Body composition of lactating women determined by anthropometry and deuterium dilution

By WILLIAM W. WONG*, NANCY F. BUTTE, E. O'BRIAN SMITH, CUTBERTO GARZA AND PETER D. KLEIN

USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas 77030, USA

1. Body fat, fat-free mass and total body water of ten lactating women were estimated from deuterium-dilution spaces and from skinfold thickness measurements. Deuterium-dilution spaces were calculated from the 6 h (equilibration) and zero-time (extrapolation) deuterium enrichments in saliva, urine, human milk and breath water vapour samples.

2. The deuterium spaces obtained by equilibration were statistically larger than those obtained by extrapolation. Isotope dilution spaces derived from deuterium enrichments in saliva, breath water vapour and human milk did not differ with the exception of the 6 h equilibration value of milk, which was greater than that estimated from saliva. Deuterium-dilution spaces estimated from urine were consistently smaller than those derived from the other biological fluids.

3. No significant differences in body fat, fat-free mass and total body water were observed between anthropometric measurements and deuterium-dilution methods, except for extrapolated values derived from deuterium enrichments in urine.

Studies of body composition in human subjects inherently lack absolute certainty, because methods by necessity must be indirect. Methodological evaluation when applied to human subjects, therefore, has been based on validations in animals and on comparisons between indirect methods. During lactation, dynamic changes occur in body composition as the body reverts to its prepartum state. Questions of relevancy arise when body composition techniques that are validated in normal adults are applied to post-partum, lactating women, because of the changes in fat distribution and metabolism that occur during lactation. In a previous publication, we concluded that equations specific for lactating women were not necessary to predict body density from skinfold thicknesses (Butte *et al*, 1985).

In the present study, estimations of body fat, fat-free mass (FFM) and total body water (TBW) of lactating women were compared by anthropometric and deuterium-dilution methods. Technical aspects of the deuterium-dilution method and their impact on the prediction of body composition compartments were investigated. Specifically, in vivo isotope fractionation factors of hydrogen in saliva, urine, human milk and respiratory water vapour were measured and incorporated into the calculation of isotope dilution space (IDS); one-point equilibration and multi-point extrapolation modes to calculate IDS were compared, and differences in IDS based on saliva, urine, human milk and respiratory water vapour were evaluated.

SUBJECTS AND METHODS

Subjects

Ten lactating women, mean age 28·4 (SD 4·2) years, and parity equal to 1·2 (SD 0·4), participated in the study. Informed written consent was obtained from each subject and the

* Children's Nutrition Research Center, 1100 Bates Street, Houston, Texas 77030, USA.

protocol was approved by the Institutional Review Board for Human Research of Baylor College of Medicine and the Clinical Investigations and Publications Committee at Texas Children's Hospital. Mean height and prepregnancy weight were 1·645 (SD 0·032) m and 56·7 (SE 4·6) kg respectively. Maternal weight at delivery was 71·2 (SD 6·4) kg, resulting in a weight gain during pregnancy of 14·5 (SE 2·9) kg. At the time of study, 3·4 (SD 1·3) months postpartum, the women weighed 61·1 (SD 7·6) kg.

Infants were term and appropriate size for gestational age. All the infants were healthy at the time of the study; five were fed on human milk exclusively and five received food supplements in addition to human milk.

Experimental design

Maternal body fat, FFM and TBW were estimated from measurements of skinfold thicknesses and deuterium-dilution spaces. The study was conducted over a 14 d period. For the first 2 d, mothers and infants were admitted to the Clinical Research Center (CRC) at Texas Children's Hospital. Anthropometric measurements were made, and the deuterium dilution procedure begun. Urine, saliva, respiratory water vapour, and milk samples were obtained for the estimation of IDS. Biological specimens were collected at the CRC by the nursing staff or at the home by the mothers after instruction in the CRC.

Anthropometry

Women were weighed on a beam balance (3PY1002; Detecto Medic, New York). Height was measured with an upright extension meter. Skinfold thicknesses were measured to the nearest 0·5 mm using Lange calipers (Cambridge Scientific Industries, Cambridge, MD) at the following sites: triceps, biceps and subscapular (Durnin & Rahaman, 1967). Because of the difficulty in obtaining accurate and reproducible skinfold thickness measurements in the suprailiac regions of post-partum women, the suprailiac skinfold thicknesses were not used in the calculation. Measurements were made by one person (NFB). Body density was predicted from Durnin & Womersley's (1974) regression equation for females of 20–29 years of age: density (g/ml) = $1·1605 - 0·0777 \times$ log (sum of three skinfold thicknesses). Body fat was calculated according to Siri (1956): body fat = $(4·95/\text{density} - 4·50)$. FFM was equal to the difference between body-weight and body fat. TBW was estimated from the anthropometrically derived value of FFM and the conversion factor of 0·73 (Forbes, 1987).

Deuterium-dilution method

Baseline samples of saliva, urine, human milk and respiratory water vapour were collected from each women before oral administration of the ^2H_2O tracer (100 mg/kg body-weight). Post-dose samples were collected at approximately 0·5, 1, 1·5, 2, 3, 6, 9 and 12 h and on days 2, 4, 6, 8, 10, 12 and 14 following administration of the ^2H_2O.

Saliva samples (1 ml) were collected from under the tongue using a plastic syringe and transferred to a crimp-top vial (Wheaton, Millville, NJ) for storage. Urine samples were collected with a specimen cup. A portion of urine (1 ml) was transferred to a crimp-top vial for storage. Human milk was expressed from the left breast using an electric breast pump (Egnell, Inc., Cary, IL). The milk samples were defatted by centrifugation; portions (1 ml) of the defatted milk were transferred to crimp-top vials for storage. For the collection of respiratory water vapour, subjects were instructed to exhale through a polyethylene tube (approximately 750 mm in length) which was partially submerged in a propan-2-ol-dry ice bath at $-60°$ for 2–5 min. Accumulated breath vapour (1 ml) was then thawed and transferred to a crimp-top vial.

Isotope-ratio measurements

The saliva, urine, human milk and respiratory water vapour samples were reduced to hydrogen gas over zinc-shot at 475° for 30 min (Wong *et al.* 1987). The ^{2}H:^{1}H ratio was measured by gas-isotope-ratio mass spectrometry (Finnigan Delta-E, Finnigan MAT, San Jose, CA). The results are expressed in delta (δ) per mil (‰) units which are defined as:

$$\delta_{ws}{}^2H, \text{‰} = \left(\frac{R_{sample}}{R_{ws}} - 1\right) \times 10^3, \qquad (1)$$

where R_{sample} and R_{ws} are the ^{2}H:^{1}H ratios of the sample and working standard respectively. The $\delta_{ws}{}^2$H values were normalized against two international water standards: Vienna-Standard Mean Ocean Water (V-SMOW) and Standard Light Antarctic Precipitation (SLAP) according to Gonfiantini (1984):

$$\delta_{normalized}, \text{‰} = \left[\frac{(\delta_{sample/ws} - \delta_{V\text{-}SMOW/ws})}{(\delta_{SLAP/ws} - \delta_{V\text{-}SMOW/ws})}\right] \times \delta^\circ{}_{SLAP/V\text{-}SMOW}, \qquad (2)$$

where $\delta_{sample/ws}$, $\delta_{V\text{-}SMOW/ws}$ and $\delta_{SLAP/ws}$ are the δ^2H values of the sample, V-SMOW and SLAP measured against the laboratory working standard respectively. The $\delta^\circ{}_{SLAP/V\text{-}SMOW}$ has a defined value of -428‰ (Gonfiantini, 1984) for ^{2}H:^{1}H isotope ratio measurements. An accuracy of $-4\cdot6$‰ and a precision of $3\cdot2$‰ (SD) were obtained on saliva, urine and human milk samples at 580‰ enrichment levels of ^{2}H (Wong *et al.* 1987).

IDS were calculated from the isotopic enrichment of deuterium in saliva, urine, human milk and respiratory water vapour as follows:

$$\text{IDS (kg)} = \frac{d}{MW} \times \frac{APE}{100} \times \frac{18\cdot02}{EOB \times R_{std}} \times \alpha, \qquad (3)$$

where d is the dose (g) of deuterium oxide, MW is the molecular weight of the labelled water (g/mol), APE is the atom percent excess of the isotope, 18·02 is the molecular weight of water (g/mol), EOB is the isotopic enrichment of the isotope over baseline value (per mil), R_{std} is the ^{2}H:^{1}H ratio of V-SMOW which has a value of 0·00015595 (De Wit *et al.* 1980) and α is the isotope-fractionation factor of the physiological fluid. The isotope-fractionation factors relative to plasma water were 1·004 for saliva (Wong *et al.* 1988), 0·993 for urine, 0·942 for breath water vapour and 1·002 for human milk. IDS was calculated from the EOB value at 6 h postdose and from the EOB value obtained by extrapolation to the zero-time intercept using all isotope measurements after 6 h.

TBW was assumed to be equal to IDS. FFM was calculated as TBW/0·73. Body fat was computed as body-weight minus FFM.

Statistics

Repeated-measures analysis of variance was used to detect significant differences between methods (Snedecor & Cochran, 1967). A multiple comparison procedure (Snedecor & Cochran, 1967) was then used to isolate the differences. To assess the agreement between methods, we used the following procedure (Bland & Altman, 1986). First, the between-method differences were plotted against the means of both methods. Regression analysis was then used to test for a relation between the differences and the means. If the slope was not significant, the relative bias (mean difference between methods) and the 95 % limits of agreement (mean difference and 2 SD of the differences) were computed. Of all differences 95 % were expected to fall within these limits. To determine if the bias (mean difference) was significantly different from zero, a paired *t* test was used. If the slope relating the between-method differences and means was significant, the 95 % limits of agreement were estimated as 2 SE of the estimate around the regression line.

Table 1. *Anthropometric indices of ten lactating women*

Subject no.	1	2	3	4	5	6	7	8	9	10	Mean	SD
Wt (kg)	66·5	62·2	70·4	62·7	51·7	72·3	52·3	56·1	64·1	52·3	61·1	7·6
Height (m)	1·645	1·689	1·676	1·638	1·606	1·626	1·619	1·689	1·594	1·664	1·645	0·034
Biceps (mm)	9·0	4·5	14·0	7·0	5·0	11·5	8·0	8·0	13·0	6·0	8·6	3·3
Triceps (mm)	23·0	12·5	30·0	23·0	15·5	27·0	19·0	17·5	23·5	20·5	21·2	5·3
Subscapular (mm)	25·5	12·0	24·0	23·5	11·0	20·5	11·0	6·5	19·0	9·8	16·3	6·9

Table 2. *Body composition compartments derived from anthropometric measurements of ten lactating women*

Subject no.	1	2	3	4	5	6	7	8	9	10	Mean	SD
Density: g/ml	1·024	1·047	1·018	1·026	1·044	1·023	1·038	1·044	1·025	1·039	1·033	0·011
Fat: kg	22·3	14·2	25·5	20·3	12·5	24·5	14·1	13·6	21·1	13·7	18·2	5·0
g/kg body-wt	335	228	362	324	241	339	270	243	329	263	294	49
FFM: kg	44·2	48·0	44·9	42·4	39·2	47·8	38·2	42·4	43·0	38·6	42·9	3·5
g/kg body-wt	665	772	638	676	759	661	730	756	670	737	706	49
TBW: kg	32·3	35·0	32·8	31·0	28·6	34·9	27·9	31·0	31·4	28·1	31·3	2·6
g/kg body-wt	485	563	466	494	554	482	533	552	490	538	516	36

FFM, fat-free mass; TBW, total body water.

Table 3. *Body composition compartments derived from deuterium-dilution method for ten lactating women*

Subject no.	1	2	3	4	5	6	7	8	9	10	Mean	SD
TBW*: kg	33·6	36·2	32·8	30·2	28·8	36·0	27·4	28·1	34·1	25·9	31·3	3·7
g/kg body-wt	504	582	466	482	558	498	524	500	532	494	514	35
FFM: kg	46·0	49·6	45·0	41·4	39·5	49·3	37·6	38·4	46·7	35·4	42·9	5·1
g/kg body-wt	691	797	639	660	764	682	718	685	729	677	704	48
Fat: kg	20·5	12·6	25·4	21·3	12·2	23·0	14·7	17·6	17·4	16·9	18·2	4·4
g/kg body-wt	309	202	361	340	236	318	282	314	271	323	296	48

TBW, total body water; FFM, fat-free mass.
* Calculated from 6 h deuterium-enrichment values in saliva.

RESULTS

The anthropometric indices of the ten lactating women are presented in Table 1. Body composition compartments calculated from anthropometric and deuterium-dilution methods are shown in Tables 2 and 3 respectively.

A comparison of the body composition compartments predicted from anthropometry and deuterium-dilution methods is summarized in Table 4. No significant differences in body fat, FFM or TBW were observed between anthropometry and deuterium-dilution methods (TBW based on 6 h saliva sample). It should be noted that however small the

Table 4. *Assessment of the differences observed between anthropometry and deuterium dilution methods for the determination of body fat, fat-free mass (FFM), and total body water (TBW) of ten lactating women*

(Mean values and standard deviations)

	Anthropometry		Deuterium dilution		Relative bias*		95% Limits of agreement†	Paired *t* test
	Mean	SD	Mean	SD	Mean	SD		
Fat:kg	18·2	5·0	18·2	4·4	0·02	2·3	−4·9–4·7	NS
g/kg body-wt	294	49	296	48	−2	40	−82–77	NS
FFM:kg	42·9	3·5	42·9	5·1	−0·02	2·3	−3·6–3·6	‡
g/kg body-wt	706	49	704	48	2	40	−77–82	NS
TBW:kg	31·3	2·6	31·3	3·7	−0·01	1·7	−2·6–2·6	‡
g/kg body-wt	516	36	514	35	2	29	−56–60	NS

NS, not significant.

* Mean between-method difference and SD.

† Mean difference and 2 SD of the differences.

‡ Since the slope relating the between-methods differences *v.* the mean values was significant, a test of the mean differences was not meaningful.
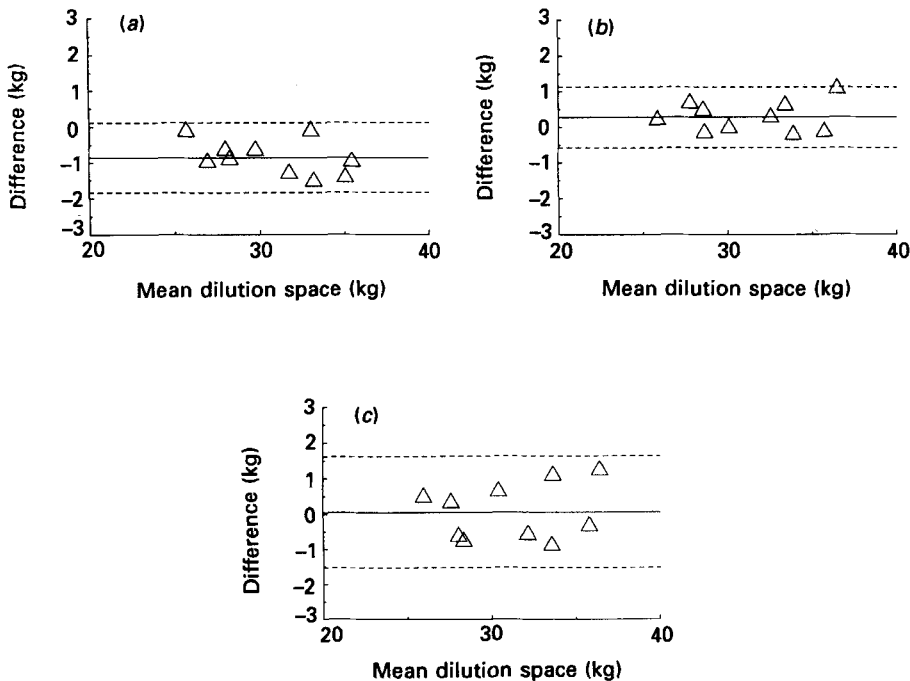

Fig. 1. Comparison of deuterium-dilution spaces derived from (*a*) urine and saliva, (*b*) human milk and saliva, and (*c*) breath water vapour and saliva, of ten lactating women. Individual differences between methods (△); relative biases (—); upper and lower 95% limits of agreement between methods (---). The ordinates represent the differences between the deuterium-dilution spaces calculated from the isotopic abundances of deuterium in the urine, human milk and breath water vapour against those derived from saliva.
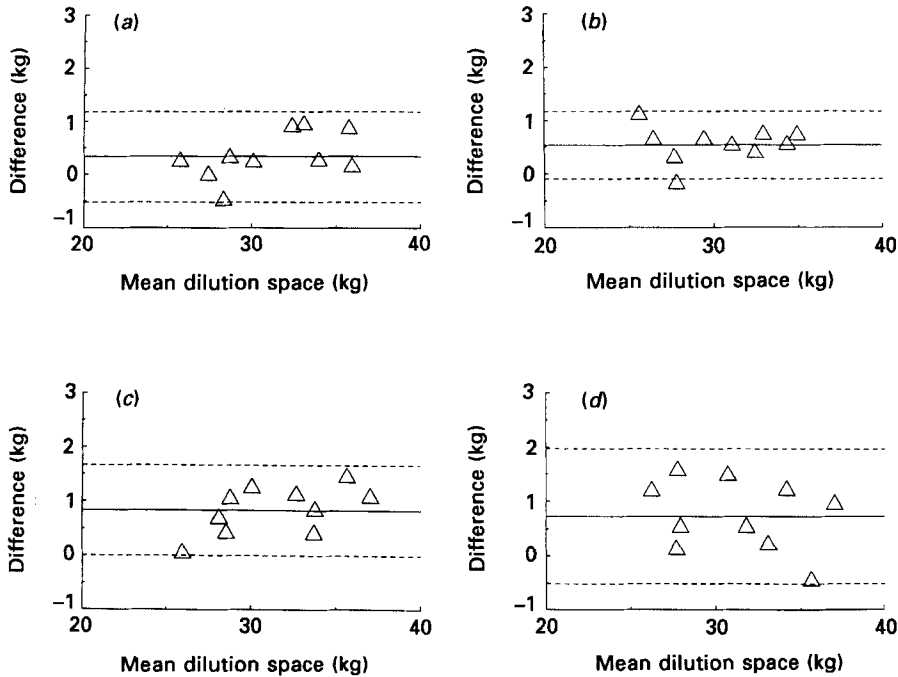
Fig. 2. Comparison of deuterium-dilution spaces calculated using the 6 h enrichment values and extrapolated zero-intercept enrichment values in (a) saliva, (b) urine, (c) human milk, and (d) breath water vapour of ten lactating women. Individual differences between methods (△); relative biases (—); upper and lower 95 % limits of agreement between methods (---). The ordinates represent the differences between the deuterium-dilution spaces derived from the 6 h and the extrapolated enrichment values.

differences between methods for FFM (kg) and TBW (kg), they tended to become smaller as the magnitude of the IDS became larger. Similar method comparisons were made between anthropometry and deuterium dilution using IDS derived from milk, breath water vapour and urine; significant differences in body compartments were demonstrated between anthropometry and deuterium dilution only when the IDS was based on urine samples calculated by extrapolation.

The IDS predicted from the four biological fluids were compared and statistically significant differences were detected (ANOVA, $P < 0.01$). Mean differences in IDS based on saliva and breath water vapour samples were not significantly different from zero, whether they were calculated from the equilibrated (mean difference 0·24 (SD 0·98) kg) or extrapolated enrichment values (mean difference 0·15 (SD 0·73) kg); nor were the mean differences in IDS derived from milk and breath significantly different from zero (mean differences 0·29 (SD 1·2) kg and 0·18 (SD 0·74) kg respectively). The IDS based on isotope enrichment of milk at 6 h was significantly greater than the estimate based on saliva at 6 h (mean difference (0·53 (SD 0·55) kg, $P < 0.02$); no significant differences in IDS were observed for the extrapolated mode of calculation between milk and saliva. The IDS based on urine samples was significantly less than that calculated for saliva, milk and breath, regardless of the mode of calculation (mean differences ranged between 0·8 and 1·3 kg, $P < 0.01$). Comparisons of IDS derived from saliva v. urine, milk or breath samples are illustrated in Fig. 1 (a–c).

Deuterium-dilution spaces calculated from equilibrated 6 h and from extrapolated zero-intercept enrichment values are compared in Fig. 2 (a–d) for saliva, urine, milk and breath

water vapour. The mean differences between these two approaches (6 h equilibrium minus the value derived from the multi-point extrapolation for calculating IDS) were 0·33 (SD 0·43) kg for saliva, 0·53 (SD 0·32) kg for urine, 0·83 (SD 0·41) kg for human milk and 0·72 (SD 0·62) kg for breath water vapour. No significant relations were seen between the differences and the mean IDS by regression analysis. The mean differences observed for the two modes of calculation were significantly different from zero in the four fluids ($P < 0.01$–0.05). The mean differences between 6 h and extrapolated values for saliva, urine, milk and respiratory water vapour were equal to 1·1, 1·8, 2·6, and 2·3 % of their respective group mean IDS.

DISCUSSION

Mean values for body fat, FFM and TBW were essentially identical when estimated by anthropometric or deuterium-dilution methods. The values obtained in the present study were similar to those reported in other studies of well-nourished lactating women (Manning-Dalton & Allen, 1983; Butte *et al.* 1984). The close agreement between methods indicates that the Durnin & Womersley (1974) and Siri (1956) equations used for the prediction of body fat from skinfold thicknesses are applicable for use in lactating women. The 95% limits of agreement between methods were relatively wide, however, which indicated that the methods could differ substantially when applied to individuals. For example, 95% of the differences between methods for predicting body fat would be within ± 8·0 %, which represents about 27% of the mean body fat observed in these women.

Although the deuterium-dilution method involves certain assumptions and errors, it is the more direct and precise of the two methods. The anthropometric approach is based on unproven assumptions that the thickness of subcutaneous fat is representative of the total body fat content and that the sites of measurement reflect the average thickness of the entire subcutaneous layer. Changes in fat distribution (Taggart *et al.* 1967) and differences in the rate of mobilization of fat from diverse adipose-tissue sites (Quandt, 1983) during lactation may account for a significant amount of the observed variability.

When the deuterium-dilution method is applied, major factors that must be considered and resolved are the biological fluid to be sampled, the schedule of sample collection, the validation of IDS and TBW equivalency, the assumed water content of FFM, and isotopic fractionation.

Various authors have shown that the IDS derived from saliva and plasma are comparable (Schoeller *et al.* 1982; Wong *et al.* 1988). In the present study we found that the IDS based on saliva, human milk and breath, calculated in the same manner, i.e. equilibration or extrapolation, were not significantly different from one another, with the exception of the saliva–milk comparison at 6 h. IDS derived from 6 h milk values was higher than that based on saliva, indicating lower enrichment values in milk; the isotope may have been diluted by pre-existing milk in the gland. IDS based on urine samples was consistently less than the IDS based on the other fluids; slight biological fractionation in the re-absorption of water from the renal tubules may account for this difference. Mean differences observed between urine and the other fluids were generally less than 1·0 kg and the 95% limits of agreement averaged ± 0·65 kg; therefore, the practicality of obtaining urine samples in some protocols may outweigh the slight loss in accuracy.

IDS may be calculated from a single enrichment value taken at equilibrium or from the zero-time intercept obtained from extrapolation of several post-dose enrichment values. In normal adults, isotope equilibrium in plasma, urine, saliva and respiratory water vapour is reached by 3 h post dose (Wong *et al.* 1988). IDS calculated from the deuterium enrichment in the 3, 4, 5 and 6 h post-dose samples were within 3% of each other. The isotopic loss

during equilibration is assumed to be negligible. The extrapolation approach accounts for isotopic loss before equilibration, but assumes that the rate of loss is mono-exponential. In our study, IDS calculated from the 6 h equilibrium enrichment were consistently higher than IDS predicted from extrapolation, and the differences between modes of calculation ranged from 1·1 to 2·6% of their respective mean IDS. The time-course of deuterium enrichment in the four fluids demonstrated that 100% equilibration had not been achieved by 6 h in all women. Pooling of water in the bladder and mammary gland may contribute to unequal distribution of isotope at 6 h post-dose. If this is the case, calculation of IDS by extrapolation may be theoretically more accurate; however, differences in IDS that range from 1·1 to 2·6% approach our analytical precision in measurements of ^{2}H:^{1}H.

The deuterium-dilution technique overestimates TBW to the degree that deuterium exchanges with non-aqueous hydrogens. Estimates of TBW by tritium have been reported to differ from those obtained by desiccation by 4–15%, depending on age and animal species (Sheng & Huggins, 1979). Error may be introduced by loss of tracer through urine, breath and skin during the equilibration period. Forbes (1987) estimated that 1·4% of the dose could be lost in the initial 5 h post-dose in a human adult. Calculation of FFM from TBW using the conversion factor, 0·73, introduces an uncertain degree of error.

In summary, deuterium-dilution and anthropometric methods provided similar mean estimates of body fat, FFM and TBW in this group of lactating females. The relatively wide 95% limits of agreement between the two methods, however, suggests that caution should be exercised when individual values are interpreted.

REFERENCES

Bland, J. M. & Altman, D. G. (1986). Statistical methods for assessing agreement between two methods of clinical measurements. *Lancet* **i**, 307–310.

Butte, N. F., Garza, C., Stuff, J. E., Smith, E. O. & Nichols, B. L. (1984). Effect of maternal diet and body composition on lactational performance. *American Journal of Clinical Nutrition* **39**, 296–306.

Butte, N. F., Wills, C., Smith, E. O. & Garza, C. (1985). Prediction of body density from skinfold measurements in lactating women. *British Journal of Nutrition* **53**, 485–489.

De Wit, J. C., Van Der Straaten, C. M. & Mook, W. G. (1980). Determination of the absolute hydrogen isotopic ratio of V-SMOW and SLAP. *Geostandards Newsletter* **4**, 33–36.

Durnin, J. V. G. A. & Rahaman, M. M. (1967). The assessment of the amount of fat in the human body from measurement of skinfold thickness. *British Journal of Nutrition* **21**, 681–689.

Durnin, J. V. G. A. & Womersley, J. (1974). Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *British Journal of Nutrition* **32**, 77–97.

Forbes, G. B. (1987). *Human Body Composition*, p. 31. New York: Springer-Verlag.

Gonfiantini, R. (1984). *Report on Advisory Group Meeting on Stable Isotope Reference Samples for Geochemical and Hydrological Investigations*. Vienna, Austria: International Atomic Energy Agency.

Manning-Dalton, C. & Allen, L. H. (1983). The effects of lactation on energy and protein consumption, postpartum weight change and body composition of well-nourished North American women. *Nutrition Research* **3**, 293–308.

Quandt, S. A. (1983). Changes in maternal postpartum adiposity and infant feeding patterns. *American Journal of Physical Anthropology* **60**, 455–461.

Schoeller, D. A., Dietz, W. D., Van Santen, E. & Klein, P. D. (1982). Validation of saliva sampling for total body water determination by $^2H_2^{18}O$ dilution. *American Journal of Clinical Nutrition* **35**, 591–594.

Sheng, H.-P. & Huggins, R. A. (1979). A review of body composition studies with emphasis on total body water and fat. *American Journal of Clinical Nutrition* **32**, 630–647.

Siri, W. E. (1956). *Body Composition from Fluid Spaces and Density. University of Colorado Donner Laboratory Physics Report.* no. 3349. Boulder, Colorado: University of Colorado.

Snedecor, G. W. & Cochran, W. G. (1967). *Statistical Methods*, pp. 299–338. Iowa: Iowa State University Press.

Taggart, N. R., Holliday, R. M., Billewicz, W. Z., Hytten, F. E. & Thomson, A. M. (1967). Changes in skinfolds during pregnancy. *British Journal of Nutrition* **21**, 439–451.

Wong, W. W., Cochran, W. J., Klish, W. J., Smith, E. O., Lee, L. S. & Klein, P. D. (1988). In vivo isotope fractionation factors and the measurement of deuterium- and oxygen-18-dilution spaces from plasma, urine, saliva, respiratory water vapor, and carbon dioxide. *American Journal of Clinical Nutrition* **47**, 1–6.

Wong, W. W., Lee, L. S. & Klein, P. D. (1987). Deuterium and oxygen-18 measurements on microliter samples of urine, plasma, saliva, and human milk. *American Journal of Clinical Nutrition* **45**, 905–913.