Comparative measurements of total body water in healthy volunteers by online breath deuterium measurement and other near-subject methods¹–³

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ABSTRACT

Background: We developed a new near-subject approach, using flowing afterglow–mass spectrometry (FA-MS) and deuterium dilution, which enables the immediate measurement of total body water (TBW) from single exhalations.

Objectives: The objectives were to show the efficacy of the new FA-MS method in measuring TBW in healthy subjects and to compare these measurements with values derived from multifrequency bioelectrical impedance analysis, skinfold-thickness (SFT) measurements, and both recent and historical published regression equations.

Design: After baseline measurement of breath deuterium abundance, 24 healthy subjects ingested 0.3 g D_2O/kg body wt. A second breath sample was taken after 3 h to measure the increase in deuterium, from which TBW was calculated. Bioelectrical impedance analysis was carried out with a multifrequency analyzer, and SFT was measured by a single trained observer. Methods were compared with the use of Pearson’s correlation coefficient and Bland-Altman analyses.

Results: TBW measures obtained by all methods were highly correlated (r = 0.95–0.98, P < 0.001), especially those between FA-MS, SFT measurement, and recent regression equations. The mean values obtained were within 2% of those published for age-matched control subjects and varied by 1–6% when all methods were compared. Systematic bias was greatest when FA-MS was compared with bioelectrical impedance analysis, which tended to underestimate TBW in smaller, female subjects. No bias related to subject size was observed in a comparison of FA-MS with SFT measurement or with more recent regression equations.

Conclusions: FA-MS is a simple and effective new approach to TBW measurement in healthy subjects. The difficulty of using population-derived equations to estimate TBW in individual subjects is emphasized.

KEY WORDS Total body water, deuterium isotope dilution, flowing afterglow–mass spectrometry, bioelectrical impedance analysis, breath test, noninvasive measurement

INTRODUCTION

Accurate measurements of total body water (TBW) are of value in many physiologic and pathophysiologic circumstances. A fundamental aspect of body composition, TBW is influenced by nutritional status (1) and water homeostasis (2). In renal medicine, where both these aspects of body composition can be adversely affected (3), TBW is of particular importance because it also determines the volume of distribution of water-soluble uremic toxins (4).

Several approaches have been taken to the measurement of TBW; most of them provide little more than an estimate, often relying on regression equations derived from population data. These approaches include skinfold-thickness (SFT) measurements (5), bioelectrical impedance analysis (BIA) (6), differential X-ray absorption (7), computerized tomography scanning (8), and indirect measures of body density such as underwater weighing or air displacement (9). However, the most direct and accurate method involves isotope dilution. In this approach, a known dose of an isotopic variant of water is ingested, and, after the elapsing of enough time to allow equilibration of the deuterium within the body water, a sample of blood, urine, or saliva is taken for analysis by stable-isotope mass spectrometry (10). Isotope dilution using D_2O and H_2^{18}O as well as radioactive tritiated water has been used extensively (10, 11).

Currently, the use of isotope dilution to measure TBW is limited to the research environment. A number of factors prevented its becoming a routine clinical method, including the inconvenience of blood sampling, the delay (typically, several days) in the results of mass spectrometric blood analysis, and the cost. A breath test using H_2^{18}O dilution and breath C^{18}O_2 analysis was developed (10), but the cost of the isotopic variant of water is prohibitive. BIA is more immediate, but it is time-consuming and its accuracy

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We showed, by using standard dilute mixtures of D₂O in isotope analysis flowing afterglow–mass spectrometry (FA-MS) of breath exhalations (14, 15). We call this new method of stable real-time, the fraction of deuterium in the water contained in single-breath exhalations (14, 15). We carried out a comparative study of the TBW in 24 healthy volunteers with the use of parallel deuterium-dilution and BIA measurements of TBW. As a step toward this goal, we carried out a comparative study of the TBW in 24 healthy volunteers with the use of parallel deuterium-dilution and BIA measurements of TBW.

SUBJECTS AND METHODS

Subjects and study design

Twenty-four healthy volunteers (12 women and 12 men, aged 28–79 y) were included in the study. The mean values of weight, height, body mass index (BMI; in kg/m²), and age are shown in Table 1 according to sex. The formulas of Watson et al (11) and Chumlea et al (18) were used to predict the subjects’ expected TBW values, to enable comparison with both FA-MS and BIA TBW determinations. Using a cross-sectional study design, each study subject underwent BIA, SFT measurements, and deuterium-dilution measurement by the new FA-MS method to determine his or her TBW. Data were usually collected on the same day, but, when this was not possible, a check was performed to verify that the subject’s body weight had not changed. No subjects were taking regular medication that might interfere with body composition. One person was taking amiloride for mild, well-controlled hypertension. Throughout the FA-MS experiments, the breath deuterium of a single, healthy control subject who had not ingested D₂O was regularly measured to verify that cross-contamination and baseline drift were not occurring. The North Staffordshire Hospital Ethics Committee approved the study, and all subjects gave written informed consent.

Total body water measurement from deuterium in breath water vapor by FA-MS

The details of the FA-MS technique are presented in several recent publications (14–17, 19). Briefly, a swarm of H₃O⁺ precursor ions is created in helium carrier gas by a weak microwave discharge. These precursor ions react with the water, HDO, H₂¹⁷O, and H₂¹⁸O molecules that occur naturally in the water contained in a breath exhalation, a small fraction of which is sampled via a calibrated capillary and introduced into the carrier-gas ion swarm. The hydrated ions H₃O⁺·(H₂O), at a mass-to-charge ratio (m/z) of 73 and their isotopic variant ions H₄DO⁺, H₅DO⁺, and H₆DO⁺ at an m/z of 74 and H₃¹⁷O⁺, at an m/z of 75 are thus formed as the H₃O⁺ ions associate with water molecules (16). With the adoption of the known fractional abundance of ¹⁸O in water vapor and with an accounting for the contribution of the isotopic ions H₅¹⁷O⁺ to the ion signal at an m/z of 74, measurement of the 74:75 ion signal ratio by quantitative mass spectrometry provides the fractional deuterium abundance in the water vapor sample (14). Correction is made for differences in the evaporation rates of water and HDO at the lung blood–breath interface (at body temperature). Examples of the raw data obtained in FA-MS are given in our previous publications (14, 15). It is sufficient to say here that the D:H in single-breath exhalations can be obtained to < 1% precision and accuracy (17). Thus, when this ratio is measured after the ingestion of an accurately measured amount of D₂O, the TBW value can be deduced to a similar precision. This means that the TBW can be measured to an accuracy within a few hundred milliliters in most cases.

Another exciting feature of the FA-MS method is its ability to explore the kinetics of water dispersal between the various body compartments. Typical time constants for dispersal (upper intestinal tract to blood; blood to muscle) are 10–20 min. Thus, by an exploitation of the high sampling frequency of breath deuterium in the FA-MS method, which can be several times/min if necessary, the breath-time profile of deuterium can be well described (15). This also allows the equilibrated breath deuterium content to be well established (14).

Baseline breath deuterium values were obtained for all 24 subjects before D₂O ingestion and for the control subject (=156 ppm, consistent with the values in local tap water). The subjects then ingested a quantity of 99.9%-pure D₂O in ~100 mL of tap water; the dose of D₂O equaled ~0.3 g · kg⁻¹ body wt. The actual individual doses of D₂O were accurately weighed, and the D₂O was washed down with an additional 100 mL of tap water. Breath samples were taken ~3 h after the D₂O ingestion, after which time our previous studies have shown that a near steady state of breath deuterium content is reached, which implies even dispersal among the body water (15). The TBW is simply measured from the increase in the breath deuterium content in relation to the volume of D₂O ingested. This is explained in more detail in our previous publications (14, 15). Finally, the TBW values were reduced by 4% to account for the H-D exchange that occurs between HDO and the carboxyl, hydroxyl, and amino groups in body proteins (10, 20).
Total body water measurement by bioelectrical impedance analysis

BIAs were made with a multifrequency analyzer (model 4200, Xitron Hydra; Xitron Technologies Inc, San Diego) (21). Subjects were asked to lie supine on a couch for 10 min. After the skin was cleansed to ensure good contact, and electrodes were attached to the right wrist and ankle in a standardized fashion: the voltage-detector electrodes were placed at the line of the joint and the current-injection electrodes were placed over the metacarpal–phalangeal and metatarsal–phalangeal joints, respectively. These electrodes were then coupled to the Xitron Hydra, which was operated from a laptop computer, to initiate and store the readings. The acquisition of data typically took 15 s.

The Xitron Hydra measures resistance and reactance, from which are calculated the reciprocal impedance at 50 programmed frequencies. These frequencies are spaced between 5 and 1 MHz for calculation of the combined extracellular and intracellular fluid volumes and thus of TBW. With the use of the Cole-Cole plot to determine the theoretical resistance at infinite frequency (12), the TBW is calculated from the formula

\[ V_{\text{TBW}} = K_p \rho_f \cdot L^2 / R \]

where \( V \) is volume, \( K_p \) is a factor relating the relative proportions of the leg, arm, torso, and height; \( \rho_f \) is the resistivity of the fluid; \( L \) is the body height; and \( R \) is the resistance. This method of measuring TBW has been validated in young and middle-aged healthy subjects with the use of isotope-dilution methods, with a correlation ranging between 0.89 and 0.99 (22, 23).

Total body water measurements by skinfold-thickness measurement

Each subject’s height was measured with the use of a wall-mounted stadiometer to the nearest 0.1 cm, and the weight was measured on a balance-beam scale to the nearest 100 g. A single trained person (BE) whose technique had been validated (CV using 4 sites within published range: 9%; 24) took SFT measurements at 4 sites (triceps, biceps, subscapular, and suprailiac) on the right side of the body with a SFT caliper (Holtain Ltd, Dyfed, United Kingdom) according to the methods described by Harrison et al (25). Three measurements were taken at each site in rotation, and the mean was used for subsequent analysis. Body density was calculated from the age- and sex- specific formulas of Durnin and Womersley (9), and the percentage total body fat was derived (26). TBW was derived from percentage body fat by calculating \( 0.73 \times (\text{weight} - \text{body fat}) \) (20).

Statistical analyses

The precision of the FA-MS measurements was calculated with the use of the SE of triplicate breath analyses that took into account the Poisson distribution of the detected ion counts. Comparisons between the methods of measuring or estimating TBW were made by calculation of Pearson’s correlation coefficient, and the slope of the intercepts of the regression lines was tested for significant differences from 1 and 0, respectively. Bias and systematic differences between methods were assessed by the method of Bland and Altman (27). Comparisons of the mean differences between methods of measuring TBW according to sex were analyzed with the use of analysis of variance to test for sex-by-method interactions.

RESULTS

Using the new FA-MS method, successful measurements of TBW were made for all of the study subjects. Typically, breath sample acquisition took \( \approx 1 \) min, and once the post-D\(_2\)O ingestion breath sample had been obtained, the TBW was calculated immediately, because the FA-MS software has been developed to give instantaneous measurement of the deuterium abundance (P Španěl, SCILIB library, version 4, 2001. Internet: http://scilib.webpark.cz). As would be expected, the baseline breath D:H essentially did not vary between subjects (± 2 ppm), and it reflected the ratio in the local water supply (± 156 ppm). One of the advantages of the FA-MS method is that, in contrast with conventional techniques, it does not require calibration (17), so in principle TBW can be measured to the required accuracy from a single post-D\(_2\)O ingestion breath sample.

FA-MS measurements of TBW for each subject are shown in Table 1, where they can be compared with estimates made with the use of BIA, SFT measurement, and the Watson (11) and Chumlea (18) formulas referred to previously. The TBW values from all 5 methods were highly correlated with each other (see Table 2 for summary), although the correlations were best in comparisons of FA-MS with the Chumlea formula and SFT measurement and least good in comparisons of BIA with the Chumlea formula and SFT measurement. The mean differences in TBW between methods are summarized in Table 3, and they vary from 1% to 6%. The best agreement was observed between FA-MS and the Chumlea formula, when both the mean differences and the spread of agreement (SD) are taken into account.

The effect of sex on the degree of the difference observed varied according to the method used. The largest discrepancies were observed in comparison between the Watson formula and other

### Table 2

Summary of correlations between the flowing afterglow–mass spectrometry (FA-MS) measurements and the estimates of total body water (TBW)\(^{\dagger}\)

<table>
<thead>
<tr>
<th>Method</th>
<th>FA-MS</th>
<th>Chumlea (18) formula</th>
<th>Watson (11) formula</th>
<th>BIA</th>
<th>SFT measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBW: D(_2)O FA-MS (kg)</td>
<td>1.00</td>
<td>0.97</td>
<td>0.97</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>TBW: Chumlea (18) formula (kg)</td>
<td>0.97</td>
<td>0.97</td>
<td>0.97</td>
<td>0.97</td>
<td>0.98</td>
</tr>
<tr>
<td>TBW: Watson (11) formula (kg)</td>
<td>0.97</td>
<td>0.97</td>
<td>0.97</td>
<td>0.97</td>
<td>0.97</td>
</tr>
<tr>
<td>TBW: BIA (kg)</td>
<td>0.97</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>TBW: SFT measurement (kg)</td>
<td>0.98</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

\(^{\dagger}\)BIA, bioelectrical impedance analysis; SFT, skinfold thickness. All values are significant, \( P < 0.001 \).
methods, whereas the least difference by sex was observed between FA-MS and SFT measurement.

Systematic bias according to subject size varied according to the methods compared (Table 4 and Figure 1). The bias was greatest when FA-MS was compared with BIA, such that the latter underestimated TBW in smaller, female subjects, whereas when the Watson formula was compared with the Chumlea formula, the former gave lower values for TBW in larger, male subjects than did the latter. When TBW, measured by either FA-MS or BIA, is related to BMI, clear separation by sex is evident (Figure 2), with different slopes of the regression lines. In other words, women generally have lower absolute TBW as well as a lower percentage body water relative to body mass than men have, as a result of women’s higher body fat content. This suggests that the relatively poor agreement between these methods of TBW determination that is seen in smaller, female subjects is related to the different fat content in women, which is not adequately accounted for by the formulas used to derive TBW from BIA. The least systematic bias was seen between the SFT measurement, FA-MS, and Chumlea formula methods.

With these observations taken together, there appear to be 2 principal reasons for disagreement between these various methods, and both are related to sex differences. On the one hand, better agreement between FA-MS and the Chumlea formula appears to result from the closer values obtained in the men, because of the relatively higher absolute values for TBW obtained by the more recent regression equations. On the other hand, agreement is influenced by the degree of body fat, especially in women in whom BIA underestimated TBW. This latter problem is less evident with the use of SFT measurement, which by definition takes fat distribution into account.

**DISCUSSION**

The primary objective of this study was to show the utility of the new, rapid FA-MS breath test in measuring TBW in human subjects. The FA-MS measurements were successfully accomplished with ease. The subjects were able to give satisfactory breath samples with minimal practice, and they found the method acceptable and preferable to blood sampling. The method also enabled immediate calculation of the TBW. This is in clear contrast to previous attempts to create a rapid method of TBW measurement (10). The only previous attempt at developing an isotope-dilution breath test used the more expensive H218O stable isotope of water to measure the 18O enrichment of CO2 in breath (10). Rather than using a near-patient device, as can be done with FA-MS, workers had to transfer samples rapidly to the laboratory, and subsequent analysis took an additional 2 h. A breath-test approach using ethanol dilution has also been attempted (28). This approach, which assumes the steady-state metabolism of alcohol and subsequent alcohol dehydrogenase in the stomach and liver, requires the subject to give repeated breath samples for at least 4 h. The subject also has to remain under observation until the potential toxic

### Table 4

Summary of correlations between differences and average estimates of total body water (TBW) by method (Bland-Altman analysis)

<table>
<thead>
<tr>
<th></th>
<th>FA-MS</th>
<th>Chumlea (18) formula</th>
<th>Watson (11) formula</th>
<th>BIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBW: Chumlea (18) formula (kg)</td>
<td>-0.32</td>
<td>0</td>
<td>-0.51</td>
<td>0.42</td>
</tr>
<tr>
<td>TBW: Watson (11) formula (kg)</td>
<td>0.39</td>
<td>0.65</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TBW: BIA (kg)</td>
<td>-0.61</td>
<td>0.31</td>
<td>0.27</td>
<td>0</td>
</tr>
<tr>
<td>TBW: SFT measurement (kg)</td>
<td>-0.15</td>
<td>0.26</td>
<td>-0.51</td>
<td>0.42</td>
</tr>
</tbody>
</table>

1 BIA, bioelectrical impedance analysis; SFT, skinfold thickness.

2 Figure 1).

3 BIA, bioelectrical impedance analysis; SFT, skinfold thickness.

4 Significantly different from women (ANOVA); 5 P < 0.017. 6 P < 0.008.
RAPID MEASUREMENT OF TOTAL BODY WATER

FIGURE 1. Bland-Altman plots (differences between values obtained by 2 measurement methods and the mean of the values for the 2 methods) to examine for bias and systematic error the methods of measuring absolute total body water in 24 healthy volunteers: flowing afterglow–mass spectrometry (FA-MS) and the Chumlea (18) formula, the Watson (11) formula, bioelectrical impedance analysis (BIA), and skinfold-thickness (SFT) measurements. Details of mean ± SD values of differences and significance of regression lines are shown in Tables 3 and 4.

effects of the alcohol have worn off. In view of the assumptions made, which may not be valid for all ages (29), the subjective placing of a regression line defining the linear rate of alcohol metabolism (which has a large effect on the extrapolated value for TBW) and the impracticalities of sample acquisition render this approach inferior to the FA-MS method presented here. The additional advantage of the FA-MS method is that instrument calibration is unnecessary. Moreover, because it can be assumed that the subjects will have a breath deuterium abundance identical to that of local tap water before the D2O dose, a single postingestion breath analysis can be made, and this enables the FA-MS method to be applied to TBW measurements in large groups, for example, in the screening of populations for body composition.

The accuracy of the new FA-MS method was established previously (17) at close to 1%. The absolute measurements of TBW obtained in this study are in general agreement with those reported in the literature (11, 18). The mean TBW reported for women, after correction for deuterium exchange between HDO and body proteins, was 32.3 kg, representing 47.5% of body weight. This value is within 1.5 kg for age-matched women reported in series by Watson et al (11), Cohn et al (30), Piers et al (13), and Chumlea et al (31). As the data for these studies were collected over the last 25 y, it would seem that this aspect of body composition has been stable for white women of European origin, but such may not be the case for men. The mean value for TBW reported here for men was 46.2 kg (54.5% of body weight), which compares well to recent population studies of Chumlea et al (45.8 kg, 55.5%; 31) and Piers et al (45.3 kg, 58%; 13) but these data appear to be different from the older data reported by Watson et al (38.2 kg; 11) and Norris (40.8 kg) (as cited in Chumlea et al, 31) in comparing age-matched groups. When we compared the TBW measurements made with FA-MS with those derived from the regression equations published by Watson et al and Chumlea et al, we found that the former gave poor agreement in the men, whereas the latter showed good agreement for both sexes. The explanation for this observation would seem to be clear. More recent estimates of TBW in men of this age group (× age: 62 y) would indicate that TBW is relatively well preserved with aging, which perhaps reflects better nutrition than was seen in earlier generations. This possibility draws attention to another risk in using population-derived regression equations to measure TBW in individual patients, namely, that populations can change with time.

FIGURE 2. Plots of absolute total body water (TBW) and TBW per body mass as measured by flowing afterglow–mass spectrometry (FA-MS) against the BMI for 12 healthy men (■) and 12 healthy women (△). Note the clear separation between the sets of data for men and women and the lower slope of the line through the set of data for women in the upper panel.
The secondary objective of this study was to compare the measurements of TBW made by the FA-MS method with estimates made by the BIA and SFT measurement methods. Despite the strong correlations found in comparisons of these methods (Table 2), significant systematic differences were observed. In the comparison of FA-MS and BIA, these differences were most marked in smaller subjects, which suggests that the algorithms used to calculate TBW by the Xitron Hydra are overcompensating for the influence of body fat in patients who are less obese. Prediction of body fat from BIA methods can vary considerably according to the regression equations used (32), with some studies showing a range of overprediction or underprediction by an average of 6% in either direction in the same women (33). Among the factors confounding the BIA approach is the effect of regional variation in fat distribution. There is a tendency for fat to be overestimated in subjects with predominantly upper-body distribution and underestimated in those with predominantly lower-body distribution (33). The systematic differences between FA-MS and SFT measurement were not influenced by sex and body size. There was, however, a consistent difference between the 2 methods, such that FA-MS gave, on average, a value for TBW that was 2 kg higher than SFT measurement. The reason for this difference is not certain, but it cannot be ascribed to sex differences. The accuracy of SFT measurement is subject to several potential variances, including the accuracy of measurements, which can vary by 9% even in experienced hands (24), the applicability of the population-derived equations for determining body density in individual persons, and the assumption that the hydration of fat-free mass is 0.73 in all persons. That figure, originally determined from cadaveric measurements, is subject to variability (4%) (20).

The comparisons made in this study between methods for determining TBW show the difficulties in using population data to derive measurements in a person. A recent study of 117 healthy Australians confirms this difference by showing that, when compared with deuterium dilution, estimates of fat mass and lean body mass from BMI, BIA, and SA cannot be used interchangeably without the risk of considerable error (13). Although the newer regression equations published by Chumlea et al clearly are an improvement, providing for the first time data for black subjects, those authors emphasize in their report that the 95% CI is such that TBW may be over- or underestimated by 6–10 L, depending on the size of the person (31). This will have a large effect on the calculated dialysis dose at the level of the individual prescription. There is, therefore, a clear need for a simple, accurate method for measuring TBW that is practically suitable for use in the clinical environment, and we believe that the new, rapid FA-MS breath measurement presented here fulfills this requirement.

The intention now is to produce a small FA-MS instrument (Trans Spectra Limited, Stoke-on-Trent, United Kingdom) for use at the laboratory bench and convenient sitting in the clinical environment (eg, in renal dialysis units) to perform D:H measurements in the water contained in single-breath exhalations. It is anticipated that any person, after slight training, could operate such an instrument and thus obtain an accurate and precise measurement of TBW. It would then be sensible to perform TBW measurements by using FA-MS in conjunction with BIA measurements. An accurate BIA algorithm could be derived for each person, and this would avoid the problem of applying population-derived formulas that clearly result in demonstrable inaccuracies in TBW determination.

REFERENCES


