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Body Water Measurements in Premature and Older Infants Using $H_2{}^{18}O$ Isotopic Determinations

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Summary

Total body water was measured by $H_2{}^{18}O$ stable isotope dilution in two groups: in premature infants without complications, who were studied from 8 d of age until discharge; and in Peruvian subjects aged 6–36 mo, who were in the long-term convalescent stage of recovery from malnutrition. Results indicated that reliable total body water estimates can be obtained from sample volumes as small as 50 μl of urine or plasma using a gas-isotope-ratio mass spectrometer equipped with an automated purification inlet system. Results from 21 studies in 10 Peruvian infants indicated substantially completed isotope equilibration in plasma by 2 h after the dose; total body water estimates from the 2-h samples averaged 98.7% (± 4.1) of 6-h values. Samples obtained at 4-h postdose gave total body water estimates that averaged 99.0% (± 2.9) of the 6-h value, showing essentially complete equilibration and reduced variability. Total body water estimates from urine samples collected 3–5 h postdose were closely correlated with 6-h, plasma-based total body water values in both premature and older infants; however, some reduction in variability was observed when urine collection was extended to 5–7 h, at which time urine-based estimates averaged 98.8% (± 2.0) and 100.7% (± 3.1) of plasma-based values for prematures and older Peruvian infants, respectively. The correlation between 5–7 h urine-based estimates of total body water with plasma-based values was $r = 0.96$ for 30 studies in prematures and $r = 0.99$ for 57 studies in older Peruvian infants. Data points adhered closely to the lines of identity in both study groups. These results suggest that noninvasive urine sampling techniques can be substituted for plasma sampling in body water studies in infants.

Abbreviation

TBW, total body water

TBW measurements in infants are useful for assessing body composition; however, infant studies require small samples, minimal invasiveness of procedures, and avoiding exposure to radioactive isotopes. The stable, naturally occurring oxygen-18 isotope (^{18}O), when present in enriched concentrations as $H_2{}^{18}O$, meets the requirements of infant body water studies because it lacks toxicity and its isotopic ratio is relatively easily measured by automated mass spectrometric techniques (3). $H_2{}^{18}O$ was used for body water studies in adults (3) and adolescents (5); however, sample volumes of 1.0–1.5 ml of urine, plasma, or saliva were

used for isotopic analysis in these studies (3, 5). Such large sample volumes may become impractical when plasma is used for TBW measurement in infants or prematures, or when the sample has to be partitioned for other analyses.

The aims of this report are 3-fold. First, we describe a method for TBW measurement using $H_2{}^{18}O$ in premature infants and in older infants. *2*) We examine whether a sample as small as 50 μl is adequate for oxygen-18 analysis. *3*) We examine the suitability of using urine rather than plasma samples for TBW studies because urine samples are less traumatic to collect in small infants and may be more practical than plasma samples in field studies.

MATERIALS AND METHODS

The subjects in this study consisted of two distinct populations. The first group consisted of premature infants without complications at the neonatal intensive care unit of the Johns Hopkins Hospital who were studied from 8 d of age until discharge. The second group consisted of Peruvian infants, 6 to approximately 36 mo of age who were in the long-term convalescent stage of recovery from malnutrition at the Instituto de Investigacion Nutricional in Lima, Peru.

Body water measurements. These older infants were studied monthly over a 3-mo period. Study procedures were similar in the two groups and were based on the same principles used in previous isotope dilution studies in infants (1), but they employed $H_2{}^{18}O$ instead of deuterium. A dosage of 0.3 g/kg of $H_2{}^{18}O$ at 20 atom % concentration (0.06 g of ^{18}O/kg) was administered to both prematures and older infants by nasogastric tube, followed by 2–3 ml of tap water to flush the tubing. The exact weight of the $H_2{}^{18}O$ dose was determined by weighing the syringe on an analytical balance before and after delivering the dose into the nasogastric tube.

In the premature infants, a baseline sample of urine was collected for determination of basal $^{18}O/^{16}O$ ratio before the administration of the isotope. In one group of four prematures in whom 11 studies were completed, a single postdose blood sample (0.3–0.5 cc) was drawn into a large-bore capillary tube at a fixed time of 6 h after the administration of $H_2{}^{18}O$. In a second group of nine prematures in whom 19 studies were completed, the postdose blood sample was drawn just before the next feeding, 1–3 h after the $H_2{}^{18}O$ dose.

In the Peruvian infant study, blood sampling was more feasible because of the infants' larger size. In addition to a baseline urine sample, blood samples were obtained immediately before the dose and after 2, 4, and 6 h. In both premature and older infant studies, the time of each urine voiding during the next 6–7 h was recorded, and samples of each void were obtained for isotopic analysis.

Adjustment for the contribution of fluids administered during the study period was made by subtracting the intrastudy fluid intake volume from the final TBW estimate. This adjusted the final estimate to the TBW status of the infant at the time of the isotope dose. Premature infants required feeding 1–2 times during the 6-h study period. Older infants, who could tolerate longer fasting, were fed only once: shortly after the $H_2{}^{18}O$ dose to allow maximum equilibration for the ^{18}O isotope and to minimize dilution of the isotope by subsequent fluid intake. No adjustments were made for sensible or insensible loss of fluid during the study period because equilibration of $H_2{}^{18}O$ in body water occurs rapidly. After equilibration, fluid losses affect both $H_2{}^{18}O$ and $H_2{}^{16}O$ in equal proportion so that the $^{18}O/^{16}O$ ratio from which TBW is calculated is not affected.

TBW was determined by application of the dilution principle. A carefully measured dose of $H_2{}^{18}O$ was administered orally and the TBW was then calculated from the change in $^{18}O/^{16}O$ ratio from the basal to the postdose sample, according to the following equation:

$$\text{TBW(kg)} = \frac{\text{Dose(g)}}{\text{MW}} \times \frac{\text{APE}}{100} \times \frac{18.02}{\text{K}}$$

in which dose is the weight of $H_2{}^{18}O$ (20 atom percent concentration) administered in grams; APE is the atom percent excess of ^{18}O in the $H_2{}^{18}O$; 18.02 is the molecular weight of water; MW is the molecular weight of the $H_2{}^{18}O$; and K is the change in the absolute ratio of $^{18}O/^{16}O$ from basal to the postdose sample. The absolute ratio of $^{18}O/^{16}O$ (R) in a sample was calculated according to the following equation:

$$\text{R(ppm)} = (\delta^{18}\text{O}/1000 + 1) \times \text{R}_{\text{PDB}}$$

R_{PDB} is the absolute ratio of $^{18}O/^{16}O$ in the standard Pee Dee Belemnite (PDB), which is defined as 2079 ppm. The $\delta\ ^{18}O$ value of the sample was defined as follows:

$$\delta^{18}\text{O(o/oo)} = \left[\frac{(^{18}\text{O}/^{16}\text{O})\ \text{sample}}{(^{18}\text{O}/^{16}\text{O})\ \text{standard}} - 1\right] \times 10^3$$

This value was determined by equilibrating a known aliquot of the sample with 20 ml of 15% CO_2 in N_2 in a 20-ml Vacutainer at 25°C for 72 h. The $^{18}O/^{16}O$ isotope ratio of the 15% CO_2 after equilibration was measured with a Nuclide 3-60 gas-isotope-ratio mass spectrometer (Nuclide Corporation, State College, PA) equipped with an automated purification inlet system (4). Corrections for abundance sensitivity, background effect, ^{13}C–^{17}O contribution to mass 46, and isotopic and atomic contribution of tank CO_2 (15%) to the $^{18}O/^{16}O$ isotopic ratio of the sample were made according to the procedure described by Craig (2).

The minimal sample volume required for $^{18}O/^{16}O$ analysis was evaluated by measuring the isotopic composition at two different volumes (0.05 and 1.0 ml) of a urine sample with natural abundance of oxygen isotopes and after spiking with a known quantity of $H_2{}^{18}O$. The mean isotopic ratios from 10 duplicate measurements for samples of the same fluid at 0.05 and 1.0 ml were compared and their differences were evaluated for statistical significance by the standard *t* test.

Special care must be taken in collecting, storing, and analyzing small postdose samples for isotope ratio measurement in order to exclude contamination by atmospheric moisture which is depleted in ^{18}O. Admixture of such moisture from improperly dried vials, syringes, etc., will result in an over estimation of TBW space. In a humid environment, this source of contamination can easily be prevented by storing vials and syringes that have been cleaned and dried in a desiccator before use for these small samples.

TBW data were analyzed in several ways. Isotope equilibration in plasma was studied in the older infants of the Peruvian study population for whom 2- and 4-h plasma samples were available for comparison with 6-h values, which were assumed to be completely equilibrated. Isotope equilibration in urine over the 5–7-h collection period was studied in both prematures and older infants, again using 6-h plasma-based TBW values as the reference point that defined "true" fully equilibrated values. The ability of urine-based TBW estimates to substitute for plasma-based values was evaluated by examining the correlation of urine- and plasma-based TBW estimates in the same infants.

All procedures in these studies were reviewed and approved by the Joint Committee on Clinical Investigation of the Johns Hopkins Medical Institutions and the Committee on Investigational Ethics of the Instituto de Investigacion Nutricional.

RESULTS

Sample volume requirement. The effects of sample volume on isotope ratio measurements are displayed in Table 1. At a 99% confidence level, the confidence limits for the differences between the means of urine samples at 0.05 and 1.00 ml are calculated to be -1.0 ± 3.1 and 0.6 ± 1.7 ppm for the basal and spiked samples, respectively. These differences constitute a maximum error of 0.2% or less with respect to the measured mean ratios; therefore, the $^{18}O/^{16}O$ ratios measured from a 0.05 ml sample are as accurate as those obtained when larger sample volumes were used.

Isotope equilibration in plasma. In 21 studies carried out in 10

Peruvian infants for whom 2-, 4-, and 6-h plasma samples were analyzed, TBW estimates from 2-h samples averaged 98.7% (± 4.1) of the 6-h values with a range of 91.8–108.1%. By the time of the 4-h sample, TBW estimates averaged 99.0% (±2.9) of the 6-h value with a range of 95.0–106.7%, indicating essentially complete equilibration. The narrower range and lower standard deviation of the 4-h values suggests reduced variability compared with the 2-h samples.

Isotope equilibration in urine. The equilibration of TBW estimates from urine samples compared with 6-h plasma-based values was studied in both prematures and older infants. Results from the 11 studies of prematures in which 6-h plasma samples were available (Table 2) indicated that equilibration of urine-based TBW values was substantially completed by 3–5 h, but that a reduction in variability was obtained by extending urine collection to 5–7 h. Comparison of urine TBW estimates with plasma-based values in older infants (Table 3) likewise indicated an elevated TBW estimate from the urine samples collected earlier in the study (1–3-h), but also confirmed that equilibration was substantially completed by 3–5 h and that some reduction in the range and variability of values was observed in urine samples collected at 5–7 h. The high TBW estimate at 1–3 h in Table 3 is due to mixing of urine produced after the $H_2{}^{18}O$ dose with residual urine in the bladder from before the dose, resulting in a dilution of the $H_2{}^{18}O$ ratio.

Correlation of urine and plasma-based TBW estimates. The correlation of final, 5–7-h urine-based estimates of TBW with plasma-based values was examined in both prematures and older Peruvian infants. For 30 studies in prematures (Fig. 1), the correlation coefficient was 0.96 with a standard deviation about the fitted line of 0.468 l and a coefficient of variation of 3.7%. For 57 studies in older infants (Fig. 2), the correlation coefficient was 0.99 with a standard deviation about the fitted line of 0.155 l and a coefficient of variation of 3.3%. In both study groups the data points adhered closely to the lines of identity.

Table 1. *Isotope ratios of urine in relation to sample volume*

Sample volume (ml)	Absolute ratios of $^{18}O/^{16}O$ in urine (ppm ± SD*)			
	Basal	*n*†	Spiked‡	*n*†
1.00	2061.4 ± 2.5	10	2162.6 ± 1.7	10
0.05	2062.4 ± 2.3	10	2161.9 ± 0.8	10

* SD, standard deviation.
† *n*, sample size.
‡ Urine spiked with $H_2{}^{18}O$ at 10.4 atom % ^{18}O.

Table 2. *Total body water (TBW) estimates from urines at 1–3, 3–5, and 5–7 h* as a percentage of 6-h plasma TBW (prematures: Baltimore)*

Time of urine sample (h)	Percentage of 6-h plasma TBW			
	Mean	SD†	Range	*n*‡
1–3	103.5	±2.8	98.7–105.8	5
3–5	99.2	±4.8	92.0–110.3	11
5–7	98.8	±2.0	95.0–101.2	11

* Excluding first voidings: adjusted for fluid intake.
† SD, standard deviation.
‡ Differences in the number of samples at different time intervals reflect the irregular voiding of infants. Because the infants generally void just before the start of the study and because the first postdose voiding is discarded, there are fewer subjects providing samples for analysis in the earlier time intervals.

Table 3. *Total body water (TBW) estimates from urines at 1–3, 3–5, and 5–7 h* as a percentage of 6-h plasma TBW (infants: Peru)*

Time of urine sample (h)	Percentage of 6-h plasma TBW			
	Mean	SD†	Range	*n*‡
1–3	130.9	±70.3	92.8–387.3	17
3–5	98.7	±3.8	91.9–110.6	26
5–7	100.7	±3.1	95.7–109.9	44

* Excluding first voidings: adjusted for fluid intake.
† SD, standard deviation.
‡ Differences in the number of samples at different time intervals reflect the irregular voiding of infants. Because the infants generally void just before the start of the study and because the first postdose voiding is discarded, there are fewer subjects providing samples for analysis in the earlier time intervals.

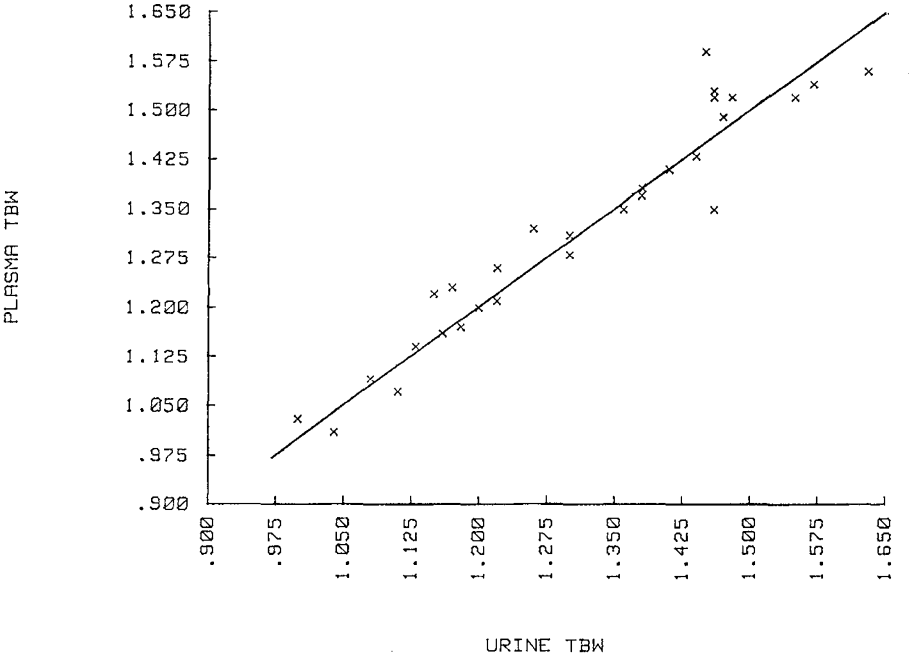

Fig. 1. Plasma *vs* urine total body water (TBW) expressed in liters for 30 studies in premature infants. The line represents identity between plasma and urine based TBW values. TBW (plasma) = 0.965 × TBW (urine) + 0.0541. The correlation coefficient (r) = 0.96.

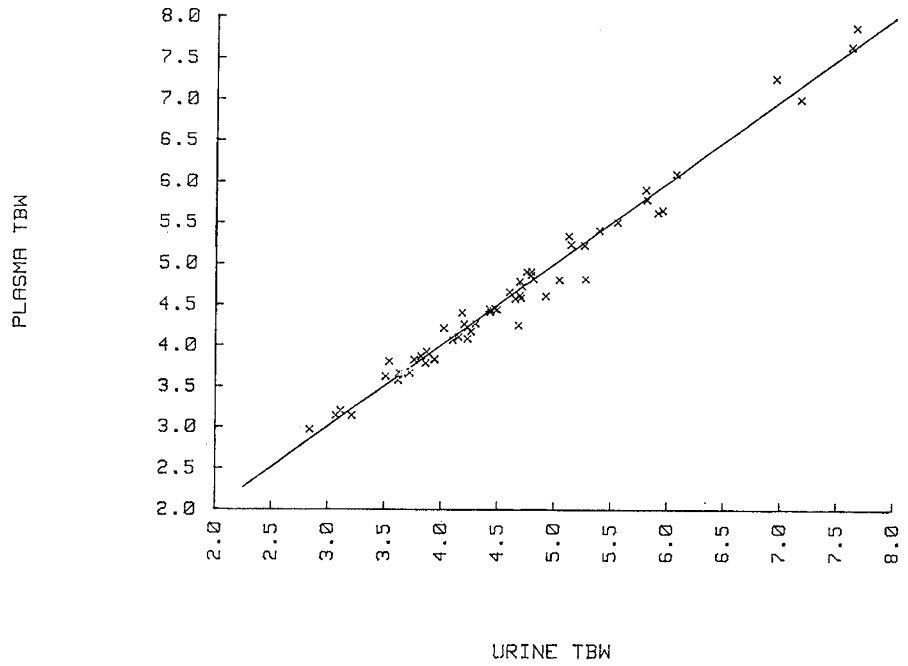

Fig. 2. Plasma *vs* urine total body water (TBW) expressed in liters for 57 studies in Peruvian infants. The line represents identity between plasma and urine based TBW values. TBW (plasma) = 0.99 TBW (urine) + 0.041. The correlation coefficient (r) = 0.99.

DISCUSSION

The results of this study indicate the feasibility of TBW assessment in infants when very small sample volumes of plasma or urine are used. Care must be taken in handling the samples to avoid contamination by water or atmospheric moisture, but reliable results are obtainable when attention is given to proper collection technique as described above. The timing of sample collection is another important issue. Studies in adults suggest that equilibration in plasma is substantially completed by 1–2 h (6). Results from this study suggest that plasma-based TBW estimates in infants approach 99% of 6-h values by 2 h. But even at 4 h, the average of the plasma-based estimates was slightly below 6-h values, suggesting that there may be some advantage to extending sample collection beyond 2–4 h.

Urine-based TBW estimates appear to equilibrate with 6-h plasma-based values by 3–5 h in both prematures and older infants; however, there was a reduction in the variability of TBW estimates when urine samples collected at 5–7 h were used. These results suggest that collecting urine samples 5–7 h after the administration of the isotope dose is the preferable procedure.

The high degree of correlation between urine and plasma TBW estimates indicates that urine sampling can serve as a reliable procedure for TBW studies in infants. This finding is of practical importance for the conduct of field studies and for TBW measurements in small infants because the collection of urine samples is often more practical and less traumatic than the collection of plasma samples.

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