Seasonal variation in natural abundance of $^2$H and $^{18}$O in urine samples from rural Nigeria

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THE DOUBLY LABELED WATER (DLW) method, developed by Lifson in the late 1940s, has been utilized for over 60 years to measure free-living energy expenditure in humans. Inherent to this technique is the assumption that natural abundances of stable isotopes $^2$H and $^{18}$O in body water remain constant over the course of the measurement period and after elimination of the loading dose of DLW will return to the same predose level. To determine variability in the natural abundances of $^2$H and $^{18}$O in humans living in a region with seasonal shifts in rain patterns and sources of drinking water, over the course of 12 mo we collected weekly urine samples from four individuals living in southwest Nigeria as well as samples of their drinking water. From ongoing regional studies of hypertension, obesity, and energy expenditure, we estimated average water turnover rate, urine volumes, and sodium and potassium excretion. Results suggest that $^2$H and $^{18}$O in urine, mean concentrations of urinary sodium and potassium, urine volume, and total body turnover differed significantly from dry to rainy season. Additionally, seasonal weather variables (mean monthly maximum temperatures, total monthly rainfall, and minimum relative humidity) were all significantly associated with natural abundances in urine. No seasonal difference was observed in drinking water samples. Findings suggest that natural abundances in urine may not remain constant as assumed, and studies incorporating DLW measurements across the transition of seasons should interpret results with caution unless appropriate doses of the tracers are used.

doubly labeled water; seasonal variation; Nigeria

$^{18}$O is a measure of CO$_2$ flux, thereby allowing for the calculation of TEE (30).

One of the key assumptions of the DLW method is that the natural abundances, i.e., baseline concentrations, of the stable isotopes $^2$H and $^{18}$O are constant in an animal’s body throughout the measurement period, i.e., typically 1–2 wk for human studies, and that body water will return to the same predose abundances after the elimination of the loading dose (22). In reality, natural abundances of the isotopes in a body vary with the source and amount of water and food consumed, as well as with changes in the proportions of oxygen and hydrogen lost through CO$_2$ production and the ratio of fractionated and nonfractionated water effluxes. Increases or decreases in isotope abundances can occur when water intake varies or when there is a change in water source. Changes in natural abundances may introduce error into the calculation of TEE (12). Of concern for human studies is when the natural abundance of one or both of the isotopes changes during the course of a measurement period. A stark example is that of measuring energy requirements during space flight. It is well established that astronauts’ drinking water has unusual isotopic abundances, and those changes in isotopic baseline must be made before or during the calculation of TEE (14, 33).

There is also the potential for changing isotopic baseline as a result of dramatic seasonal effects, resulting in sudden changes in sources of drinking water, such as those that can occur in regions experiencing clearly demarcated rainy and dry seasons. We sought to determine whether the season of DLW measurement in the tropics, i.e., rainy or dry, affects the natural abundances of deuterium and $^{18}$O and whether or not changes in water source could explain any identified effect. Using data collected over the course of two research studies conducted in rural southwestern Nigeria, we documented natural abundance of the isotopes in urine over the course of a year, average water turnover rates (8, 19), and average urine volumes by season (34).

METHODS

In conjunction with a concurrent study of TEE and weight change among rural Nigerians (8), weekly drinking water and urine samples were collected over the course of a year by four volunteers residing in the same community. The TEE and weight change study also allowed the calculation of water turnover rates by season. Urine volume and electrolyte concentrations were obtained from a study on sodium and blood pressure conducted in the same community (34). The studies were approved by the Institutional Review Board of Loyola University Chicago, Stritch School of Medicine, and the Ethics Committee of

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the University College Hospital, University of Ibadan (Nigeria); written, informed consent was obtained from all participants.

The studies were conducted in a village in rural southwest Nigeria, Igbo-Ora, located at latitude 7°N, where Loyola University Chicago Department of Public Health Sciences maintained a free-standing research site from the mid-1990s to 2009 (20). Drinking water for residents of the village is obtained from three primary sources: wells, boreholes, and collected rainwater. The source of drinking water tends to vary seasonally, with wells and collected rainwater being predominant during the rainy season and boreholes used more during the dry season. Nigeria has a tropical climate marked by consistently high temperatures (34/19°C, max/min) and wet and dry seasons associated with the Intertropical Convergence Zone. Whereas much of the country will have one rainy and dry season annually, in the southwestern part of the country (including the study area), a short decline in rainfall and intensity can occur in late July and August because of the Intertropical Convergence Zone (1, 23). The result is a long rainy season approximately from March to early July, a short dry period (or “little dry season”) in late July and August, a shorter rainy period in September and October, and a long dry season from November to February. For the year of data collection, there was no observable little dry season.

Four participants from the two studies collected weekly samples of both their drinking water and urine between October 1, 2001 and September 16, 2002; samples were not collected during the month of December 2001 and half of January 2002, as the research staff was on holiday, resulting in a total of 40 wk of sample collection. Participants also recorded the source of the drinking water sample weekly, i.e., well, borehole, or collected rainwater. The first urine voided on Monday mornings was collected along with a sample of the current source of drinking water. Duplicate 5-ml aliquots of each sample were prepared and stored in cryovials with Teflon O-rings. Samples were frozen at −4°C and stored at the University College Hospital in Ibadan until being shipped to the University of Wisconsin, Madison for analysis in October 2002.

Drinking water and urine samples from every second week were analyzed for abundance of deuterium and 18O at the Stable Isotope Core Laboratory in the Department of Nutritional Sciences, University of Wisconsin, Madison. Both urine and water samples were treated with 200 mg carbon black and filtered using 0.45-μm sterile filters. Isotopes were analyzed using isotope ratio mass spectrometry as previously published (12). Daily temperatures and rainfall estimates during the same time period were recorded by two separate observers in the villages. These data were verified, and monthly averages of rainfall, maximum temperature, minimum temperature, minimum relative humidity, as well as the dates for the initiation and cessation of the annual rains were obtained from the Statistics Section of the International Institute of Tropical Agriculture, Ibadan, Nigeria, located ~60 km from Igbo-Ora.

The average rate of water turnover by season was estimated using data from the parent project in which TEE was measured using DLW in 149 women (8). Total water turnover was calculated as the product of deuterium elimination rate (kD) and the deuterium dilution space (ND), and corrected for isotope fractionation (FD). The average value of FD (9) was 0.98. Volume of urine produced and total urinary sodium (Na+) and potassium (K+) excretion were obtained during a separate project on hypertension and electrolyte excretion (34) in 756 adults from the same village. Mean volumes and electrolyte excretion of three 24-h urine collections were used in the present analyses.

Data were analyzed using STATA (ver 12.0; College Station, TX). Average deuterium and 18O abundances for both urine and drinking water samples were calculated by season and expressed relative to standard mean ocean water (6). Covariation of the isotopic abundances over the course of the year was defined as having an intradividual Pearson correlation coefficient ≥0.75 (12). Student’s t-tests were used to compare isotopic abundances, water turnover rates, urine volumes, and electrolyte concentrations between the rainy and dry seasons.

RESULTS

The four participants (3 men and 1 woman), were 57.5 ± 6.5 yr old and all resided in the village for the entire study period. For each participant, 21 urine and 21 drinking samples were analyzed. The natural abundances of deuterium and 18O of these urine samples are presented in Fig. 1. Although it appears that there are two outliers (one for subject 1 and the other for subject 4), there was no known physiological or environmental explanation, and they were included in all analyses. On the basis of daily rainfall patterns as recorded by local volunteers, March 12, 2002 was designated as the start of the rainy season; thus samples collected between October 1, 2001 and March 11, 2002 were determined to be from the dry season, and those from March 12, 2002 to the end of the study were from the rainy season. There was a marked difference in rainfall pattern indicating the occurrence of one short dry season (Fig. 2); therefore, a single dry and single rainy season were defined for analyses (October 1 to March 11 and March 12 to September

Fig. 1. Deuterium and 18O abundances in urine from 4 adults in rural southwest Nigeria sampled between October 1, 2001 and September 16, 2002. The slope coefficients ± SE for participants 1 to 4 were 6.00 ± 0.29, 3.84 ± 0.22, 5.52 ± 0.26, and 5.35 ± 0.50, respectively, per ml, parts per thousand.
The mean abundances for both deuterium and $^{18}$O for the urine and two drinking water sources are presented by season in Table 1. Both isotopes were significantly more enriched in the urine samples during the dry compared with the rainy season ($P < 0.001$). The greatest variance in the urinary isotopes appeared to occur from January to May when the dry season was ending and the rains began to increase (Fig. 2). Mean monthly abundances of both deuterium and $^{18}$O in urine were also positively associated with mean monthly maximum temperatures ($r = 0.55$, $P = 0.08$ and $r = 0.86$, $P < 0.001$ respectively) and significantly inversely associated with rainfall ($-0.74$ and $-0.79$; $P < 0.001$) and minimum relative humidity ($-0.87$ and $-0.91$; $P < 0.001$).

The mean rate of total body water turnover was significantly higher ($P < 0.001$) in the dry season (0.13 ± 0.02 pools/day) than in the rainy season (0.11 ± 0.02 pools/day; $P < 0.001$), as was absolute volume of daily water turnover, 3.8 ± 0.8 vs. 3.5 ± 0.7 l ($P < 0.02$). Average total urine volumes were significantly lower in the dry (1.34 ± 0.50 l/day) compared with the rainy season (1.53 ± 0.50 l/day; $P < 0.001$). Whereas urinary sodium concentrations were not different between dry and rainy seasons (117 ± 4 vs. 121 ± 2 meq/day, respectively), urinary potassium was significantly higher in the dry season, 49.9 ± 1.8 vs. 43.1 ± 0.9 meq/day ($P < 0.001$). Both the higher urinary potassium concentrations and a lower urine volume/water turnover rate indicate more concentrated urine being observed during the dry season.

In Fig. 3, the difference between the abundance of the isotopes in urine and drinking water is plotted against the rate of water turnover. Although the rate of water turnover only explains 25% of the variation in deuterium abundance and 7% of the $^{18}$O abundance, these data support the concept that baseline urinary isotope abundances reflect water turnover.

**DISCUSSION**

The natural abundances of deuterium and $^{18}$O in humans, as measured in urine, do vary significantly between dry and rainy seasons in this tropical region, with both being more enriched during the dry season. On the basis of our findings, the observed urinary differences in abundance between the seasons are not due to changes in the isotopic abundance of drinking water but rather likely due to changes in water turnover that are potentially influenced by environmental factors, including temperature and relative humidity (28).

The DLW method has been widely used to measure TEE in multiple populations around the world; it has also been used to establish energy intake requirements for individuals of all ages and phase of life. Because the technique is based on the assumption that natural abundances within the body remain constant throughout the measurement period, knowing if and
possibly when baseline abundances vary could better inform the use of this method in the tropics. Rural southwest Nigeria is a compelling area to evaluate natural abundances of stable isotopes because of its relatively warm year-round temperatures and distinct seasonal changes in rainfall and sources of drinking water. As hypothesized, measured natural abundances of deuterium and \(^{18}\)O did indeed differ significantly by season, however, only in urine and not well and borehole drinking water sources. It is unknown whether the addition of rainwater as a drinking water source during the rainy season may have played a role in the observed seasonal difference urine isotopes, as so few samples of rainwater were collected in this study. In studies of water movement and budget, the isotopic variance in rainwater is used to identify water from a precipitation event as opposed to ground water (11, 16). This suggests that, when used as a primary water source during only one season, rainwater might be associated with a shift in a body’s natural abundances of deuterium and \(^{18}\)O.

During the study period, it appeared as if the greatest variance in urinary isotopes from the four participants occurred during the transition from dry to rainy seasons when rains began to increase in intensity. The weather-related variables of mean monthly maximum temperatures, rainfall, and minimum relative humidity were all significantly associated with natural abundance in urine and provide additional evidence that, with the climatic region of the study, seasonal weather patterns over the year can affect isotope abundance within the body. Physiologically, weather can influence daily obligatory water loss by increasing transcutaneous evaporation during extended periods of high temperatures and low humidity and as a result may impact deuterium and \(^{18}\)O abundances in the body. This would also explain the seasonal differences in turnover rate, urine volume, and K⁺ concentrations observed in the study. In studies of the isotopic compositions of water, the most significant fractionalations are associated with phase changes (i.e., condensation and evaporation) (16), and it would thus follow that such variations could similarly occur as those phase changes manifest from the human body. Obviously a larger number of study participants could help clarify the relationship between our measured environmental variables and natural abundances, but the observed seasonal variances of our measured variables do appear to be associated with changes in water turnover rates (28).

If mean monthly maximum temperatures, rainfall, and minimum relative humidity are indeed associated with fluctuations of natural abundances within the body, then other regions of the world, and particularly those with distinct seasons (e.g., higher-latitude climates with cold winters), may experience a similar or more pronounced isotopic variation. For example, increasing latitudes in the Northern Hemisphere have increasing ranges of monthly temperatures, which, as our results indicate, could potentially affect or add variance to natural abundances within participants of studies performed in those areas. This may particularly be the case during times of seasonal transition (months of September to October, March to May, etc.) and in regions farther away from larger bodies of water (e.g., inland vs. coastal areas) when temperatures and precipitation may fluctuate more. Likewise a subject’s daily exposure to environmental temperatures, humidity, and rainfall may also influence natural abundances. The four study participants lived in and worked in a rural area of Nigeria where air conditioning was uncommon, and these subjects were likely more directly exposed to environmental variables associated with weather than individuals residing and working in more urbanized climate-controlled environments. Such differences in local environmental exposures (occupational, rural vs. urban, etc.) may also introduce more variance into natural abundance measurements.

However, potential error resulting from natural fluctuations in abundance can be reduced when deuterium and \(^{18}\)O are dosed in a manner such that enrichments have a ratio similar to the ratio of the anticipated abundance changes in urine. Natural changes in abundances create similar errors in the elimination

### Table 1. Mean abundances for deuterium and \(^{18}\)O for urine and drinking water sources by season

<table>
<thead>
<tr>
<th>Source</th>
<th>Dry (n=10)</th>
<th>Rainy (n=6)</th>
<th>P Value</th>
<th>Dry (n=10)</th>
<th>Rainy (n=6)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>0.47 (2.24)</td>
<td>-2.19 (2.29)</td>
<td>&lt;0.001</td>
<td>-0.56 (0.46)</td>
<td>-0.93 (0.49)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Well water</td>
<td>-11.79 (4.00)</td>
<td>-12.61 (4.83)</td>
<td>0.49</td>
<td>-2.96 (0.35)</td>
<td>-2.90 (0.53)</td>
<td>0.65</td>
</tr>
<tr>
<td>Borehole water</td>
<td>-12.71 (1.87)</td>
<td>-11.56 (3.89)</td>
<td>0.39</td>
<td>-3.38 (0.50)</td>
<td>-3.22 (0.52)</td>
<td>0.48</td>
</tr>
<tr>
<td>Rain water</td>
<td>-6.33 (6.92)</td>
<td>---</td>
<td>---</td>
<td>-2.18 (0.83)</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

Applicable values are presented as means (SE). Number of samples analyzed (by season): urine (dry) n = 35, urine (rainy) n = 45; well water (dry) n = 23, well water (rainy) n = 34; borehole water (dry) n = 11, borehole water (rainy) n = 10; rain water (rainy) n = 6. Rain water was not used as drinking water source during dry season.

Fig. 3. Relationship between water turnover, presented as the natural log (Ln) of daily deuterium flux and the abundance of deuterium and \(^{18}\)O in urine relative to drinking water, permil, parts per thousand. D/H, deuterium/hydrogen.
rates of both isotopes, and thus these errors cancel when the difference of the two elimination rates is calculated. Anyone performing studies in which samples are collected weeks after the predose sample should be careful not to underestimate the scale and effect of natural isotopic variations over time, which may be significant, as the results of this study suggest. Ultimately such an underestimation may lead to error as great as 30% (27). The doses of $^{18}$O and $^2$H-enriched water should yield a postdose enrichment in parts per thousand units of 8 to 1 of $^{18}$O to $^2$H enrichment, respectively (4), although there is very little effect for doses in the ratio of between 6 and 12 to 1 (12). In our laboratory, we aim for a postdose $^{18}$O enrichment of 90 parts. With the use of 10 atom percent (AP) $^{18}$O water and 99.9 AP $^2$H water, respectively, this corresponds to a dose weight of 1.8 g of 10 AP $^{18}$O water per kilogram of total body water and 0.13 g of 99.9 AP $^2$H$_2$O per kilogram total body water.

Although it appears from this study that natural abundances of $^2$H and $^{18}$O in urine may change over the scope of the year, how this information will affect the results of other studies requires further inquiry. In general, energy expenditure studies will utilize the DLW method for a much smaller 1–4 wk measurement period (35), which may or may not be too short a time period for significant isotopic variance to occur. Often, however, the exact dates of DLW measurements are not reported, so critical consideration of any potential effect of seasonality becomes difficult. Such information would appear to be particularly relevant for TEE studies in areas such as parts of Cameroon (2, 3), Brazil (25), India (13, 5), Peru (10), and Kenya (24) that experience similar wet and dry seasonality as our study site. Overall, our results suggest that, at least in southwest Nigeria, the assumption that natural abundances will return to predose measurements during DLW measurements may not be correct, and the accuracy of this method may depend on the timing of measurements, particularly during the transition between dry and rainy seasons. Results of energy expenditure studies that utilize DLW measurements for human TEE calculations during a period of seasonal transition or over a longer period of time should be interpreted with caution. The potential error, however, can be minimized by dosing the two stable isotopes in an appropriate ratio such that errors in the $^2$H and $^{18}$O elimination rates cancel when the difference of the rates is calculated.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS