casein and soy diets were 27.4 ± 0.2 ‰ and 26.8 ± 0.7 ‰, respectively. Animals in both dietary groups had ad libitum access to the same water source (δ^2H value of -32.9 ± 2.1 ‰ and $\delta^{18}O$ value of -5.8 ± 0.2 ‰).

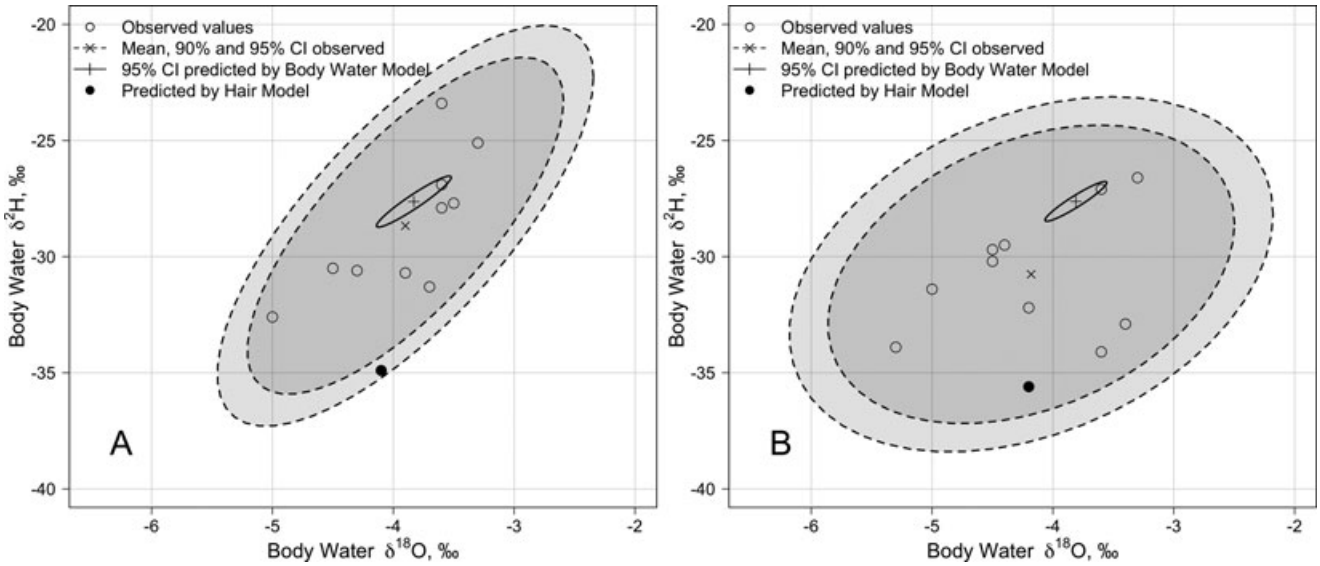

Fig. 2. Measured and predicted δ^2H and δ^{18}O values (‰) of body water from nonhuman primates fed two different diets, (**A**) TAD casein and (**B**) TAD soy. Open circles represent measured values. Ellipses are the normal probability contours of the isotope distributions for the measured (dashed lines) and predicted (solid line) values by the Primate Body Water Model. Filled circle represents the body water value predicted as an intermediate step in the Hair Model.

The δ^2H values of the body water of primates fed TAD-casein and TAD-soy were -28.7 ± 3.0 ‰ and 30.8 ± 2.6 ‰, respectively. These δ^2H values were not statistically different ($t = 1.7$, $P = 0.11$, df = 17.7). The δ^{18}O values of the body water of primates fed TAD-casein and TAD-soy were -3.9 ± 0.5 ‰ and -4.2 ± 0.7 ‰, respectively. These δ^{18}O values were not statistically different from each other ($t = 1.02$, $P = 0.32$, df = 17.0).

As would be predicted by the hair model, given similar body water values of animals in the two dietary groups, there was no statistically significant difference in the isotopic composition of hair between the two dietary groups. The δ^2H values of the hair of primates on the casein diet were -90.4 ± 2.8 ‰ and -89.5 ± 1.6 ‰ for those on the soy diet ($t = -0.83$, $P = 0.42$, df = 15.0). The δ^{18}O values of hair of primates fed TAD-casein and TAD-soy were 12.1 ± 0.6 ‰ and 12.4 ± 0.5 ‰, respectively ($t = -1.40$, $P = 0.18$, df = 16.4).

Model Predictions

Body water and Hair Model predictions

The δ^2H and δ^{18}O values of body water, predicted by the Primate Body Water Model, for animals on the casein and soy diet were not statistically different from the measured values (Fig. 2; Table II; TAD-casein measured δ^2H vs. predicted δ^2H values $t = -1.1$, $P = 0.30$, df = 9.3; TAD-casein measured δ^{18}O vs. predicted δ^{18}O values $t = -0.41$, $P = 0.70$, df = 9.71; TAD-soy measured δ^2H vs. predicted δ^2H values $t = -3.76$, $P = 0.05$, df = 9.2; TAD-soy measured δ^{18}O vs. predicted δ^{18}O values $t = -1.70$, $P = 0.12$, df = 9.3). The body water values predicted as an intermediate step in the Hair Model were contained within the 95% confidence interval (CI) of the observed isotope distributions (Fig. 2); although, for the casein diet, the predicted value was not contained within the 90% CI (Fig. 2A).

The fractional contribution of body water to the gut water pool is represented in the Hair Model by the term g_1. Ehleringer et al. [2008] set the value for g_1 to 0.57 in agreement with a previously published observation [Malagelada et al., 1976]. Here, we tested Hair Model predictions for $g_1 = 0.57$ and $g_1 = 1.0$, which seemed more biologically reasonable given the rapid exchange across intestinal walls. Finally, leaving $g_1 = 1.0$, we combined the body water and hair models, using body water model estimates to predict the isotopic composition of hair.

TABLE II. Measured and Predicted δ^2H and δ^{18}O Values (‰) of Body Water from Nonhuman Primates.

	measured body water		predicted using body water model		predicted using hair model	
Diet	δ^2H	δ^{18}O	δ^2H	δ^{18}O	δ^2H	δ^{18}O
Casein	-28.7 ± 3.0	-3.9 ± 0.6	-27.6 ± 0.4	-3.8 ± 0.1	-34.9	-4.1
Soy	-30.8 ± 2.6	-4.2 ± 0.7	-27.6 ± 0.3	-3.8 ± 0.1	-35.6	-4.2

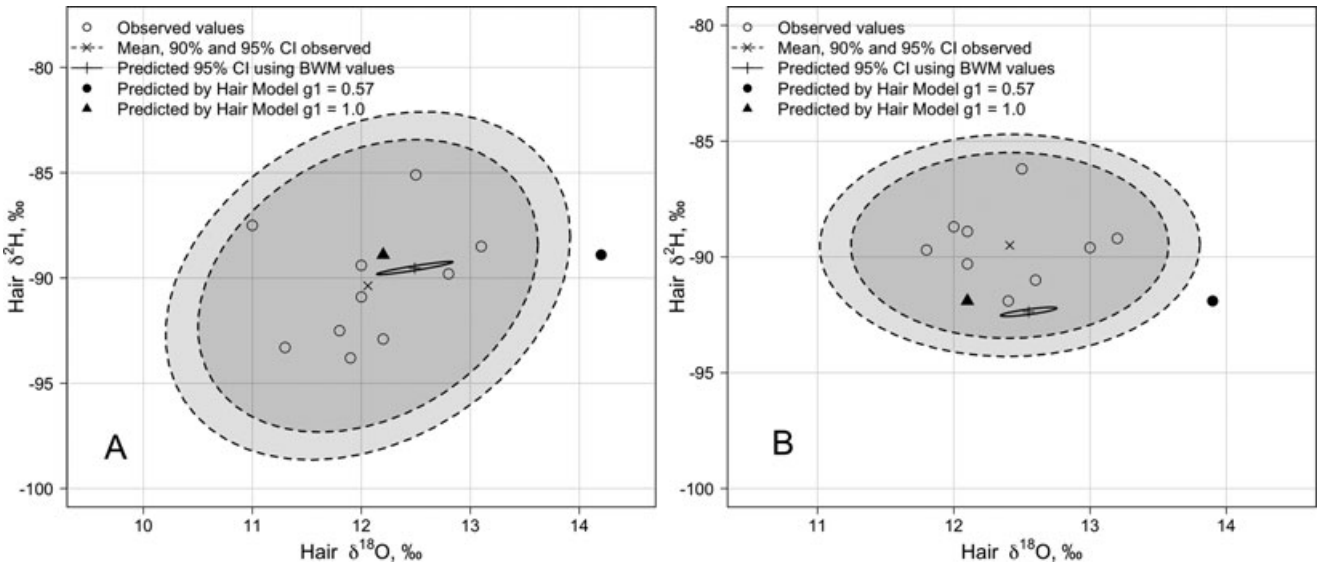

Fig. 3. Measured and predicted δ^2H and $\delta^{18}O$ values (‰) of hair from nonhuman primates fed two different diets, (**A**) TAD casein and (B) TAD soy. Open circles represent measured values. Ellipses are the normal probability contours of the isotope distributions for the measured (dashed lines) and predicted (solid line) values using the Body Water Model estimates as an intermediate step. The filled circle and triangle represent the hair values predicted by the Hair Model with $g_1 = 0.57$ and 1.0, respectively.

The δ^2H and $\delta^{18}O$ values predicted by the Hair Model using $g_1 = 0.57$ were not contained within the 95% CI of the observed isotope distributions (Fig. 3; Table III). When g_1 was set to 1.0, the predicted values for both diets were contained within the 90% CI of the distributions and were similar to the measured observed isotope values (Fig. 3; Table III). The δ^2H and $\delta^{18}O$ values of hair predicted using the combined Hair Model and Body Water Model for animals on two different diets were not statistically different from the observations (Fig. 3; Table III; TAD-casein measured δ^2H vs. predicted δ^2H values $t = -0.93$, $P = 0.37$, df = 9.0; TAD-casein measured $\delta^{18}O$ vs. predicted $\delta^{18}O$ values $t = -2.10$, $P = 0.06$, df = 9.6; TAD-soy measured δ^2H vs. predicted δ^2H values $t = 4.25$, $P = 0.048$, df = 12.0); TAD-soy measured $\delta^{18}O$ vs. predicted $\delta^{18}O$ values $t = -0.88$, $P = 0.40$, df = 8.3).

Calculating the carbonyl oxygen–water fractionation factor in the gut (α_{ow})

In setting $g_1 = 1$, we effectively are stating that the isotopic composition of body water ($\delta^{18}O_{wb}$) was equal to gut water ($\delta^{18}O_{wg}$). This assumption allowed us to calculate α_{ow}, or the ^{18}O fractionation associated with a carbonyl oxygen–water interaction in the gut. We calculated α_{ow} to be 1.0164 ± 0.0005 in this nonhuman primate system, which is in close agreement with the calculation of Kreuzer-Martin et al. [2003] in microbial spore cell walls. This suggests robustness in this fractionation value across biological organisms.

DISCUSSION

Using body water and hair samples from a nonhuman primate system, we evaluated the robustness of semimechanistic models of the δ^2H and $\delta^{18}O$ values of body water [Bryant & Froelich, 1995; Kohn, 1996] and hair [Bowen et al., 2009; Ehleringer et al., 2008; Podlesak et al., 2008]. Both models performed well (Tables II and III; Fig. 2 and 3), suggesting that broader applications of the models to other primates and to reconstructing human dietary patterns and movements may be justified. A significant improvement in model predictions came as a result of

TABLE III. Measured and Predicted δ^2H and $\delta^{18}O$ Values (‰) of Hair from Nonhuman Primates.

Diet	Measured hair		Predicted using hair model			Predicted using body water Model intermediate value	
	δ^2H	$\delta^{18}O$	δ^2H	$g_1 = 0.57$ $\delta^{18}O$	$g_1 = 1.0$ $\delta^{18}O$	δ^2H	$\delta^{18}O$
Casein	-90.4 ± 2.8	12.1 ± 0.6	-88.9	14.2	12.2	-89.5 ± 0.1	12.5 ± 0.1
Soy	-89.5 ± 1.6	12.4 ± 0.5	-91.9	13.9	12.1	-92.4 ± 0.1	12.5 ± 0.1

describing gut water as isotopically similar to that of body water, which makes biological sense because of rapid water exchanges across the intestinal walls, and in this system because of the low water content of the food. There were no notable improvements to the δ^2H and $\delta^{18}O$ estimates of hair keratin when the outputs of the Primate Body Water Model were used in place of body water calculations within the Hair Model (when g_1 was equal to 1).

The incorporation of physiological (i.e., mass, metabolic rate, water intake) and dietary details (i.e., macronutrient density, food water content) into body water modeling allows for the impact of variables to be isolated. For example, O'Grady et al. [2010] demonstrated elevated water fluxes associated with diabetes could be detected through measurement of the isotopic composition of body water. In this study, the isotopic values of body water from streptozotocin (STZ)-induced diabetic mice approached that of drinking water, due to abnormally high rates of water flux. O'Grady et al. [2010] were able to predict the H and O isotope values of body water in control versus diabetic mice modifying only water flux rates in the body water model. Defining the influence of particular variables, such as water flux, on the isotopic composition of body water may improve explanations of within-population variance, where diet and water inputs are similar.

In addition to evaluating the robustness of the body water and hair models, the results of this study indicate that estimates of the $\delta^{18}O$ values of hair were improved by increasing the fractional contribution of body water to gut water (g_1) from 0.57 to 1.0. This model parameter adjustment suggests that the gut water pool is similar in composition to body water in nonhuman primates. Given the physiological similarities between human and nonhuman primates, it is probable that model predictions for human hair would be improved if g_1 were close to or equal to 1.0. By defining g_1 as 1.0, we were able to calculate the oxygen–isotope fractionation associated with the carbonyl oxygen–water interaction in the gut, α_{ow}. Interestingly, our calculated value for this nonhuman primate system (1.0164) agreed well with the calculation of α_{ow} for microbial spore cell walls [Kreuzer-Martin et al., 2003].

We found that current models for both the isotopic composition of body water and hair are accurate for the population of nonhuman primates considered. Investigating within-population variance in the isotopic composition of primate body water and hair would require more detailed information about the individuals studied and their environment. While much of isotopic modeling has been done using unconstrained systems, controlled study systems can shed light on many parameter estimates that cannot be measured directly. Further constraining model variables will only improve model estimates and simultaneously increase knowledge of the metabolic processes involved in shaping the flow of hydrogen and oxygen atoms into biologic tissues.

ACKNOWLEDGMENTS

We would like to acknowledge two reviewers that comment on an earlier version of the manuscript. We would like to thank Melissa Ayers and Joel Collins for assistance with sample collection and Anthony N. Macharia for initial processing of food samples. We would like to thank all SIRFER members for their help during isotope analyses. We also thank Ellen Miller. Funding for this study was provided by an IC postdoctoral fellowship [SPO] and a National Center for Research Resources (P40 RR021380 [JDW]). CHR was funded by NSF-RTG. The monkeys from which samples were obtained are part of a National Center for Research Resources (P40 RR021380) supported cynomolgus macaque (*M. fascicularis*) breeding colony.

REFERENCES

Ayliffe LK, Chivas AR. 1990. Oxygen isotope composition of the bone phosphate of Australian kangaroos: potential as a palaeoenvironmental recorder. Geochim Cosmochim Acta 54:2603–2609.

Bedaso Z, Wynn JG, Alemseged Z, Geraads D. 2010. Paleoenvironmental reconstruction of the Asbole fauna (Busidima Formation, Afar, Ethiopia) using stable isotopes. Geobios 43:165–177.

Bol R, Marsh J, Heaton THE. 2007. Multiple stable isotope (^{18}O, ^{13}C, ^{15}N and ^{34}S) analysis of human hair to identify the recent migrants in a rural community in SW England. Rapid Commun Mass Sp 21:2951–2954.

Bowen GJ. 2010. Isoscapes: spatial pattern in isotopic biogeochemistry. Ann Rev Earth Pl Sc 38:161–187.

Bowen GJ, Chesson L, Nielson K, Cerling TE, Ehleringer JR. 2005a. Treatment methods for the determination of δ^2H and $\delta^{18}O$ of hair keratin by continuous-flow isotope-ratio mass spectrometry. Rapid Commun Mass Sp 19:2371–2378.

Bowen G, Ehleringer J, Chesson L, Stange E, Cerling T. 2007. Stable isotope ratios of tap water in the contiguous United States. Water Resour Res 43:W03419:1–12.

Bowen GJ, Ehleringer JR, Chesson LA, Thompson AH, Podlesak DW, Cerling TE. 2009. Dietary and physiological controls on the hydrogen and oxygen isotope ratios of hair from mid-20th century indigenous populations. Am J Phys Anthropol 139:494–504.

Bowen GJ, Wassenaar LI, Hobson KA. 2005b. Global application of stable hydrogen and oxygen isotopes to wildlife forensics. Oecologia 143:337–348.

Brattström O, Wassenaar LI, Hobson KA, Åkesson S. 2008. Placing butterflies on the map – testing regional geographical resolution of three stable isotopes in Sweden using the monophagus peacock Inachis io. Ecography 31:490–498.

Bryant JD, Froelich PN. 1995. A model of oxygen isotope fractionation in body water of large mammals. Geochim Cosmochim Acta 59:4523–4537.

Cerling TE, Harris J, Leakey M, Mudida N. 2003. Stable isotope ecology of northern Kenya with emphasis on the Turkana Basin. In: Leakey MG, Harris JM, editors. Lothagam: the dawn of humanity in Eastern Africa. New York: Columbia University Press. p 583–603.

Cerling TE, Hart JA, Hart TB. 2004. Stable isotope ecology in the Ituri Forest. Oecologia 138:5–12.

Cerling TE, Mbua E, Kirera FM, Manthi FK, Grine FE, Leakey MG, Sponheimer M, Uno KT. 2011. Diet of Paranthropus boisei in the early Pleistocene of East Africa. Proceedings of the National Academy of Sciences 108:9337–9341.

Cerling TE, Wittemyer G, Rasmussen HB, Vollrath F, Cerling CE, Robinson TJ, Douglas-Hamilton I. 2006. Stable isotopes in elephant hair document migration patterns and diet changes. Proceedings of the National Academy of Sciences of the United States of America 103:371–373.

Chamberlain CP, Blum JD, Holmes RT, Feng X, Sherry TW, Graves GR. 1997. The use of isotope tracers for identifying populations of migratory birds. Oecologia 109:132–141.

Chenery CA, Lamb AL, O'Regan HJ, Elton S. 2011. Multitissue analysis of oxygen isotopes in wild rhesus macaques (*Macaca mulatta*). Rapid Commun Mass Sp 25:779–788.

Chesson LA, Podlesak DW, Cerling TE, Ehleringer JR. 2009. Evaluating uncertainty in the calculation of nonexchangeable hydrogen fractions within organic materials. Rapid Commun Mass Sp 23:1275–1280.

Coplen TB. 2011. Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. Rapid Commun Mass Sp 25:2538–2560.

Craig H. 1961. Isotopic variations in meteoric waters. Science 133:1702–1703.

Dansgaard W. 1954. The O18-abundance in fresh water. Geochim Cosmochim Acta 6:241–260.

Daux V, Lécuyer C, Héran M-A, Amiot R, Simon L, Fourel F, Martineau F, Lynnerup N, Reychler H, Escarguel G. 2008. Oxygen isotope fractionation between human phosphate and water revisited. J Hum Evol 55:1138–1147.

Ehleringer JR, Bowen GJ, Chesson LA, West AG, Podlesak DW, Cerling TE. 2008. Hydrogen and oxygen isotope ratios in human hair are related to geography. Proceedings of the National Academy of Sciences of the United States of America 105:2788–2793.

Fourie NH, Lee-Thorp JA, Ackermann RR. 2008. Biogeochemical and craniometric investigation of dietary ecology, niche separation, and taxonomy of Plio-Pleistocene cercopithecoids from the Makapansgat Limeworks. Am J Phys Anthropol 135:121–135.

Fraser I, Meier-Augenstein W, Kalin RM. 2006. The role of stable isotopes in human identification: a longitudinal study into the variability of isotopic signals in human hair and nails. Rapid Commun Mass Sp 20:1109–1116.

Gretebeck RJ, Schoeller DA, Socki RA, Davis-Street J, Gibson EK, Schulz LO, Lane HW. 1997. Adaptation of the doubly labeled water method for subjects consuming isotopically enriched water. J Appl Physiol (Bethesda, Md.: 1985) 82:563–570.

Hobson KA, Bowen GJ, Wassenaar LI, Ferrand Y, Lormee H. 2004. Using stable hydrogen and oxygen isotope measurements of feathers to infer geographical origins of migrating European birds. Oecologia 141:477–488.

Hobson KA, Wassenaar LI. 1997. Linking breeding and wintering grounds of neotropical migrant songbirds using stable hydrogen isotopic analysis of feathers. Oecologia 109:142–148.

Horita J, Wesolowski DJ. 1994. Liquid-vapor fractionation of oxygen and hydrogen isotopes of water from the freezing to the critical temperature. Geochim Cosmochim Acta 58:3425–3437.

Kendall C, Coplen TB. 2001. Distribution of oxygen-18 and deuterium in river waters across the United States. Hydrol Processes 15:1363–1393.

Key C, Ross C. 1999. Sex differences in energy expenditure in non-human primates. Proc R Soc Lond B Biol Sci 266:2479–2485.

Kohn M. 1996. Predicting animal $\delta^{18}O$: Accounting for diet and physiological adaptation. Geochim Cosmochim Acta 60:4811–4829.

Kreuzer-Martin HW, Lott MJ, Dorigan J, Ehleringer JR. 2003. Microbe forensics: oxygen and hydrogen stable isotope ratios in *Bacillus subtilis* cells and spores. Proceedings of the National Academy of Sciences of the United States of America 100:815–819.

Levin NE, Cerling TE, Passey BH, Harris JM, Ehleringer JR. 2006. A stable isotope aridity index for terrestrial environments. Proceedings of the National Academy of Sciences 103:11201–11205.

Luz B, Kolodny Y. 1985. Oxygen isotope variations in phosphate of biogenic apatites, IV. Mammal teeth and bones. Earth Planet Sci Lett 75:29–36.

Malagelada JR, Longstreth GF, Summerskill WH, Go VL. 1976. Measurement of gastric functions during digestion of ordinary solid meals in man. Gastroenterology 70:203–210.

Meier-Augenstein W, Fraser I. 2008. Forensic isotope analysis leads to identification of a mutilated murder victim. Sci Justice 48:153–159.

Mützel Rauch E, Lehn C, Peschel O, Hölzl S, Rossmann A. 2009. Assignment of unknown persons to their geographical origin by determination of stable isotopes in hair samples. Int J Legal Med 123:35–40.

Nardoto GB, Silva S, Kendall C, Ehleringer JR, Chesson LA, Ferraz ESB, Moreira MZ, Ometto JPHB, Martinelli LA. 2006. Geographical patterns of human diet derived from stable-isotope analysis of fingernails. Am J Phys Anthropol 131:137–146.

O'Connell TC, Hedges REM. 1999. Isotopic comparison of hair and bone: archaeological analyses. J Archaeol Sci 26:661–665.

O'Grady SP, Wende AR, Remien CH, Valenzuela LO, Enright LE, Chesson LA, Abel ED, Cerling TE, Ehleringer JR. 2010. Aberrant water homeostasis detected by stable isotope analysis. PLoS ONE 5:e11699:1–7.

Pflug K, Schuster K, Pichotka J, Forstel H. 1979. Fractionation effects of oxygen isotopes in mammals. In: Stable isotopes: proceedings of the third international conference. New York: Academic Press. p 553–561.

Podlesak DW, Torregrossa AM, Ehleringer JR, Dearing MD, Passey BH, Cerling TE. 2008. Turnover of oxygen and hydrogen isotopes in the body water, CO2, hair, and enamel of a small mammal. Geochim Cosmochim Acta 72:19–35.

Pontzer H, Wrangham RW. 2004. Climbing and the daily energy cost of locomotion in wild chimpanzees: implications for hominoid locomotor evolution. J Hum Evol 46:315–333.

Quade J, Cerling TE, Andrews P, Alpagut B. 1995. Paleodietary reconstruction of Miocene faunas from Paşalar, Turkey using stable carbon and oxygen isotopes of fossil tooth enamel. J Hum Evol 28:373–384.

R Development Core Team. 2008. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Available from: http://www.R-project.org.

Ramos R, González-Solís J, Ruiz X. 2009. Linking isotopic and migratory patterns in a pelagic seabird. Oecologia 160:97–105.

Rubenstein DR, Hobson KA. 2004. From birds to butterflies: animal movement patterns and stable isotopes. Trends Ecol Evol 19:256–263.

Schoeller DA, Ravussin E, Schutz Y, Acheson KJ, Baertschi P, Jequier E. 1986. Energy expenditure by doubly labeled water: validation in humans and proposed calculation. Am J Physiol - Regul, Integr Comp Physiol 250:R823–R830.

Sharp ZD, Atudorei V, Panarello HO, Fernández J, Douthitt C. 2003. Hydrogen isotope systematics of hair: archeological and forensic applications. J ArchaeolSci 30:1709–1716.

Smith CC, Morgan ME, Pilbeam D. 2010. Isotopic ecology and dietary profiles of Liberian chimpanzees. J Hum Evol 58:43–55.

Sponheimer M, Lee-Thorp JA. 2001. The oxygen isotope composition of mammalian enamel carbonate from Morea Estate, South Africa. Oecologia 126:153–157.

Thompson AH, Chesson LA, Podlesak DW, Bowen GJ, Cerling TE, Ehleringer JR. 2010. Stable isotope analysis of modern human hair collected from Asia (China, India, Mongolia, and Pakistan). Am J Phys Anthropol 141:440–451.

Wagner JD, Jorgensen MJ, Cline JM, Lees CJ, Franke AA, Zhang L, Ayers MR, Schultz C, Kaplan JR. 2009. Effects of soy vs. casein protein on body weight and glycemic control in female monkeys and their offspring. Am J Primatol 71:802–811.

Wassenaar LI, Hobson KA. 1998. Natal origins of migratory monarch butterflies at wintering colonies in Mexico: new isotopic evidence. Proc Nat Acad Sci USA 95:15436–15439.

Welch BL. 1947. The generalization of "student's" problem when several different population varlances are involved. Biometrika 34:28–35.

West JB, Bowen GJ, Cerling TE, Ehleringer JR. 2006. Stable isotopes as one of nature's ecological recorders. Trends Ecol Evol 21:408–414.

West JB, Bowen GJ, Dawson TE, editors. 2010. Isoscapes: understanding movement, pattern, and process on earth through isotope mapping. New York, NY: Springer. p. 495.

White TD, Ambrose SH, Suwa G, Su DF, DeGusta D, Bernor RL, Boisserie J-R, Brunet M, Delson E, Frost S, et al. 2009. Macrovertebrate paleontology and the pliocene habitat of Ardipithecus ramidus. Science 326:67–93.

Yeakel JD, Bennett NC, Koch PL, Dominy NJ. 2007. The isotopic ecology of African mole rats informs hypotheses on the evolution of human diet. Proc R Soc B Biol Sci 274:1723–1730.