Deuterium Dilution: The Time Course of $^2\text{H}$ Enrichment in Saliva, Urine, and Serum, Catherine M. Jankowski,* Bakary J. Sonko, Wendolyn S. Gozansky, and Wendy M. Kohrt (University of Colorado Health Sciences Center, Denver, CO; * address correspondence to this author at: University of Colorado Health Sciences Center, Division of Geriatric Medicine, 4200 East Ninth Ave., Box B179, Denver, CO 80262; fax 303-372-2920, e-mail catherine.jankowski@uchsc.edu)

Total body water (TBW) volume can be measured in humans by administering an oral dose of an isotope tracer, such as $^2\text{H}_2\text{O}$ or $^2\text{H}^1\text{H}^1\text{O}$, and measuring the isotope enrichment in saliva, urine, or serum samples. Although serum has long been considered the medium of choice for measuring isotope enrichment because of rapid tracer equilibration (1), urine or saliva sampling is less burdensome to the patient. However, the measurement of isotope enrichment was found to be less reliable in urine than in serum, possibly because of a longer time period for isotopic equilibration of the bladder contents relative to serum (2). Schoeller et al. (1) suggested that saliva was an acceptable medium for measurement of TBW using $^2\text{H}_2\text{O}$ because isotopic equilibration occurred within 3 h and provided results similar to those obtained in serum. In another study, the enrichments of $^2\text{H}$ in saliva and plasma were identical and reached a plateau 2 h after administration of an oral 10-g dose of $^2\text{H}_2\text{O}$ (3).

To our knowledge, there have been no within-subject comparisons of the time to achieve a $^2\text{H}$ enrichment plateau in saliva, urine, and serum samples obtained simultaneously. The purpose of this study, therefore, was to determine the time course for isotope equilibration of $^2\text{H}$ in saliva, urine, and serum and the level of agreement in $^2\text{H}$ enrichment among these media. This information can be applied to protocols for measurement of TBW in clinical and research settings (4, 5).

Five healthy, weight-stable, nonobese women [mean (SD) body mass index, 22.1 (2.0) kg/m$^2$], 20 to 43 years of age, provided written informed consent and completed the study. The study was approved by the Colorado Multiple Institutional Review Board. The participants fasted for 8 h before arrival at the University of Colorado Health Sciences Center General Clinical Research Center at 0800. After collection of baseline saliva, urine, and blood samples (2.0 mL each), participants ingested 0.05 g/kg of body mass $^2\text{H}_2\text{O}$ (99.9 atom%; Isotech Inc.). The dose container was rinsed with 50 mL of tap water, and the rinse was ingested. Samples of all media were collected every 30 min from 0.5 to 6 h and then stored at $-80\,^\circ\text{C}$ until analysis by the University of Colorado Health Sciences Center Mass Spectrometry Laboratory. The participants consumed no other fluids or food for the first 4 h after $^2\text{H}_2\text{O}$ dosing and then ingested 236.5 mL/h of caffeine-free beverages during hours 5 and 6.

Isotope enrichment of the samples was determined by a VG Optima isotope ratio mass spectrometer (IRMS; Fison Instruments, VG Isotech), which is a magnetic sector dual-inlet instrument equipped with three Faraday collectors to detect the various masses of interest. The hydrogen analysis system applies the platinum equilibration technique (6). In brief, duplicate 0.5-mL aliquots were transferred to 4-mL clear glass vials with catalyst-coated glass rods. Sample equilibration was achieved in 2 h at 44 $^\circ\text{C}$. The sampled gas was dried and then released to the pressure transducer of the IRMS for sample size determination. The values of interest in this determination were the masses of 2 ($^2\text{H}$) and 3 ($^2\text{H}^1\text{H}$) and the ratio of mass 3 to mass 2. The data obtained from the hydrogen analysis system were drift-corrected off-line and normalized with respect to the Vienna-Standard Mean Ocean Water/Standard Light Antarctic Precipitation scale. The final results were expressed as units of $\delta$ calculated as:

$$\delta = \frac{(R_s - R_i) \times 10^3}{R_i}$$

where $R_s$ and $R_i$ are the isotopic ratios of the sample and reference, respectively (7), corrected for predose background enrichments. The IRMS has an external precision ($n = 20$) of 0.5% or better for $^2\text{H}$ enrichment.

The standard recommendation for initiating sample collection for the determination of TBW is 3 h after isotope ingestion (8). Therefore, the mean steady-state isotope enrichment was calculated from samples obtained during the fourth hour of the procedure (180, 210, and 240 min). The time to achieve a plateau in isotope enrichment for each medium was defined as the point at which the enrichment was within 2% of the steady-state value.

The $^2\text{H}$ enrichment concentrations in saliva, urine, and serum were compared by a two-way (media $\times$ time) repeated-measures ANOVA for four postdose time intervals: hour 3 after dosing (120, 150, 180 min); hour 4 after dosing (180, 210, and 240 min); hour 5 after dosing (240, 270, and 300 min); and hour 6 after dosing (300, 330, and 360 min). The $^2\text{H}$ enrichments in saliva and urine were also expressed as a percentage of the serum enrichments at each of the four postdose time intervals.

Participant 4 was able to provide urine samples at only four time points (1.0, 1.5, 4.5, and 6 h). Two saliva samples, one urine sample, and one serum sample were not collected from two other participants. For all time points up to 5.5 h, $^2\text{H}$ enrichment in urine tended to be lower and the variability greater than in saliva or serum (see Table 1 in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol50/issue9/). When individual


data were plotted (Fig. 1), it was apparent that the $^2$H enrichments in urine for participant 5 were low compared with saliva and serum or when compared with enrichments in all media obtained from the other participants.

The results that follow exclude all data for participant 5 unless otherwise noted.

Isotope equilibration, defined as the interval over which the enrichment was within 2% of the mean enrichment in hour 4 for each medium (Table 1), spanned $2.0 \sim 4.0$ h in saliva, $2.5 \sim 5.0$ h in urine, and $1.5 \sim 6.0$ h in serum (Fig. 1). There were no significant differences in the isotope enrichments in the three media from 2 to 6 h ($P > 0.05$). For participant 5, isotope enrichment in urine failed to reach a plateau by 5 h (Fig. 1, bottom). During hours 3 to 6 after dosing, mean $^2$H enrichment in saliva was $96.4 \sim 101.9$% of serum concentrations, and $^2$H enrichment in urine was $101.4 \sim 106.3$% of serum concentrations (see Table 2 in the online Data Supplement).

Although this study included only a small number of participants, it provided a direct comparison of the time course of changes in $^2$H enrichment in serum, saliva, and urine under the same experimental conditions. In agreement with previous studies (3, 9, 10), we found that $^2$H enrichment in serum achieved a plateau within 2 h of oral isotope ingestion and remained stable for several hours. Furthermore, from 2 to 6 h after dosing, isotope enrichment in saliva and urine was not significantly different from serum concentrations.

The time to equilibrium of $^2$H enrichment varied among the three media. The enrichment plateau was achieved earliest in serum (1.5 h), followed by saliva and urine (2.0 and 2.5 h, respectively), and the decrease in enrichment began earlier in saliva (4.0 h) than in urine or serum (5.0 and 6.0 h, respectively). Our findings suggest that sampling of any of these media 2.5 to 4.0 h after dosing would provide steady-state isotope enrichments, although we found that the best agreement was between saliva and serum enrichments in samples collected 4–5 h after dosing. Lukaski and Johnson (3) found good agreement between saliva and plasma enrichment 2 h after $^2$H ingestion.

The greater variability in urine $^2$H enrichment that we initially found throughout the time course was attributable largely to the very low urine enrichment in one participant. Retrospectively, this participant was found to

---

Table 1. Steady-state $^2$H enrichments (180–240 min).

<table>
<thead>
<tr>
<th>Participant</th>
<th>Saliva ($^2$H enrichment, $%$)</th>
<th>Urine ($^2$H enrichment, $%$)</th>
<th>Serum ($^2$H enrichment, $%$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>570.03 (4.98)</td>
<td>578.23 (3.71)</td>
<td>557.99 (0.30)</td>
</tr>
<tr>
<td>2</td>
<td>547.54 (2.38)</td>
<td>567.53 (4.95)</td>
<td>557.75 (1.95)</td>
</tr>
<tr>
<td>3</td>
<td>572.22 (2.49)</td>
<td>583.89 (1.52)</td>
<td>565.15 (5.50)</td>
</tr>
<tr>
<td>4</td>
<td>575.51 (3.58)</td>
<td>NA</td>
<td>568.98 (2.12)</td>
</tr>
<tr>
<td>5</td>
<td>577.14 (0.78)</td>
<td>468.03 (13.06)</td>
<td>580.72 (1.32)</td>
</tr>
</tbody>
</table>

Mean (SD)$^a$: 568.49 (12.04) 549.42 (54.68) 565.72 (9.95)

Mean (SD)$^b$: 566.32 (12.73) 576.55 (8.31) 561.96 (6.16)

$^a$ NA, no specimen available.

$^b$ Mean (SD) for all available data.

$^c$ Mean (SD) omitting participant 5.
have voiding dysfunction and a history of vesicoureteral reflux. The failure of urine enrichment to plateau given the history of voiding dysfunction raises concern regarding the frequency of such responses, particularly in older women, in whom a high prevalence of lower urinary tract symptoms would be expected, and older men, in whom benign prostatic hyperplasia is common. Therefore, screening for voiding dysfunction should be considered if urine is analyzed for measurement of TBW.

Our results in clinically euvoletic individuals may not apply to persons with hypervolemic states such as renal or heart failure, where up to 6 h may be required for equilibration in urine or serum (2). Although the measurement of TBW is generally performed in the research setting, an important application of these data is to determine prediction equations for clinical use, such as calculating dialysis dosing (4).

The results of this study suggest that all three media are acceptable choices for measuring $^2$H enrichment but that urine sampling is likely to have increased variability. Because, with few exceptions, the enrichment concentrations in saliva were closer to those in serum than were the urine enrichment concentrations for each individual at each hourly time interval, saliva appears to be the more accurate alternative to serum enrichment measures. Steady-state enrichment concentrations were achieved within 2.5 h of ingestion of the isotope, suggesting that this is the minimum time interval for isotope equilibration in healthy young adults. It is recommended that three or more samples be obtained, at time intervals sufficiently long (e.g., 30 min) to verify whether a steady-state enrichment concentration was achieved.

Financial support for this research was provided by NIH Grants R01 AG18857, M01 RR00051, P30 DK48520, and T32 AG00279 (to C.M.J.).

References