Influence of delayed isotopic equilibration in urine on the accuracy of the $^{2}\text{H}_2^{18}\text{O}$ method in the elderly

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Blanc, Stéphane, Amanda S. Colligan, Jillian Trabulsi, Tamara Harris, James E. Everhart, Doug Bauer, and Dale A. Schoeller. Influence of delayed isotopic equilibration in urine on the accuracy of the $^{2}\text{H}_2^{18}\text{O}$ method in the elderly. J Appl Physiol 92: 1036–1044, 2002. First published November 2, 2001; 10.1152/japplphysiol.00743.2001.—Isotopic determination of total energy expenditure (TEE) by the doubly labeled water (DLW) method may be affected by urine retention in the elderly. The isotopic enrichments in urine and plasma sampled simultaneously 4 h post-DLW dose were compared in a subset of 281 subjects [139 women, 142 men, 75 ± 3 (SD) yr] of the 3,075 participants in the Health, Aging, and Body Composition study. Based on analytic precisions, a ±2% urine-plasma difference was set as the cut-off value. Ten percent of the population presented a difference lower than −2%, suggesting a delay in urine isotopic equilibration. This −13 ± 10% urine-plasma difference was not linked to analytic errors, illnesses, the sampling time, or the time and quantity of water intake, suggesting that urine retention may be the main factor. The consequences are an 18 ± 13 and 21 ± 16% overestimation of the total body water and the TEE, respectively. Unexpectedly, 21% of the population presented a urine-plasma difference higher than ±2% that resulted, however, in a nonsignificant TEE underestimation of −3 ± 5%. In conclusion, the delayed isotopic equilibration observed in urine reduces the accuracy of the DLW method in the elderly. It is recommended, when blood sampling is impossible, to adopt the intercept method with urine sampling 24 h postdose.

energy requirements; deuterium; oxygen-18

TO MAINTAIN A DEGREE OF FITNESS and health compatible with an autonomous lifestyle, it is essential to establish accurate estimates of energy requirements. When the energy balance equation is maintained, the measure of a subject’s total energy expenditure (TEE) gives a proxy estimate of his or her energy requirements. The gold standard for TEE measurement in free-living people is the doubly labeled water (DLW) method (19). The technique is based on the exponential elimination of the stable isotopes $^2\text{H}$ and $^{18}\text{O}$ after a bolus dose of water labeled with both isotopes. The $^2\text{H}$ are lost as water, whereas the $^{18}\text{O}$ are lost both as water and as CO$_2$. Thus the excess disappearance rate of $^{18}\text{O}$ relative to $^2\text{H}$, after correction for isotopic fractionation, is a measure of the CO$_2$ production rate (18, 19). This latter can be converted to an estimate of TEE using a known or estimated respiratory quotient and the classic indirect calorimetry equations (23).

The isotope disappearance rates are typically calculated from enrichment measured in spot urine or plasma samples taken at the start and end of a 1- to 3-wk elimination period. Plasma is considered the most desirable sample source because it reflects the rapidly exchanging body pool, but this invasive sampling limits some of the method’s advantages. Conversely, urine can be collected noninvasively, the sample volume is rarely limiting, and it is excellent from an analytic perspective (13). However, with urine samples, there is concern about whether water stored in the bladder is in complete equilibrium with the body water and thus whether the sample actually represents an integrated sample collected over a previously indeterminate period (21). Consequently, errors can be introduced both from the timing of the sample and from the determination of the isotope equilibration, impinging on the accuracy of the TEE estimates.

In young human subjects, these errors are minimized because the bladder is completely emptied after the first void before the DLW urine collection. This has been demonstrated in the validation studies in which the technique has shown a precision of 4–9% and an accuracy of 1% in young cohorts when urine was sampled (12, 18, 19). In the elderly, however, voiding may be incomplete because of urine retention. With advancing age, the occurrence of postvoid residual urine volume (PRUV) increases in men and women. A PRUV >50 and 100 ml has been observed with a prevalence of 25% (8) and 12–34% (9, 15), respectively, among persons at least 65 yr of age. The same range of prevalence has also been reported for PRUV >250 ml in an insti-
tutional setting (27). When energy requirements are measured in the elderly by the DLW method using urine sampling, the presence of a PRUV may delay the isotopic equilibration and skew the DLW-derived CO₂ production rate estimates. For example, if we assume a prevoid volume of 150 ml, then a PRUV of 100 ml would still introduce a 30% error in the isotope enrichment of the third postdose void.

The DLW protocol conducted in the Health, Aging, and Body Composition (Health ABC) study allowed us to test the hypothesis that a delayed isotopic equilibration in urine, theoretically attributable to urine retention, may biased the TEE estimates. Based on the 322 subjects of the Health ABC energy expenditure sub-study, we have specifically determined 1) the proportion of the population presenting a delay in equilibration of labeled water in urine compared with plasma and 2) the extent to which such a putative delay may affect the TEE measurements.

MATERIALS AND METHODS

Subjects

The Health ABC study involves a cohort of 3,075 participants. Entry criteria included age (70–79 yr old), ability to walk one-quarter mile without aid and to climb a flight of stairs, and residence within 50 miles of either Pittsburgh, PA or Memphis, TN. The TEE was measured by the DLW method on a subset of 322 subjects of this cohort. TEE substudy subjects were initially selected at random with stratification for race and gender to ensure roughly equal participation (Table 1). Because participation rates were low, especially among African-American men, the pool of prospective participants was expanded during the last 4 mo to include all appropriate Health ABC participants. The TEE study was performed between late 1998 and early 2000. Participants were excluded if they had traveled >200 miles away from home before or during the TEE measurements, were receiving supplemental oxygen, had recent blood trans-fusion or intravenous fluid administration, or used insulin. The subjects were fully informed of the purpose and potential risks of the experimental protocol, and individual informed written consents were obtained before the study, which was approved by the Internal Review Boards at the University of Tennessee-Memphis and the University of Pittsburgh.

Protocol

Subjects were enrolled at either the Pittsburgh or Memphis Field Center. They arrived fasted on day 0, produced a baseline urine sample, and received a dose of DLW for the measurement of total body water (TBW) and TEE. The isotope equilibration was measured in two sequential urine samples and one blood sample collected over a 4-h period.

Table 1. Anthropometric data

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population, n</td>
<td>142</td>
<td>139</td>
</tr>
<tr>
<td>African-American/Caucasian, n</td>
<td>71/71</td>
<td>59/80</td>
</tr>
<tr>
<td>Age, yr</td>
<td>75.1±3.1</td>
<td>74.8±3.0</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>82.1±13.4</td>
<td>71.2±15.3</td>
</tr>
<tr>
<td>Body mass index, kg/m</td>
<td>27.4±4.3</td>
<td>27.4±5.4</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of subjects.

Fig. 1. Time line of the sampling protocol of the equilibration period. DLW, doubly labeled water. Voids 1, 2, and 3: urine samples collected at 2, 3, and 4 h, respectively, after dosing.

after which the subjects were excused from the clinics. One hour after the dose, the participants were allowed a liquid meal (237 ml; Boost Plus, Mead Johnson Nutritional, Evansville, IN), orange juice (200 ml), coffee (200 ml), or water (150 ml). These drinks were not allowed the last hour of equilibration unless the subject was unable to provide a void. The type, quantity, and time of liquid ingestion were recorded to correct the TBW estimates. Subjects returned on day 15 to provide urine samples for DLW analysis. Specific details are provided below.

TEE Measurements

DLW procedures. The TEE was determined using the two-point DLW method according to Schoeller and coworkers (18, 19). After the subjects provided baseline urine samples, a premixed 2 g/kg estimated TBW dose of DLW was administered to the subjects. The dose was composed of 1.9 g/kg estimated TBW of 10% H₂18O (Isotec, Miamisburg, OH) and 0.12 g/kg estimated TBW of 99.9% 2H₂O (Cambridge Isotope Laboratories, Andover, MA). Because of the large number of subjects, four different dose types were prepared before the study. These doses were based on a gender-dependent range of body weight and were calculated to ensure an in vivo enrichment of ~95 and 660 delta per mil (‰) for oxygen-18 and deuterium, respectively [Δ‰ = (Rsample/Rstandard – 1) × 1,000, where R is the ratio of 2H to 1H]. After dosing, three urine samples were collected at ~2, 3, and 4 h and are called void 1, void 2, and void 3, respectively. Immediately after void 3, a 5-ml blood sample was collected and then centrifuged for 10 min at 3,500 rpm for plasma separation. Fifteen days after dosing, the subjects returned to the laboratories, and urine samples were collected from two consecutive voids. Urine and plasma were stored at −20°C in cryogenically stable tubes until analysis by isotope ratio mass spectrometry. The time line of the equilibrium period is presented in Fig. 1.

Sample preparations and mass spectrometry analyses. Urine samples (5 ml) were treated with dry carbon black (200 mg) and filtered (0.45 μm, Cameo 25Gas, Osmonics, Minnetonka, MN). Water from plasma samples was extracted by centrifugation (4°C, 1 h at 10,000 rpm) on regenerated cellulose filters (YM-50, Centricron, Bedford, MA).

For deuterium analyses, 1 ml of cleaned sample was placed in a sealed vial (Target I-D vials, National Scientific, Lawrenceville, GA). An autosampler injected 0.8 μl of the sample into a quartz tube packed with chromium metal powder (Fischer Scientific Chemical, Itaska, IL) maintained at 850°C to reduce the water to hydrogen gas. The gas was then analyzed using a dual-inlet isotope ratio mass spectrometer (Delta Plus Mass Spectrometer, Finnigan MAT, San Jose,
CA). The results are expressed relative to a standard mean ocean water scale using two laboratory standards having a 8% vs. standard mean ocean water of +679 and −49 (16). Analyses were performed in duplicate and repeated if the SD exceeded 3 8%. Tests using waters of known abundances demonstrated that the filtration of plasma on cellulose filters results in a loss of deuterium isotope enrichment. This loss is independent of the volume filtered and was 0.6% of enrichment. The deuterium enrichments of the plasma samples were accordingly corrected. For oxygen-18 analyses, 1 ml of cleaned sample was transferred into a 3-ml Vacutainer (Becton Dickson, Franklin Lakes, NJ) to equilibrate with 1 ml of CO2 at 30°C for at least 24 h. About 20 µl of equilibrated CO2 were analyzed on a Delta-S isotope ratio mass spectrometer (Finnigan MAT) through a continuous-flow inlet system developed in the laboratory (17). Analyses were performed in triplicate and repeated if the SD exceeded 0.5‰.

**DLW calculations.** For each subject, the TEE was calculated independently using the void 2, void 3, and plasma isotopic enrichments. The dilution spaces for 2H and 18O were calculated from the baseline and urine or plasma sample according to Coward (3). The TBW was calculated from the average of the dilution spaces of deuterium and oxygen-18 after correction for isotope exchange by 1.041 and 1.007, respectively (11). The CO2 production was estimated according to Schoeller et al. (19), and the TEE was derived using Wier’s equation (23) and assuming a respiratory quotient of 0.86.

**Data Organization and Statistical Analyses**

The oxygen-18 enrichment above baseline in plasma was analyzed in the overall population and was used as reference for the body fluid isotopic enrichment. Enrichments of void 2 and void 3 were expressed as a percentage relative to plasma. Therefore, negative values corresponded to urine enrichments lower than plasma and were hypothesized to be attributable to a delay in equilibration because of PRUV. Considering a urine vs. plasma difference of ±2% as the limit for acceptability based on analytic considerations (16), three categories were produced: values below −2%, values within the range of ±2%, and values higher than 2%. Deuterium plasma enrichments were analyzed when the void 2 and void 3 vs. plasma differences were not within the range of ±2% and, additionally, in a subset of subjects of the central range to compare the TEE estimates. Both isotopes should satisfy a urine vs. plasma enrichment of ±2% to be included in the central range that was defined above.

Given the above-described data organization and to ensure a homogeneous statistical analysis, 41 subjects were excluded from the present study. The reasons for these exclusions were a missing plasma or urine sample (n = 22), no final visit at the field centers (n = 8), an enriched baseline urine sample (n = 1), a suspected incomplete dose administration (n = 2), and a discordance between isotopes of the above ±2% agreement cut-off value (n = 8).

The population variables were analyzed through descriptive statistics of oxygen-18 enrichments. A percentile analysis was performed to evaluate the skewness of the data. The effects of relevant variables (time of plasma sampling, quantity of water intake, time of water intake) that may explain the differences observed between plasma and urine enrichments were tested by use of a multiple stepwise regression analysis with an F to remove of 3.996 and an F to enter of 4.000. The group of individuals presenting a delay in isotope equilibration was analyzed separately. A factorial ANOVA was performed 1) for comparison of the above variables among the three ranges of urine vs. plasma differences, 2) to detect differences related to a significant range-by-gender interaction, and 3) to characterize the errors introduced by the delayed equilibration in the TEE and TBW estimates. Lastly, a χ2 test was used to detect differences in the medical history of the subjects as segregated among the three groups. All statistics were performed using StatView 5.01 (SAS Institute, Cary, NC). Values are expressed as means ± SD, unless otherwise stated, and P < 0.05 was considered significant.

**RESULTS**

**Behavior of the Population**

In Fig. 2, the individual oxygen-18 enrichments of void 3 vs. plasma are presented. We observed few extreme values around the mean; however, the data are clearly negatively skewed, suggesting that the few extreme values are large. The negative skewness of the...
population segregated by gender is presented through percentile analysis in Fig. 3. We noted that, for both men and women, the bias between void 2 or void 3 and plasma at the 50th percentile is slightly greater than zero.

Determants of Urine vs. Plasma Differences

The correlation between deuterium and oxygen-18 differences of urine vs. plasma was highly significant for both void 2 and void 3 (Fig. 4). Because the two isotopic analyses were largely independent and, therefore, the analytic errors are not expected to correlate, such a relation suggests that the differences were physiological rather than analytic.

Table 2 reports the sampling times, the time and quantity of water intake, and void 2 and void 3 vs. plasma differences for the three ranges defined above. The lower range group of subjects exhibited urine oxygen-18 enrichment lower than that of plasma. This defines a delay in the isotopic enrichment assumed to be due to PRUV. From Table 2, the subjects of the lower range included 10% of the population and was composed of 64% men. The relative enrichment differences observed were higher in men than in women ($-16.5 \pm 9.8$ vs. $-10.9 \pm 5.9%; F = 12.0, P < 0.001$). The two other ranges did not display any gender differences.

The results of the multiple stepwise regression analysis show that the differences observed between void 3 and plasma in the lower range of agreement were not attributable to the sampling time of plasma or to the time and quantity of water intake (Table 3). In the same way, the $\chi^2$ test did not indicate that diseases or urinary symptoms were different among the groups (Table 4). From the medical history, the use of diuretics was higher in the delayed equilibration group than in the two other groups. However, it is unlikely that this explains the lower urine enrichment compared with plasma, because we might expect that diuretics would minimize the importance of PRUV by promoting urine production.

The observation of a large number of subjects (21%) presenting urine isotopic enrichment higher than plasma was unexpected. Compared with the subjects of the lower range, the difference between isotopic enrichment of body fluids was, however, of a lesser extent ($2.6 \pm 2.9$ vs. $-13.1 \pm 0.2%$). From the regression analysis, it appears that the time of plasma sampling was a significant determinant of this difference with a negative $\beta$-coefficient (Table 3). However, the plasma sampling time only accounted for 24% of the variation, and thus most of the difference is unexplained. Additionally, the subjects' medical histories do not give any insight regarding this group.

Consequences of Delayed Equilibration on TEE and TBW Estimates

The errors introduced in TEE and TBW calculations are presented in Fig. 5. The subjects of the lower range, with a delay in the isotope equilibration, overestimated TBW and TEE when calculations were made from urine samples compared with plasma. We observed that void 2 overestimated TEE by $45 \pm 37\%$ compared with the TEE of the central range group, for which the difference was $-5 \pm 5\% (P < 0.0001)$. In the same way, estimates from void 3 overestimated the TEE by $21 \pm 16\% (P < 0.0001$ vs. subjects of central range display-
1040  

URINARY RETENTION AND DLW ACCURACY

Table 2. Range of $^{18}O$ agreement between urine and plasma sample

<table>
<thead>
<tr>
<th>n</th>
<th>Lower $&lt; -2%$</th>
<th>Control $\pm 2%$</th>
<th>Upper $&gt; +2%$</th>
<th>F-ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women/men</td>
<td>10/18</td>
<td>109/95</td>
<td>20/29</td>
<td></td>
</tr>
<tr>
<td>Postdose sampling time, h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Void 1</td>
<td>$1.6 \pm 0.7$</td>
<td>$1.9 \pm 0.8$</td>
<td>$1.5 \pm 0.6$</td>
<td>5.8</td>
</tr>
<tr>
<td>Void 2</td>
<td>$2.8 \pm 1.4$</td>
<td>$2.9 \pm 1.0$</td>
<td>$2.4 \pm 0.7$</td>
<td>4.2</td>
</tr>
<tr>
<td>Void 3</td>
<td>$3.5 \pm 0.7$</td>
<td>$3.9 \pm 0.8$</td>
<td>$3.5 \pm 0.7$</td>
<td>5.6</td>
</tr>
<tr>
<td>Plasma</td>
<td>$3.6 \pm 0.7$</td>
<td>$3.9 \pm 0.8$</td>
<td>$3.5 \pm 0.7$</td>
<td>5.5</td>
</tr>
<tr>
<td>Total water intake, %TBW</td>
<td>$1.3 \pm 0.6$</td>
<td>$1.8 \pm 0.8$</td>
<td>$1.9 \pm 1.1$</td>
<td>4.9</td>
</tr>
<tr>
<td>Time of water intake before plasma, h</td>
<td>$1.6 \pm 0.8$</td>
<td>$1.2 \pm 0.8$</td>
<td>$1.2 \pm 0.7$</td>
<td>2.5</td>
</tr>
<tr>
<td>Void 2 vs. plasma, %</td>
<td>$-22.1 \pm 12.0$</td>
<td>$2.2 \pm 4.3$</td>
<td>$6.1 \pm 4.6$</td>
<td>359.2</td>
</tr>
<tr>
<td>Void 3 vs. plasma, %</td>
<td>$-13.1 \pm 10.2$</td>
<td>$0.9 \pm 2.1$</td>
<td>$2.6 \pm 2.9$</td>
<td>259.4</td>
</tr>
<tr>
<td>Void 2 vs. void 3, %</td>
<td>$-9.3 \pm 9.5$</td>
<td>$1.4 \pm 4.1$</td>
<td>$3.0 \pm 3.9$</td>
<td>66.6</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of subjects. TBW, total body water; voids 1–3, urine samples collected 2, 3, and 4 h, respectively, after dosing. See text for explanation of groups.

Table 3. Multiple stepwise regression analysis of the determinants of void 3 vs. plasma $^{18}O$ differences

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>Plasma sampling time, h</th>
<th>Water intake, %TBW</th>
<th>Time of water intake vs. void 3, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower Partial $F$ ratio</td>
<td>0.640</td>
<td>1.185</td>
<td>1.194</td>
</tr>
<tr>
<td>Partial $F$ ratio</td>
<td>14.240</td>
<td>0.214</td>
<td>1.092</td>
</tr>
<tr>
<td>Normal and upper Partial $F$ ratio</td>
<td>14.240</td>
<td>0.214</td>
<td>1.092</td>
</tr>
<tr>
<td>Enrolment</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Time of water intake before plasma, h</td>
<td>$-0.38 \pm 0.10$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Determinants of Differences Between Urine and Plasma Isotope Enrichment

As expected, the subjects in the central range displayed small differences ($-2 \pm 2\%$) between the TEE estimates from simultaneous sampling of plasma and urine at 4 h postdose. In this group, the 5% difference observed between plasma and void 2 estimates of TEE reflects the fact that a postdose equilibration of 3 h is not necessarily sufficient to reach the isotopic plateau of equilibrium.

The presence of an upper range group of subjects representing 21% of the population and characterized by higher urinary isotope enrichments than plasma was unexpected. This resulted in a 3 ± 5% underestimation of the TEE that was not significantly different from that of the central range group. The prevalence of this upper range group can be linked to several factors, such as the time and quantity of water intake, the sampling time of body fluids, and, lastly, any pharmacological drugs that potentially affect the water balance.

During the equilibration period, the subjects were allowed the option of a liquid meal and drinking coffee, orange juice, or water in an effort to maintain urine production. This is an unusual situation during a DLW sampling period.

DisCUSSION

Accurate energy requirements can be derived from the free-living energy expenditure measured by the DLW method. In the elderly, the prevalence and severity of urinary retention may have a profound impact on the accuracy of the method by delaying the isotope equilibration, when urine is sampled. Based on results from 281 subjects of the Health ABC study, we were able to demonstrate that the equilibration of isotopes in urine is delayed in 10% of the population and that such a delay induced a 21 ± 16% overestimation of the TEE estimates. Based on comparable prevalences and theoretical expectations regarding the modeling of PRUV on isotope equilibration and on the fact that no tested variables explained the isotopic delay, we conclude that the differences observed between urine and plasma isotopic enrichments can reasonably be attributable to PRUV.

Table 4. Summary of the multiple stepwise regression analysis of the determinants of void 3 vs. plasma $^{18}O$ differences

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>Plasma sampling time, h</th>
<th>Water intake, %TBW</th>
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<tr>
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<tr>
<td>Enrolment</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Time of water intake before plasma, h</td>
<td>$-0.38 \pm 0.10$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. In indicates the variables significantly included in the model based on a partial $F$ ratio to remove and to enter of 3.996 and 4.000, respectively.
The protocol, because the subjects are generally fasting during this period. The effect of food or fluid intake during the equilibration period has been previously reported. On one hand, Drews and Stein (5) investigated the consequences of large volumes of water intake (700–1,000 ml) and reported that TEE was overestimated by 20% when body fluid was sampled within 1 h of ingestion. On the other hand, Calazel et al. (2) reported the consequence of feeding (744 kcal) and noted no effect, regardless of the feeding time during equilibration. This was confirmed by Westerterp et al. (24). Our experiment supports the studies of Calazel et al. (2) and Westerterp et al. (24), because neither the water intake nor the time of intake explained the void-plasma differences.

The second factor that may have caused the observed higher urine enrichment than plasma is the time of body fluid sampling. Wong et al. (26) indicated that samples taken 4–6 h postdose were comparable across fluid sources. These observations, however, may support one possible explanation of the relatively higher isotope enrichment of urine compared with plasma observed in the present study. The upper range group of subjects is the one for whom all of the fluid sampling, voids as well as plasma, were significantly closest to the dose time (ranging from 11 to 20% earlier than the central range group). It is, therefore, acceptable to argue that these subjects did not reach equilibrium at the sampling time and that urine isotopic enrichment reflects the pulse overshoot of the isotope recorded in the plasma. However, because of a lag in the urine response due to a delay in isotopes passing from the plasma into the urine pool, plasma enrichment appears lower than that recorded in urine.

Long-term use of medications, especially those used to treat cardiovascular diseases, may affect the water and electrolyte balances. These drugs include diuretics, conversion enzyme inhibitors, and any steroids that interfere with the adrenal function. However, from the statistical analyses, no disease and medication appeared to explain the results of the upper range group. Finally, the higher enrichment of urine may result from an analytic problem. We did observe and samples did not stabilize until three voids postdose. Wong et al. (26) indicated that samples taken 4–6 h postdose were comparable across fluid sources. These observations, however, may support one possible explanation of the relatively higher isotope enrichment of urine compared with plasma observed in the present study. The upper range group of subjects is the one for whom all of the fluid sampling, voids as well as plasma, were significantly closest to the dose time (ranging from 11 to 20% earlier than the central range group). It is, therefore, acceptable to argue that these subjects did not reach equilibrium at the sampling time and that urine isotopic enrichment reflects the pulse overshoot of the isotope recorded in the plasma. However, because of a lag in the urine response due to a delay in isotopes passing from the plasma into the urine pool, plasma enrichment appears lower than that recorded in urine.

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Table 4. Clinical characteristics of patients in the 3 groups of urine vs. plasma isotope enrichments

<table>
<thead>
<tr>
<th></th>
<th>Lower &lt; -2%</th>
<th>Normal ± 2%</th>
<th>Upper &gt; +2%</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>28</td>
<td>204</td>
<td>49</td>
</tr>
</tbody>
</table>

Present Characteristics, n

Medical history
- Cerebrovascular disease 1 17 4
- Congestive heart failure 0 2 0
- Myocardial infarctus 0 8 1
- Hypertension 9 74 18
- Pulmonary disease 4 21 6
- Diabetes 8 22 2
- Benign prostatic hyperplasia 7 45 9
- Cured prostate cancer 1 6 1

Medication
- ß-Adrenergic blockers 3 31 7
- Calcium blockers 3 46 1
- Antiarrhythmics 2 2 2
- Diuretics* 17 60 9
- Natriuresis 0 13 4
- Vasodilator/ACEI 4 31 8
- Drugs for prostate disease 6 22 4

Urinary history
- Urine leak 6 84 19
- 3 Mictions during the night 1 6 2
- Urination < 2 h 1 5 1
- Enlarged prostate 8 49 10
- Emergency treatment for urination 2 3 1
- Difficulty to postpone urine 2 8 1
- Push/strain to urinate 1 5 1
- Start/stop when urinating 2 3 0
- Feeling of bladder not empty 3 19 5
- Weak urinary stream 2 4 0

n, No. of subjects. ACEI, angiotensin-converting enzyme inhibitor.
* Frequency higher in the lower group, (P < 0.05).

Fig. 5. Total body water (A) and total energy expenditure (B) from void 2 and void 3 expressed as percentage of plasma estimates within each range of agreement [lower (n = 28), normal (n = 96), and upper range (n = 49)]. Values are means ± SE. * P < 0.05 vs. normal range (paired least significant difference Fisher’s test results from F-ANOVA).
correct for a 0.6% dilution of plasma during exclusion filtration, and it is possible that a few filters or some other steps introduced a larger dilution.

Our observation of higher urine enrichment than plasma seems to be mainly attributable to a sampling of body fluids out of the equilibrium period and corresponds to the bladder integration of the isotope pulse overshoot occurring in the plasma pool. Nevertheless, even if the group represents 21% of the population and warrants consideration, the differences between isotope enrichment were statistically similar to those of the central range (2.6 ± 2.9 vs. 0.9 ± 2.1%).

The group with low-isotopic enrichment in urine is a far more important group with regard to the accuracy of the DLW method. We hypothesize that these subjects may reflect the incidence of PRUV in the elderly. The primary aims of the Health ABC study were not dedicated to investigate the consequences of urine retention on the DLW method, and thus no ultrasound evaluation of PRUV was performed. This is clearly the principal limitation of our ability to relate the isotopic delay with PRUV. The results demonstrated that neither analytic problems, illnesses, the plasma sampling time, nor the time and quantity of water intake was a determinant of the differences observed between plasma and urine isotope enrichments in those who displayed slow equilibration. The absence of a relationship between the PRUV and the continence status has been previously reported (4).

The prevalence and severity of PRUV in the elderly has been well documented, but the definition of a “normal” volume is not clear cut: PRUV measurements are not easy and appear to be variable (8). Yet the range of volume reported is larger than what would explain the DLW results. A PRUV prevalence of >100 ml has been reported to vary among 12% (15), 29% (6), and 38% (27) in hospitalized patients or subjects in long-term care institutions. These data, however, may not apply to our study of ambulatory, free-living, elderly people. Ouslander et al. (9) studied a geriatric outpatient population composed of 263 subjects and observed that 34% presented a PRUV >100 ml. Of these subjects, 70% were men. A gender difference was also observed in the study of Bonde et al. (1) that examined 140 ambulatory subjects of 75 yr of age, selected at random from a central Danish persons register. In men, the median PRUV was 90 ml and ranged from 10 to 1,500 ml. Among women, the median was 45 ml and ranged from 0 to 180 ml. Therefore, the prevalence and severity of PRUV is far from trivial and appears similar across institutionalized and ambulatory subjects. The 10% prevalence reported in our study, based on isotope data, is clearly in the lower range of the literature. Three factors may be implied in this low prevalence: 1) the selection process that favors active and healthy subjects, 2) the consumption of diuretics that favor isotopic equilibration through urine production, thus underscoring the urine retention problem in the DLW method, and 3) a slow isotope exchange across the bladder. Indeed, the errors observed in the TBW and TEE calculations are far from being negligible (18 and 21%, respectively), and the prevalence (18 vs. 10%) and severity (~16 vs. ~11%) are higher in men than in women. Moreover, recent data suggest little variation in the distribution of PRUV across age groups (6). Obviously this point warrants further urological confirmation but may imply that the results of our experiment are not restricted to the elderly.

Overall, the problem of urine retention would not be important if the urine in the bladder was in rapid isotopic equilibration with the remainder of the body water. By using a dog model in which the bladder was isolated by severing the ureters, Jonhson et al. (7) found that urine is in isotopic equilibrium with the remainder of the body water pool with a half-life of ~3 h. This would have reduced the influence of PRUV, because urine samples are generally collected between 4 and 8 h. Popit et al. (cited in Ref. 21) found differences in the enrichments of simultaneously collected urine and blood from tenrecs (Echinops telfairi), indicating that the urine in the bladder is not in complete exchange with the body water and thus represents an integrated sample over some unknown previous time period. These data, taken with ours, suggest a slow isotopic equilibrium across the bladder. Thus, despite variable prevalences, the problem of urine retention in the elderly is far from trivial, and the consequences for the DLW method appear not to be compensated for by dynamic isotope exchange across the bladder. The severity of the error introduced in the DLW-derived TEE estimates of the present study emphasize the importance of PRUV to studies of energy expenditure and body composition, especially given the fact that our prevalence is in the lower range of those published.

Proposed Solution to Overcome the Urine Retention Problem

Solutions to the problem of PRUV include blood or saliva sampling. Both samplings are, to some extent, a...
limitation to the method. Blood collection is not always possible, and saliva is sensitive to isotopic fractionation, and thus may not be as precise (18). Moreover, with saliva, drinks could not be allowed during the final portion of the equilibration period, which can be problematic, depending on the population tested. Lastly, many medications prescribed in the elderly reduce saliva and thus may interfere with sampling. Another solution related to DLW can be proposed. The isotope pool spaces can be calculated by two different approaches: the plateau and intercept methods (21). The previous reports on energy requirement measurements of elderly people by the DLW method are based on either intercept (10, 22) or plateau (14, 25) calculations, but, to our knowledge, no studies have compared these methods in elderly people.

When blood sampling is impossible, the intercept method should reduce the problem of delay in isotope equilibration. Essentially, the initial enrichment is obtained by backextrapolation from the points that make up the washout curve. Therefore, it is not necessary to time the initial sample to coincide exactly with the plateau period. A direct corollary is that the intercept estimate is not as critically dependent on the rate of elimination of deuterium label distribution in the body. Some calculations support this hypothesis. From the deuterium elimination rate, we calculated a water turnover of 2.9 g/day. This estimate is not as critically dependent on the rate of urine production as determined by the doubly labeled water study. From the deuterium elimination rate, we calculated a water turnover of 2.9 g/day. This estimate is not as critically dependent on the rate of urine production as determined by the doubly labeled water study.

Conclusion

Based on the 281 elderly subjects of the Health ABC study, we observed that 10% of the population present a delay of oxygen-18 and deuterium equilibration in urine compared with plasma. This difference of about 13% is significant and is not linked to analytic errors, the sampling times, the time and quantity of water intake, or illness, suggesting that PRUV is likely to be the main factor. Moreover, 64% are men and present a higher effect than that observed in women, indicating a severity and prevalence consistent with the literature on PRUV. This urine retention induces a dramatic TBW and TEE overestimation of 18 and 21%, respectively. Therefore, special attention should be paid to the use of urine samples when the DLW method is used in the elderly. When plasma sampling is not possible, we suggest the intercept method to calculate the DLW variables with the first urine sampling occurring 24 h postdose.

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REFERENCES

12. Ravussin E, Harper IT, Rising R, and Bogardus C. Energy expenditure by the doubly labeled water: validation in lean and


