

## Measurements of $^2\text{H}$ and $^{18}\text{O}$ in body water: analytical considerations and physiological implications

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Measurement of energy expenditure with doubly-labelled water and of body composition and breast milk output with  $^2\text{H}$  or  $^{18}\text{O}$  requires accurate and precise techniques for measuring isotopic enrichments. The possibility of an inaccuracy in measurements of  $^2\text{H}$  and  $^{18}\text{O}$  isotopic enrichment arising from the matrix in biological fluids was investigated (1) by simulating a dilution experiment in both water and urine samples and (2) by reconstituting urine samples, ranging from 10 to 60 g/kg in solid concentration, from freeze-dried urinary solids mixed with either natural abundance or doubly-labelled water. Current techniques involved in measuring  $^2\text{H}$  and  $^{18}\text{O}$  isotopic enrichments were used (reduction of the samples to  $\text{H}_2$  gas with either Zn or U, and  $\text{CO}_2/\text{H}_2\text{O}$  equilibration or direct measurement of mass 20:18 ratios on water vapour for  $^{18}\text{O}$  analysis). All four methods accurately measured serial dilutions in both urine and water. Dilution space calculated from isotopic enrichments, compared with the water content of urine (determined by freeze-drying and accounting for exchangeable isotopes) was overestimated by about 2.4% by the Zn technique whereas other methods were accurate. The urinary solids content of a water solution was related to that inaccuracy. The use of the Zn technique with biological samples is likely to create biases in  $^2\text{H}$  distribution space. Examination of recent literature supports this view. Caution should therefore be used when physiological conclusions have to be made from the relative size of  $^2\text{H}$  and  $^{18}\text{O}$  distribution spaces.

Doubly-labelled water: Total body water: Deuterium measurement:  $^{18}\text{O}$  measurement

The dilution of isotopically labelled water with body water to determine, for example, body composition, breast milk output and energy expenditure is a commonly used technique. However, although it is well established that available measurement techniques are accurate and precise for International Water Standards (Coleman *et al.* 1982; Wong *et al.* 1984, 1987, 1992; Kendall & Coplen 1985; Tanweer *et al.* 1988) and the dilution of isotopes in water (Halliday & Miller 1977), with the exception of the studies of Wong *et al.* (1987, 1992), there is no corresponding mass of data to indicate that isotope dilution can be measured correctly in body water and biological fluids such as plasma, saliva, urine or milk.

In a typical isotope experiment it is required to use the relationship: amount of isotope given equals amount of isotope found in body water. The amount of isotope found is the product of the isotope concentration in the body and body water mass (N); measurements of isotope concentration in a body fluid are therefore required. The amount of the isotope in a dose given to a subject cannot be known unless it is also measured with the same techniques as those used in the measurements of isotope enrichments in body water before ( $\hat{c}_p$ ) and after ( $\hat{c}_s$ ) isotope administration; (for definition of units of isotopic enrichments

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used in this paper, see p. 5). Typically, this measurement ( $\partial_a$ ) is made on a sample of the dose ( $D_w$ ) diluted in natural abundance water (T) having an enrichment ( $\partial_t$ ), in a proportion roughly equivalent to the expected dilution of the dose ( $D_u$ ) in N.

Implicit in the relationship derived to calculate body water with these methods:

$$N = \frac{D_u T(\partial_a - \partial_t)}{D_w(\partial_s - \partial_p)} \quad (1)$$

is the assumption that there is no fundamental difference between the measurement of the isotopic enrichment in the water sample and the measurement of the isotopic enrichment in a body fluid.

Measurements of turnover rate (F) require that N is multiplied by a rate constant for exponential isotope loss ( $k$ ) ( $F = kN$ ). Unlike the measurement of N, the measurement of  $k$  does not depend upon comparison between isotope enrichment in water and isotope enrichment in a physiological fluid but merely requires the precise measurement of serial dilution. It is clear that if body water can be measured correctly, it is probable that rate constants will be measured correctly. However the converse is not true. The ability to measure  $k$  correctly does not provide any security that N is being measured correctly because this requires comparisons between measurements made in water and measurements made in a body fluid.

Accordingly, the present work describes attempts to show that currently used methods for the measurements of isotopic enrichment in biological fluids produce values that will not compromise the calculations of  $k$  and N.

## MATERIALS AND METHODS

### *Simulation of isotope dilution experiments*

Samples of enriched water and urine were prepared to simulate isotope disappearance curves and to assess the accuracy of isotopic distribution space measurements.

A weighed amount ( $D_w$ ) of water enriched in both  $^2\text{H}$  and  $^{18}\text{O}$  was made up to 250 g with natural abundance water (Cambridge Tap Water;  $C_0$ ). A portion of this enriched sample ( $C_{100}$ ) was further diluted in  $C_0$  to give solutions enriched above natural abundance at levels of 80, 60, 40 and 20% ( $C_{80}$ ,  $C_{60}$ ,  $C_{40}$  and  $C_{20}$ ). The procedures were repeated with a weighed amount of enriched water ( $D_u$ ) similarly diluted with natural abundance urine. Serial dilutions were made by weighing the diluants to  $\pm 10^{-3}$  g and  $D_w$  and  $D_u$  to  $\pm 10^{-5}$  g.

### *Preparation of reconstituted urine samples and freeze-drying*

Freeze-drying was used to measure the water content of urine samples and to prepare urine solids for reconstituted urine samples. The latter were used to measure the effect on estimates of isotopic enrichment of the solids contained in urine.

Approximately 5 g urine were frozen as a thin shell in a spherical bottle and freeze-dried for 24 h (Virtis Freeze Mobile 6; Virtis Company Inc, Gardiner, NY, USA). The water and solid content of each sample was determined gravimetrically.

Two sets of reconstituted urine samples were prepared, ranging from 10 to 60 g solids/kg. One set was made from background (natural abundance) urine solids and background water (BS/BW) and the other was made from background solids and enriched water (BS/EW).

### *Isotopic ratios measurements*

The reduction of water or urine samples to  $\text{H}_2$  gas was performed with either a U or Zn technique.

The U reduction was carried out with an on-line system (Aqua Sira, VG Isotech, Middlewich). The samples are heated and vacuum-distilled on-line before admission to a U furnace coupled directly to a mass spectrometer (Wong *et al.* 1984; Barrie & Coward, 1985). The Zn technique used was a modified version of the method described by Coleman *et al.* (1982) and further investigated by Kendall & Coplen (1985). A  $4\ \mu\text{l}$  sample was reduced with either Indiana Zn (Friends of Biogeochemistry, Indiana University Foundation) in amounts ranging from 100 to 300 mg or with 250 mg Zn shot (AnalaR; BDH, Poole, Dorset).  $^2\text{H}:^1\text{H}$  ratios measurements of Zn-generated  $\text{H}_2$  gas were performed on a Sira 10 (VG Isotech) equipped with a split flight tube.

$^{18}\text{O}:^{16}\text{O}$  isotopic ratios were measured either with an on-line Aqua Sira procedure or with the conventional  $\text{CO}_2\text{-H}_2\text{O}$  equilibration technique. In the Aqua Sira procedure, water vapour is let directly into a mass spectrometer and  $^{18}\text{O}:^{16}\text{O}$  ratios are measured as the mass 20:18 ratio. The  $\text{CO}_2\text{-H}_2\text{O}$  equilibration was fully automated on an Isoprep 18 (VG Isotech) where 3 ml samples in conical flasks were equilibrated by shaking with  $0.4 \times 10^5$  Pa  $\text{CO}_2$  for 3 h at  $25^\circ$  (Wong *et al.* 1987). The equilibrated gases were sequentially analysed in a Sira 10 mass spectrometer.

All isotopic enrichments are expressed as delta per mil ( $\delta$ ) relative to the International Standard Vienna Standard Mean Ocean Water (SMOW), i.e:

$$\delta = \left( \frac{R_s}{R_{\text{SMOW}}} - 1 \right) \times 10^3,$$

where  $R_s$  and  $R_{\text{SMOW}}$  are the heavy:light isotopes ratios in a sample and SMOW respectively. Isotopic ratios in SMOW are 155.76 ppm for  $^2\text{H}:^1\text{H}$  and 2005.2 ppm for  $^{18}\text{O}:^{16}\text{O}$  (Gonfiantini, 1978).

Precision of the measurements, calculated as the standard deviation of the differences between duplicate measurements divided by  $\sqrt{2}$ , was: with the U technique  $1.2\ \delta$  at natural abundance and  $2.7\ \delta$  at enriched levels (of about  $850\ \delta$ ), and with the Zn technique  $0.7$  and  $3.2\ \delta$ . For  $^{18}\text{O}$  it was: with the Aqua Sira procedure  $0.3$  and  $0.5\ \delta$  (at about  $200\ \delta$ ), and with the equilibration method  $0.05$  and  $0.4\ \delta$  respectively.

#### *Measurements of the concentrations of urinary solids*

Urea and creatinine were measured using standard enzymic techniques adapted for use on the Cobas Fara (Roche, Welwyn Garden City, Herts.). Reagents from the urea kit were used to measure ammonia concentrations on a UV spectrophotometer (SP8-100 Pye Unicam, Cambridge, Cambs.).

#### *Statistical methods*

All results are expressed as mean and standard deviation (SD) unless stated otherwise. Comparison of means used analysis of variance while comparison of slopes was performed by analysis of covariance.

### RESULTS

#### *Disappearance curves*

The progressively diluted samples  $C_{100}$ ,  $C_{80}$ ,  $C_{60}$ ,  $C_{40}$  and  $C_{20}$  were considered to simulate an isotope disappearance in a labelling experiment in which  $C_{100}$  was exponentially reduced to  $C_0$  with a slope of  $0.12$ . Thus each solution ( $C_x$ ) represented a particular time point (starting at  $t = 0$  for  $C_{100}$  and finishing at  $t = \text{infinity}$  for  $C_0$ ) according to the equation:

$$C_x = C_{100}e^{-0.12t}.$$

Table 1. *Simulation of  $^2\text{H}$  disappearance curves from measurements made using the uranium and the zinc methods*

Sample type	Method	Slope†	SE	Intercept ( $\bar{\delta}$ )†	SD
Water	Uranium	0.1207	0.001	818.2	3.3
Urine	Uranium	0.1206	0.001	823.6	4.3
Water	Zinc	0.1193	0.001	818.9	5.2
Urine	Zinc	0.1193	0.001	810.2***	4.3
	Theoretical	0.1200			

† Slopes and intercepts were computed with the least squares method. The slopes are not significantly different from the theoretical slope. The intercept data only allow the methods to be compared. \*\*\*  $P < 0.001$  for the comparison between intercepts of the urine curves.

Table 2. *Simulation of  $^{18}\text{O}$  disappearance curves from measurements made using the Aqua Sira procedure and the equilibration method*

Sample type	Method	Slope†	SE	Intercept ( $\bar{\delta}$ )†	SD
Water	Aqua Sira	0.1197	0.0003	182.8	0.4
Urine	Aqua Sira	0.1202	0.0003	187.3	0.3
Water	Equilibration	0.1198	0.0002	183.5	0.3
Urine	Equilibration	0.1208	0.0002	187.8	0.3
	Theoretical	0.1200			

† Slopes and intercepts were computed with the least squares method. The slopes are not significantly different from the theoretical slope. The intercept data only allow the methods to be compared.

The least squares method was used to compute slopes and intercepts of isotope disappearance curves using the measured enrichments of each solution net of measured background. These computed intercepts were used to calculate the isotope distribution spaces using equation (1); urine intercept =  $\delta_s - \delta_p$ , water intercept =  $\delta_a - \delta_l$ .

The true dilution space was taken as the water content of the urine sample, calculated from its weight before and after freeze-drying, with the addition of an extra amount arising from the presence of exchangeable H in urine solids. According to Culebras & Moore (1977) H exchanges between water and biological material occur for compounds containing hydroxyl or amino groups. Thus  $^2\text{H}$  will, when added as water to a urine sample, equilibrate with both the water and the exchangeable H in urinary solids. The extra space due to  $^2\text{H}$ - $^1\text{H}$  exchanges in non-aqueous compounds was therefore calculated from the measurements of urea, creatinine and ammonia in the urines. There is no exchangeable O in urinary solids, thus the true  $^{18}\text{O}$  distribution space was considered to be the dilution space obtained from freeze-drying.

$^2\text{H}$  measurements. For the Zn method the values at each computed time were duplicates obtained with 4  $\mu\text{l}$  sample and 200 mg Indiana Zn. U values were duplicates. Table 1 displays the features of the simulations for both water and urine, using either the U or the Zn method: in no case were the slopes significantly different from the theoretical one.

Since  $D_w$  and  $D_u$  were different the comparison of the intercepts had to be restricted to the techniques. The two methods gave equivalent intercepts for water samples: Zn, 818.9 (SD 5.2)  $\bar{\delta}$  and U, 818.2 (SD 3.3)  $\bar{\delta}$  (not significant). However, there were significant differences between the intercepts with urine samples when the U and Zn methods were compared: Zn, 810.2 (SD 4.3)  $\bar{\delta}$  and U, 823.6 (SD 4.3)  $\bar{\delta}$  ( $P < 0.001$ ).

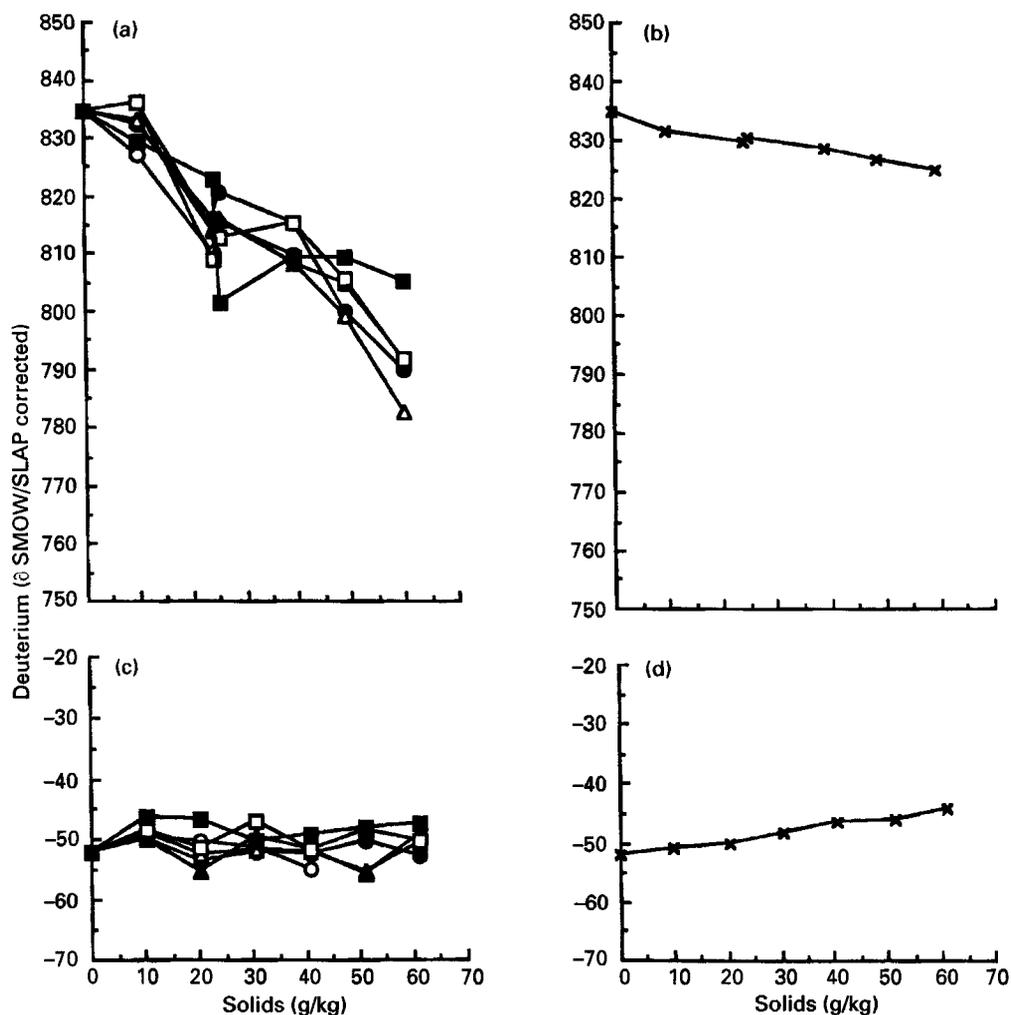


Fig. 1. Effect of increasing solids concentration on  $^2\text{H}$  enrichments of background solids (BS)/enriched water (EW) (a–b) and BS/background water (BW) (c–d) reconstituted samples analysed with either uranium ( $\times$ ) or with different amounts of zinc ( $\circ$  100 mg,  $\bullet$  150 mg,  $\triangle$  200 mg,  $\blacktriangle$  250 mg,  $\square$  300 mg or  $\blacksquare$  BDH-250 mg). All values are  $\delta$  units relative to Standard Mean Ocean Water (SMOW).

Distribution spaces calculated from these values show that the Zn method leads to a significant overestimation for  $^2\text{H}$  by 2.4 (SD 0.33) % compared with the true value obtained by freeze-drying: 246.3 (SD 0.5) g ( $P < 0.05$ ). This 'Zn-space' was significantly larger than that obtained with the U technique: 252.3 (SD 0.8) v. 247.9 (SD 1.6) g ( $P < 0.01$ ). The 'U-space' was not significantly different from the true dilution space.

$^{18}\text{O}$  measurements. Table 2 displays the features of the simulation for both water and urine, using either the Aqua Sira procedure or the equilibration method: the slopes were not significantly different from the theoretical one, whatever the method employed for the duplicate measurements.

The intercepts of the water and the urine disappearance curves were not significantly different when the two methods were compared. The isotope distribution spaces were not

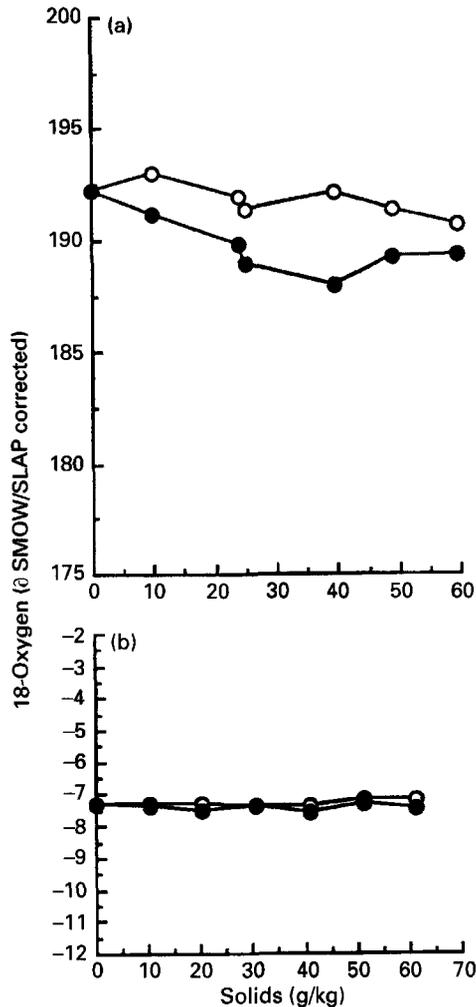


Fig. 2. Effect of increasing solids concentration on  $^{18}\text{O}$  enrichments of background solids (BS)/enriched water (EW) (a) and BS/background water (BW) (b) reconstituted samples analysed with either the Aqua Sira procedure (○) or with the equilibration technique (●). All values are  $\delta$  units relative to Standard Mean Ocean Water (SMOW).

significantly different from the space obtained by freeze-drying, 243.7 (SD 0.5) g ( $n$  9), Aqua Sira, 243.5 (SD 0.19) g and equilibration, 243.7 (SD 0.1) g (not significant).

#### *Reconstituted urine samples*

$^2\text{H}$  measurements. The relationships between the  $^2\text{H}$  enrichments of the two sets of reconstituted urine samples and their solid concentration are displayed in Fig. 1.

Fig. 1, panels (a) and (b), represents the  $^2\text{H}$  enrichments of reconstituted BS/EW (the samples made from enriched water and natural abundance urine solids). The enrichments of these samples measured in duplicate with the Zn technique are shown on panel (a). Increases in the concentration of solids significantly decreased the enrichments irrespective of the amounts of Zn used. The average slope (for all the data obtained with Indiana Zn) was  $-7.36$  (SE 0.43)  $\delta$  units per 10 g/kg solid concentration ( $P < 0.01$ ) whereas with Zn shot

the slope was  $-5.07$  (SE  $1.03$ )  $\delta$  units per  $10$  g/kg solid concentration ( $P < 0.01$ ). The precision of the measurements (for each amount of Zn used), although decreasing with the amount of solids, remained within a reasonable range ( $3.2$   $\delta$  at  $0$  solids,  $7.5$   $\delta$  at  $40$  g/kg). There was no reduction of the  $50$  and  $60$  g/kg samples with  $100$  mg Zn.

Panel (b) of Fig. 1 represents the enrichments of the same samples measured in triplicate with the U technique. There was a significant decrease of the  $^2\text{H}$  enrichment with the solid concentration ( $P < 0.01$ ). The slope was significantly different from those obtained with Zn ( $-1.49$  (SE  $0.13$ )  $\delta$  units per  $10$  g/kg solid concentration,  $P < 0.01$ ).

Panels (c) and (d) in Fig. 1 represent the  $^2\text{H}$  enrichments of reconstituted BS/BW (the samples made from background water and natural abundance urine solids) measured after reduction either with Zn or U. There was no significant relationship between the  $^2\text{H}$  enrichments measured with the Zn technique and the solid concentration (panel (c)). In contrast such a relationship was found if the measurements were performed with the U technique, the slope being  $0.92$  (SE  $0.26$ )  $\delta$  units per  $10$  g/kg solid concentration ( $P < 0.001$ ).

$^{18}\text{O}$  measurements. Fig. 2 represents the  $^{18}\text{O}$  enrichments of reconstituted BS/EW samples (panel (a)) and of BS/BW (panel (b)) measured with both techniques. There was a significant relationship between the enrichments and the solids but the slopes obtained with the two methods were not significantly different: Aqua Sira,  $-0.28$  (SE  $0.10$ ), equilibration,  $-0.51$  (SE  $0.20$ ).

No such relationship was found at natural abundance levels whatever the method used.

#### DISCUSSION

The present work has shown that when the most frequently used biological matrix (urine) is analysed for  $^2\text{H}$  enrichments the values obtained depend upon both the method and the solids content of the sample. In contrast,  $^{18}\text{O}$  measurements are not significantly affected by the same factors. For a urine sample of an average solids concentration of about  $40$  g/kg the Zn method will underestimate  $^2\text{H}$  enrichments in comparison with a pure water standard by about  $2.4\%$ . If  $^2\text{H}$  distribution space is calculated from the same data, values will be overestimated by this amount.

It could be argued that these findings might be unique to specific aspects of the present authors' technique. However, the literature (Wong *et al.* 1987) describing the use of a Zn method for urine samples also shows that for the urine samples studied, analytical values were about  $1\%$  lower than those expected, whereas  $^{18}\text{O}$  values agreed to  $0.1\%$ . The interpretation of this data in the present context is difficult because the specific nature of the urine samples used is not known. It is also interesting to note that when a series of urine samples derived from subjects taking part in a multicentre study on the doubly-labelled water technique were analysed, the range of inter-laboratory variation was greatest for  $\text{N}_d$  and progressively less for  $k_d$ ,  $k_o$  and  $\text{N}_o$ . For one subject only, five 'experienced' laboratories were asked to provide data and the ranges of values were  $\pm 3.5$ ,  $2.1$ ,  $0.7$  and  $0.4\%$ , respectively (S. B. Roberts, personal communication).

There are other data for  $^2\text{H}$  analyses in physiological water samples that are informative. If only one isotope (commonly  $^2\text{H}$ ) is used for the measurement of body water and body composition, conclusions about the adequacy of the method can only be made by comparison of the results with those obtained from other methods of measuring body composition (Fuller *et al.* 1992). However, the now-frequent use of  $^2\text{H}_2\text{O}$  and  $\text{H}_2^{18}\text{O}$  in the doubly-labelled water method has provided a large amount of valuable data (summarized in Table 3) on  $^2\text{H}$  and  $^{18}\text{O}$  distribution spaces in the body. This may be of more than physiological interest. It is difficult to avoid the conclusion that in laboratories where the Zn method has been used to measure total body water the ratio of isotope distribution

Table 3. Published  $^2\text{H}$ : $^{18}\text{O}$  distribution space ratios

Method	Sample	Ratio	SD	n	Subjects	Reference
Zn/CO <sub>2</sub>	Plasma	1.006	NA	9	Preterm infants	Jensen <i>et al.</i> (1989)
Zn/CO <sub>2</sub>	Urine	1.010	0.007	12	Preterm infants	Jensen <i>et al.</i> (1992)
Zn/CO <sub>2</sub>	Urine	1.010	0.063	41	Women	Forsum <i>et al.</i> (1992)
Zn/CO <sub>2</sub>	Urine	1.012	0.020	10	Infants*	Butte <i>et al.</i> (1990)
Ur/Water	Urine	1.019	0.068	23	Women	Forsum <i>et al.</i> (1992)
Ur/CO <sub>2</sub>	Plasma	1.021	0.014	7	Women and men	Schoeller <i>et al.</i> (1980)
Zn/CO <sub>2</sub>	Urine	1.023	0.039	10	Infants*	Wong <i>et al.</i> (1990)
Zn/CO <sub>2</sub>	Urine	1.023	0.020	10	Infants*	Butte <i>et al.</i> (1990)
Zn/CO <sub>2</sub>	Urine	1.024	NA	40	Infants*	Butte <i>et al.</i> (1992)
Zn/CO <sub>2</sub>	Urine	1.025	0.020	10	Infants*	Butte <i>et al.</i> (1990)
Ur/Water	Urine	1.025	0.012	4	Preterm infants	Roberts <i>et al.</i> (1986)
Zn/CO <sub>2</sub>	Urine	1.025	0.029	10	Infants*	Wong <i>et al.</i> (1990)
Zn/CO <sub>2</sub>	Urine (distilled)	1.027	0.020	6	Women	Casper <i>et al.</i> (1991)
Ur/CO <sub>2</sub>	Urine	1.028	0.049	8	Women and men	Schoeller <i>et al.</i> (1980)
Ur/Water	Urine	1.028	0.009	14	Senile dementia women	Coward & Cole (1991)
Ur/CO <sub>2</sub>	Saliva	1.030	0.017	6	Men	Schoeller <i>et al.</i> (1986)
Ur/Water	Urine	1.030	0.024	29	Gambian men	Coward & Cole (1991)
Zn/CO <sub>2</sub>	Urine	1.031	0.020	10	Infants*	Butte <i>et al.</i> (1990)
Ur/CO <sub>2</sub>	NA	1.032	0.018	27	Women and men	Racette (1991)
Zn/CO <sub>2</sub>	Urine (distilled)	1.032	0.010	6	Anorexic women	Casper <i>et al.</i> (1991)
Ur/Water	Urine	1.034	0.012	36	Lactating women	Coward & Cole (1991)
Ur/Water	Urine	1.034	0.011	16	Men	Coward & Cole (1991)
Ur/Water	Urine	1.035	0.020	136	Children†	Coward & Cole (1991)
Ur/CO <sub>2</sub>	Urine	1.035	0.033	4	Cerebral palsy or myelo-dysplasia children†	Bandini <i>et al.</i> (1991)
Ur/Water	Urine	1.036	0.010	16	Women	Coward & Cole (1991)
Ur/Water	Urine	1.036	0.014	13	Women and men	Pullicino <i>et al.</i> (1993)
Ur/Water	Urine	1.036	0.012	42	Pregnant women	Coward & Cole (1991)
Ur/Water	Urine	1.037	0.011	17	Women	Coward & Cole (1991)
Ur/CO <sub>2</sub>	NA	1.038	0.016	5	Children†	Schoeller (1988)
Ur/Water	Urine	1.039	0.011	36	Children†	Livingstone <i>et al.</i> (1992)
Ur/Water	Urine	1.040	0.013	8	Obese women	Coward & Cole (1991)
Zn/CO <sub>2</sub>	Urine	1.041	NA	11	Obese women	Krietzman <i>et al.</i> (1992)
Zn/CO <sub>2</sub>	Urine	1.041	0.052	10	Men	Wong <i>et al.</i> (1988)
Zn/CO <sub>2</sub>	Urine	1.043	0.075	63	Pregnant women	Forsum <i>et al.</i> (1992)
Zn/CO <sub>2</sub>	Urine	1.047	0.009	12	Children†	Goran <i>et al.</i> (1992)
Zn/CO <sub>2</sub>	Plasma	1.048	0.018	20	Women and men	Cochran <i>et al.</i> (1988)
Zn/CO <sub>2</sub>	Urine	1.048	0.021	31	Women	Goldberg, (unpublished results)
Zn/CO <sub>2</sub>	Urine	1.049	0.009	12	Obese and non-obese women and men	Ravussin <i>et al.</i> (1991)
Zn/CO <sub>2</sub>	Urine	1.050	0.011	13	Women and men	Goran <i>et al.</i> (1992)
Zn/CO <sub>2</sub>	Urine	1.050	0.022	11	Women and men	Goran <i>et al.</i> (1992)
Zn/CO <sub>2</sub>	Urine	1.051	0.015	18	Women and men	Goran <i>et al.</i> (1992)
Zn/CO <sub>2</sub>	Urine	1.052	0.032	10	Women	Wong <i>et al.</i> (1988)
Zn/CO <sub>2</sub>	NA	1.056	NA	6	Women and men	Wong <i>et al.</i> (1987)
Ur/Water	Urine	1.062	0.074	46	Pregnant women	Forsum <i>et al.</i> (1992)

Zn, zinc method; Ur, uranium method; CO<sub>2</sub>, CO<sub>2</sub>-H<sub>2</sub>O equilibration method; Water, Aqua Sira procedure; NA, not available.

\* Infants < 1 year old.

† Children > 1 year old.

spaces ( $N_D/N_O$ ) is higher than when a U method is used. In no study with U did the average ratio exceed 1.04 and in studies with Zn, with the exception of very young children and when samples were distilled, average ratios were never less than 1.04. Data from infants do not show this trend but this is likely to be because infants do not produce concentrated urine samples.

In contrast to these difficulties, it is clear that whatever the method used and whether  $^2\text{H}$  or  $^{18}\text{O}$  is analysed the methods scale correctly when serial dilutions of isotope are made using a single sample of urine. Thus, slopes of isotope disappearance curves will be measured correctly if all urine samples are of the same solids concentration, but it is likely that  $^2\text{H}$  disappearance curves could be subject to random error as a consequence of normal random variation in the solids content of the urine samples collected in the course of an experiment.

In addition to the purely analytical considerations this work has physiological implications for both body composition studies and the doubly-labelled water method. Speakman (Speakman *et al.* 1993) has proposed a revision of Schoeller's original calculation for  $\text{CO}_2$  production using doubly-labelled water (Schoeller *et al.* 1986) that involves using an average distribution space ratio of 1.04 rather than 1.03. This revision was suggested partly because recent data have moved the accumulated population mean value to 1.04 from 1.03. Reference to Table 3 (for adults) and the present work show that this could be a result of the increased use of the Zn method for  $^2\text{H}$  analysis.

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