Measurement of total body water using ^{2}H dilution: impact of different calculations for determining body fat

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This study estimated total body water (TBW) in four groups (twelve per group; sedentary and highly trained men and women) at the time of ^{2}H dosing (T_0) and after a 3·5 h equilibration period (T_{eq}). Standard TBW calculations were employed at T_0 (no correction for disproportionate urinary tracer loss) and T_{eq} (correction for urinary tracer loss only), plus those calculations that corrected for a disproportionate urinary tracer loss and insensible tracer loss respectively. The measurement of body density enabled the four TBW estimates to be compared for the determination of three-compartment % body fat (BF). The very small difference between the standard and corrected T_0 TBW data was not significant ($P=0.914$) and no Group × TBW interaction was identified ($P=0.125$). These results reflect the closeness of the ^{2}H concentration in the urine produced during the equilibration period and the T_{eq} saliva samples. The associated mean % BF values were essentially identical. Although correcting for insensible ^{2}H losses in addition to urinary losses at T_{eq} produced a statistically significant ($P<0.001$) lower mean TBW (about 200 g) than the standard calculation, this translated to a small difference in % BF (0·3). The larger difference (about 500 g, $P<0.001$) between the two (T_0, T_{eq}) corrected TBW calculations was also associated with a small body composition difference (0·1 % BF), which was less than the propagated error (0·3 % BF) for the three-compartment body composition model. Corrections to the standard calculations of TBW at T_0 and T_{eq} for a protocol employing a brief equilibration period (3·5 h) were therefore of marginal use for improving the accuracy of % BF estimates. The TBW difference over time (T_0 *v*. T_{eq}) also had little impact on % BF values.

Hydrometry: Isotopic dilution: Multicompartment body composition model

Investigators are becoming increasingly aware that the traditional two-compartment (fat mass and fat-free mass (FFM)) body composition models that involve the measurement of body density (BD) via hydrodensitometry and total body water (TBW) via isotopic dilution produce large errors. Hydrodensitometry assumes that the density of FFM is 1·100 g/cm^3. However, this assumption is often invalidated because of the acutely variable TBW, which comprises by far the largest percentage and has the lowest density of the four FFM components (water, protein, bone mineral, non-bone mineral). The isotopic dilution method is also sensitive to variability in TBW because it assumes that the FFM hydration is constant at

72 %. Withers *et al.* (1999) estimated that the extremes of FFM hydration for the normal population yield errors of 6·7 and 4·8 % body fat (BF) for the hydrodensitometry and isotopic dilution methods, respectively. Siri (1956) initially identified this problem and proposed a three-compartment (fat mass, TBW, fat-free dry solid) model, which accounts for inter-individual variability in FFM hydration. The superiority of three-compartment estimates of body composition over the two-compartment models therefore relies upon the accurate measurement of TBW via isotopic dilution. However, using ^{2}H$_2$O, which is the preferred tracer for TBW measurements because it is inexpensive and stable, invalidates the assumptions of the dilution

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Abbreviations: BD, body density; BF, body fat; FFM, fat-free mass; T_0, time of ^{2}H dosing; T_{eq}, time of collecting equilibrium body fluid samples; TBW, total body water.

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principle. ^2H not only distributes itself in the TBW pool, but it also exchanges with the non-aqueous H of proteins, carbohydrates and fats. It has been estimated that this exchange results in an overestimation of the TBW pool by about 4 % (Schoeller, 1996). Inaccuracies may also occur because of the addition of water to the body pool during the equilibration period. This contaminates those estimates of TBW established at the time of ^2H dosing (T_0). Schoeller *et al.* (1985) reported a small TBW over-estimation (0·3 %), which was attributed to metabolic water and the exchange of ^2H_2O with ambient water vapour, for a 4·0 h equilibration protocol. However, these authors did not consider the errors that may occur at T_0 if the urinary and insensible ^2H losses over the equilibration period occur disproportionately to the equilibrium levels. Estimates of TBW may also be established at the time of collecting equilibrium body fluid samples (T_{eq}). These estimates may suffer inaccuracies due to ^2H losses over the equilibration period. When urinary ^2H losses are accommodated, the TBW may be overestimated by about 1·4 % due to the loss of tracer via insensible routes (Schoeller *et al.* 1985).

Data were collected in the current study to establish if the standard calculations of TBW at T_0 and T_{eq} could be improved significantly by introducing the following corrections: (1) adjusting departures of urinary tracer levels from the equilibrium concentration as tracer loss or gain for the calculation of TBW at T_0; (2) accommodating insensible tracer losses in addition to urinary losses for the determination of TBW at T_{eq}. Three-compartment % BF estimates were also generated to clarify the value of implementing the aforementioned corrections across individual subjects with varying amounts of BF and TBW. The impact of the equilibration period on TBW and % BF was also determined by comparing the corrected T_0 and T_{eq} values.

Methods

Subjects

Forty-eight young (18–36 years old) men and women were recruited to participate in the present study, which involved the determination of body composition via a three-compartment model requiring measurements of BD and TBW. The subjects were non-obese and reported mass stability (within ± 2 kg) for the preceding 2 years. All were in good health, reporting no medication usage except for the contraceptive pill, and the women were eumenorrhoeic. There were twelve subjects in each of the

following four groups: sedentary men and women and highly trained men and women. The sedentary subjects reported abstinence from physical training for the previous 2 years, whereas those who were highly trained had prepared for middle-distance running events or triathlons at state and/or national level for at least the preceding 2 years. Table 1 shows the descriptive statistics for each group of subjects. Written informed consent was obtained from the subjects after they had reviewed written information describing the study. The present study was approved by the Flinders Medical Centre's Committee on Clinical Investigation.

Protocol

Subjects attended one testing session involving the measurement of BD and TBW by underwater weighing and isotopic dilution respectively. Measurements commenced in the morning when the subjects were 12 h post-prandial, euhydrated and had not exercised for 24 h. The effect of fluid retention by the women was minimised by not testing them either during the 7 d preceding menstruation or during menstruation. On arrival at the laboratory the subjects were asked to void and then nude mass was measured. This was followed by the collection of saliva, ^2H dosing and underwater weighing. Subjects were asked to void 3·5 h after the ^2H dose when nude mass was measured again and equilibrium saliva samples were taken.

Body density

BD was measured by underwater weighing at residual volume. Corrections were made for water density and the ventilated residual volume, which was determined by He dilution with the subjects immersed in water to neck level and in the same posture as during the underwater mass determinations. This methodology has been fully described elsewhere (Withers *et al.* 1996). Our latest precision data for two trials on six subjects yielded intraclass correlation coefficient 0·998 and technical error of measurement 0·3 % BF.

Total body water

This was measured by ^2H dilution. A saliva sample was collected on arrival at the laboratory to determine the background ^2H concentration. A dose of 40 mg ^2H_2O/kg, which was adjusted to about 100 ml with distilled water, was then

Table 1. Descriptive statistics for age, height, body mass and BMI for the trained and sedentary subjects

(Mean values and standard deviations)

Group	n	Age (years)		Height (m)		Mass (kg)		BMI (kg/m^2)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Trained males	12	22·3	5·1	1·752	0·057	67·87	5·30	22·1	1·0
Sedentary males	12	24·7	4·5	1·781	0·085	73·33	9·70	23·2	3·4
Trained females	12	23·8	5·7	1·694	0·089	57·51	5·93	20·0	1·4
Sedentary females	12	22·4	2·8	1·631	0·056	55·39	3·92	20·9	1·7

drunk through a straw. The container was rinsed three times with about 30 ml distilled water that was also ingested by the subject. An equilibrium saliva sample was taken 3·5 h later. Precautions were taken to minimise isotopic fractionation during the collection of all saliva samples. The subjects were not allowed to eat, drink or exercise during the intervening period.

The ^2H concentrations in the doses, saliva and urine samples were determined on a V.G. Micromass 602 D (Micromass Ltd, Manchester, UK) isotope ratio MS, which was calibrated against Vienna Standard Mean Ocean Water and International Atomic Energy Agency enriched standards 302A and 302B (Analytical and Quality Control Services, Vienna, Austria). The standard deviations for repeated trials on the background and two enriched standards were ≤1·0 and about 3·0‰ respectively. The TBW was calculated at T_0 and T_{eq} in accordance with the recommendations of Schoeller et al. (1986), who advocate a 4% correction factor for the exchange of ^2H_2O with labile H of protein and other body constituents. Corrected TBW values for T_0 were also generated by treating departures of ^2H concentration in the urine collected over the equilibration period from the equilibrium saliva level as tracer loss or gain. Corrected T_{eq} values were furthermore generated by accounting for evaporative tracer loss in addition to urinary losses. This correction assumed that: ^2H was lost in proportion to equilibrium levels; insensible water loss was the difference between the body mass at T_0 and the sum of the body mass at T_{eq} and the urine collected over the equilibration period. Our latest reliability data for two TBW trials on consecutive days (n 5) produced an intraclass correlation coefficient 0·999 and a technical error of measurement 0·25 kg.

Body fat

A three-compartment model was used to determine % BF. This model delineates three body compartments, namely the fat mass, TBW and fat-free dry mass whose densities at 36°C are assumed to be 0·9007 (Fidanza et al. 1953), 0·9937 (Lentner, 1981) and 1·569 (Brozek et al. 1963) g/ cm³ respectively. Substitution of these values into the following formula:

$$\frac{1}{BD} = \frac{FM}{FM \ density} + \frac{TBW}{H_2O \ density} + \frac{FFDM}{FFDM \ density},$$

produces:

$$\% \ BF = \frac{211·5}{BD} - 78·0\left(\frac{TBW}{body \ mass}\right) - 134·8,$$

where FM is fat mass and FFDM is the fat-free dry mass.

Data analysis

Differences between the standard and corrected TBW estimates at T_0 across the four groups were examined using a 2 (TBW: standard and corrected) × 4 (Group: trained and sedentary males, trained and sedentary females) factorial design ANOVA with repeated measures across TBW.

Tukey honestly significant difference post–hoc tests were applied in the event of a significant ($P \leq 0.05$) F ratio for the group main effect. This analysis was repeated using the T_{eq} data. In the absence of a significant Group × TBW interaction, the corrected T_0 and T_{eq} TBW data were pooled and the difference analysed via a paired t test ($P \leq 0.05$).

Results

Table 1 contains the descriptive statistics for the forty-eight subjects. The TBW data derived for T_0 and T_{eq} using the standard and corrected calculations are presented in Table 2. Both calculations of TBW at T_0 produced essentially identical values for each of the four groups: The 2 × 4 repeated-measures ANOVA did not, therefore, result in a statistically significant main effect (TBW calculation: $P=0.914$) and interaction (TBW calculation × Group: $P=0.125$). The same analysis for the standard calculation of TBW at T_{eq} produced significantly greater ($P<0.001$) values than the corrected calculation method across all four groups but the interaction was not significant ($P=0.130$). The mean TBW for the combined groups was 200 g greater for the standard calculation at T_{eq} compared with the corrected calculation at T_{eq}. This statistically significant difference between the TBW calculations resulted in a relatively small % BF difference (mean value for all subjects 0·3 % BF). A comparison of the corrected TBW values determined for T_0 and T_{eq} revealed that a significant (about 500 g; $P<0.001$) amount of water was lost over the 3·5 h equilibration period. However, the body composition difference between T_0 and T_{eq} as a result of this water loss was minor (mean value for all subjects 0·1 % BF).

Discussion

The accurate determination of TBW at T_0 is based on the assumption that any tracer losses during the equilibration period occur in proportion to the equilibrium concentration of the tracer in the body. However, it would seem reasonable to consider that the urine voided during the equilibration period, which was being produced continuously while tracer absorption was occurring, would contain less tracer than the TBW equilibrium concentration. Nevertheless, the results shown in Table 2 indicate very little difference ($P=0.914$) between the standard TBW values derived at T_0 and those corrected for disproportionate urinary tracer loss. This applied to all groups (Group × TBW interaction: $P=0.125$) and reflected the closeness of the ^2H concentrations in the equilibrium saliva samples and the urine that was collected over the 3·5 h equilibration period. The % BF data obtained from the two estimations of TBW were essentially identical. Measurements of urinary tracer loss would therefore appear unjustified when TBW is estimated at T_0.

The extent to which insensible ^2H losses detract from the accuracy of the TBW estimates at T_0 could not be quantified in the current study. However, insensible water loss in our subjects only ranged from 50–250 g over the 3·5 h equilibration period. Moreover, the rapid distribution of

Table 2. Total body water (TBW) and three-compartment percentage body fat (BF) estimates determined at the time of ^2H dosing (T_0) and at the equilibrium time 3·5 h later (T_{eq})*
(Mean values and standard deviations)

		TBW (kg)								Three-compartment % BF							
		T_0				T_{eq}				T_0				T_{eq}			
		Standard†		Corrected‡		Standard§		Corrected‖		Standard		Corrected		Standard		Corrected	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
All subjects	48	36·91	6·72	36·91	6·72	36·60	6·67	36·40	6·60	19·6	7·9	19·6	7·9	19·4	8·0	19·7	7·9
Trained men	12	43·24	3·60	43·23	3·59	42·89	3·42	42·62	3·42	11·9	2·7	12·0	2·8	11·7	2·8	12·0	2·8
Sedentary men	12	41·08	4·57	41·08	4·56	40·74	4·73	40·51	4·62	21·7	8·0	21·7	8·1	21·6	8·2	21·9	8·1
Trained women	12	34·70	3·50	34·68	3·48	34·32	3·32	34·19	3·29	16·2	2·3	16·2	2·3	16·1	2·3	16·3	2·3
Sedentary women	12	28·62	2·08	28·64	2·08	28·44	2·14	28·30	2·13	28·4	4·7	28·4	4·7	28·3	4·8	28·5	4·8

* For details of subjects and procedures, see Table 1 and p. 326.
† TBW calculations assumed that any urinary or insensible tracer loss occurred in proportion to the equilibrium tracer concentration and therefore did not influence the TBW estimates.
‡ TBW calculations treated deviations of urinary tracer concentration from the equilibrium value as tracer loss or gain.
§ TBW calculations ignored insensible tracer loss, but corrected for urinary losses over the equilibration period.
‖ TBW calculations corrected for insensible and urinary tracer losses.

^2H into the urine, which was identified by the afore-mentioned data, suggest that insensible ^2H losses also quickly reach levels commensurate with the equilibrium concentration. One final consideration in relation to insensible ^2H losses is the lower propensity of ^2H_2O to enter the vapour state compared with unlabelled water. Wong et al. (1988) quantified this property by determining plasma ^2H concentration:^2H concentration in respiratory water vapour. Their value of 0·944 agreed closely with an in vitro established vapour:liquid value of 0·941 reported by Schoeller (1996). While a fractionation ratio needs to be considered when estimating TBW from ^2H measurements of respiratory water vapour, it would provide a negligible correction to the amount of tracer accounted for in a small volume (50–250 g) of water lost insensibly.

Finally, the estimation of TBW at T_0 may be over-estimated due to the addition of water to the body pool following a ^2H dose. Our protocols preclude the ingestion of water during the equilibration period and Schoeller et al. (1985) have demonstrated that overestimations due to metabolic water and the exchange of ^2H_2O with ambient water are negligible (about 0·2 % in 3·5 h). In our subjects, the aforementioned error would translate to about 60 g. The mass of the dose solutions and rinsings (about 200 g) used in the present study, which constitute an addition to the TBW pool at T_0, were subtracted from the TBW values.

When the TBW is estimated at T_{eq}, which was 3·5 h following the ^2H dose in the present study, the dose needs to be adjusted to account for the tracer lost over the equilibration period. While investigators usually account for urinary tracer losses, insensible losses are generally ignored. Table 2 indicates that the accommodation of only urinary tracer losses leads to an overestimation of TBW by about 200 g (0·2 % BF underestimation). This represents a relatively small error, albeit a statistically significant ($P < 0·001$) one, which applied across all the groups (Group × TBW interaction: $P = 0·13$). However, protocols that are associated with greater insensible water losses than were experienced in the present study may be prone to physiologically significant errors.

A statistically significant ($P < 0·001$) difference was identified between the two corrected calculations of TBW at T_0 and T_{eq}. Although this difference (about 500 g) was also physiologically significant, the impact on the % BF estimates was relatively minor (0·1). The difference between T_0 and T_{eq} was less than the reported propagated error (0·3 % BF; Withers et al. 1999) for our three-compartment body composition technique. However, the aforementioned difference would be amplified when longer equilibration periods are employed or the ambient conditions are conducive to high rates of insensible water loss. If this were the case, it would be prudent to measure TBW at T_0, which would best reflect the euhydrated state given that no fluid replacement occurs during the equilibration period.

In summary, our present results suggest that the correction of TBW values at T_0 and T_{eq} for disproportionate urinary tracer loss and insensible tracer loss respectively were of marginal use in improving the accuracy of % BF estimates using the three-compartment body composition model. Furthermore, the statistically significant TBW

difference between the two corrected methods translated to a % BF difference that was less than the propagated error for the three-compartment body composition model. However, protocols employing longer equilibration periods and producing greater insensible water losses than were experienced in the present study would amplify the % BF differences.

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