Evaluation of between-methods agreement of extracellular water measurements in adults and children\(^1\)–\(^3\)


ABSTRACT

Background: Extracellular water (ECW), a relevant molecular level component for clinical assessment, is commonly obtained by 2 methods that rely on assumptions that may not be possible to test at the time the measurements are made.

Objective: The aim of the current study was to evaluate the degree of agreement between ECW assessment by the sodium bromide dilution (ECW\(_{\text{NaBr}}\)) and total body potassium (TBK; whole-body \(^{40}\)K counting) methods (ECW\(_{\text{TBK-TBW}}\)) in an ethnically mixed group of children and adults.

Design: ECW was measured with the ECW\(_{\text{NaBr}}\) and ECW\(_{\text{TBK-TBW}}\) methods in 526 white and African American males and females (86 nonobese children, 193 nonobese adults, and 247 obese adults). Fat mass was assessed with dual-energy X-ray absorptiometry. Multiple regression analysis was used to examine the variables related to between-ECW method differences.

Results: Significant but generally small group mean (±SD) differences in ECW were found in the obese adults (1.28 ± 2.54 kg) and children (−0.71 ± 1.78 kg). The magnitude of the differences was related to mean ECW in obese adults, children, and nonobese adults, and the relations between these variables were modified by sex for nonobese adults. ECW differences were also dependent on age, weight, sex, and race or on interactions between these variables.

Conclusions: Overall, although good between-method agreement was found across the 3 groups, the degree of agreement varied according to subject characteristics, particularly at the extremes of ECW and body weight. We advance a possible mechanism that may link subject characteristics with the degree of agreement between ECW measurement methods and their underlying assumptions. Am J Clin Nutr 2008;88:315–23.

INTRODUCTION

Extracellular water (ECW), the major compartment of extracellular fluid (ECF), is the aqueous component that surrounds cells and reflects clinical status in patients with acute or chronic diseases. ECW varies widely with age (1, 2), and among race groups, with the largest age-related differences observed in African Americans (3). Monitoring the ECW compartment, in combination with other nutritional measures, may be of interest in both healthy subjects and, particularly, in patients with clinical conditions (4–6). Moreover, and recently, reference values for ECW were proposed for healthy adults across the adult life span (7).

ECW is distributed in plasma; interstitial, lymph, and connective tissues; and transcellular water compartments (8, 9). One approach to distinguishing ECW from intracellular water (ICW) is based on compartment-specific cation/anion patterns, potassium is primarily found in the ICW compartment, whereas chloride is found primarily in the ECW compartment (2). Several methods exist to measure the distribution of ion-related markers, but the dilution of sodium bromide in the bromide space is typically the most used technique (10). A second noninvasive approach is to estimate total body water (TBW) by isotope dilution combined with total body potassium (TBK) measurement by whole-body \(^{40}\)K counting (ie, ECW = TBW – ICW). Because of the absence of radiation hazard and the ease of administration, ECW estimations obtained from the sodium bromide dilution technique (ECW\(_{\text{NaBr}}\)) and the TBK-TBW method are appropriate for use in children and adults.

Although multiple methods exist to assess ECW, subjects vary widely in age, body mass, ethnicity, sex, and health status, and the degree of influence of these variables on the fundamental assumptions of the ECW estimation method has not been well studied. For instance, sodium bromide may not distribute uniformly in the various subcompartments of ECW, and ECW\(_{\text{TBK-TBW}}\) may not present a stable TBK concentration between the ICW and ECW compartments. Consequently, estimated ECW volumes may differ depending on how subject characteristics interact with the specific ECW technique and its respective assumptions (11).

The primary aim of the current study was to investigate, in an ethnically mixed subject group of nonobese children and adults and obese adults, the degree of agreement and differences in ECW measurements obtained from the sodium bromide dilution and TBK-TBW methods to identify subject characteristics and ranges of variables where the methods were in good agreement.

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and to examine the association of variables such as age, ethnicity, and sex with between-method differences with a view to whether these associations may reveal which variables critically affect agreement.

SUBJECTS AND METHODS

Protocol and subjects
Subjects were a convenience sample of 526 subjects participating in other unrelated investigations (12, 13). Two race groups, whites and African Americans, were identified by self-report. Classification into racial groups required similar parent and grandparent race. Subjects with a history of high blood pressure and/or use of medication for the treatment of high blood pressure were excluded. Body-composition studies were carried out over a single day. Three subgroups of subjects were identified: 86 nonobese children, 193 nonobese adults, and 247 obese adults. In the nonobese adult category, subjects with a body mass index (in kg/m²) <18.5 or >30 were excluded. For the child subgroup, the cutoff values developed by Cole et al (14) were used to exclude those identified as obese. A health screening was carried out in these subjects to ensure that they were in good health. The obese adult group consisted of individuals with a body mass index >30 and with no major diseases other than obesity.

There were 4 main evaluations: TBK was measured by whole-body ⁴⁰K counting, TBW was measured by deuterium or tritium dilution, ECW was estimated by sodium bromide dilution, and fat mass (FM) was estimated by dual-energy X-ray absorptiometry (DXA). All of the measurements were made for each subject on the same day, after an overnight fast, at the Body Composition Unit of St Luke’s–Roosevelt Hospital in New York City. All of the original investigations were approved by the Institutional Review Board of St Luke’s–Roosevelt Hospital.

Body-composition measurements

Anthropometric measurements
Body weight was measured to the nearest 0.1 kg (Weight Tronix, New York, NY) and height to the nearest 0.5 cm with a stadiometer (Holtain, Crosswell, United Kingdom).

Fat mass
FM was measured by using a series of pencil-beam dual-photon systems and related software manufactured by GE Lunar Corporation (Madison, WI), specifically Lunar Prodigy (GE Medical) and Lunar DPX version 3.6, which incorporated pediatric software version 3.8G, which was used to analyze all of the DXA scans on children. Body-composition data collected with different DXA systems were translated to common values with cross-calibration equations using the procedure reported by Russell-Aulet et al (15). The cross-calibrations involved scanning samples of healthy subjects using old and new DXA systems in random order within the same day. Appropriate calibration equations were then developed for each of the relevant components.

Total body water
Deuterium (²H₂O) or tritium (³H₂O) dilution volumes were measured with CVs of 1.5% and 2.0%, respectively. The dilution volumes were then used to calculate TBW, as described by Schoeller (10): TBW (L) = ³H₂O dilution space × 0.96. However, this correction was not applied for the deuterium dilution space, as reported elsewhere (16–18). TBW was then converted to water mass by using a water density of 0.9937 kg/L, assuming an average body temperature of 36 °C.

Total body potassium
Total-body ⁴⁰K mass was estimated from the measured 1.46 MeV γ-ray decay of naturally occurring ⁴⁰K in subject’s body. The subject’s ⁴⁰K was determined by counting for 9 min in a 4π whole-body counter (19). TBK mass was then calculated as TBK = ⁴⁰K/0.000118 (19). The raw count is corrected for body mass as described elsewhere (20). The TBK counter was calibrated daily by counting a standard of known activity (each standard bottle contains 5000 counts/min of ⁴⁰K) and adjusting all subsequent patient readings on that day by the deviation from that standard and for background counts. The reproducibility and accuracy of this system have between-measurement CVs of 1.5% and 2.3%, respectively (21).

Extracellular water measured with the TBK-TBW method
Potassium is present in ECW and ICW at relatively stable concentrations of 4 and 152 mmol/kg H₂O, respectively (22). The ECW can be derived from TBK and TBW (23, 24) as follows:

\[
TBK = 152 \times ICW + 4 \times ECW \quad (1)
\]

\[
TBW = ICW + ECW \quad (2)
\]

where TBK and TBW are in mmol and kg, respectively. Resolving these 2 simultaneous equations, ECW mass can be calculated from measured TBK and TBW as follows:

\[
ECW = (152 \times TBW - TBK)/148 \quad (3)
\]

Extracellular water measured with the sodium bromide dilution method
The subject was asked to drink 5 g of a 4 mol/L solution of sodium bromide. The sodium bromide concentration in plasma was measured by HPLC before and 3 h after tracer administration. The volume of ECW was calculated as follows:

\[
ECW(L) = \left[\text{dose/}[\text{post-plasma bromide}]_{\text{PLASMA}} \right] \times 0.90 \times 0.95 \quad (4)
\]

where 0.90 is a correction factor for intracellular bromide (Br⁻), found mainly in red blood cells, and 0.95 is the Donnan equilibrium factor (10). ECW was also converted to kg by multiplying the ECW values in liters by 0.9937 kg/L, assuming an average body temperature of 36 °C. The CV for sodium bromide dilution is 2.9% (11).

Propagated measurement error
Measurement precision is high for TBK and TBW, the 2 components of the TBK-TBW method. We accounted for the propagated error of these 2 measurements using the dilution technique for TBW (CV: 1.5% to 2.0%) and whole-body counting for TBK (CV: 1.5%). Therefore, considering equation 3 coefficients, the respective CV for each measurement,
and TBK and TBW expressed in the same units, the propagated measurement error for ECW_{TBK-TBW} can be estimated for the whole subjects in this study by assuming an average body composition (mean TBW and TBK of 34.7 kg and 2768.1 mmol, respectively) and the stated measurement precisions. Accordingly,

\[ \sigma_{ECW}^2 = \sigma_{TBW}^2 + \sigma_{TBK}^2 \]  
\[ \sigma_{ECW}^2 = (152/148 \times 0.02 \times 34.7)^2 + (1/148 \times 0.015 \times 2768.1)^2 \]  
\[ \sigma_{ECW}^2 = 0.587 \]  
so SD = 0.77 kg

Statistical methods

All group results are expressed as means ± SDs. The analyses were carried out using the statistical program SPSS version 14.0 (SPSS Inc, Chicago, IL). P < 0.05 was considered significant for individual tests. Independent-sample t tests were used to compare whites with African Americans and males with females within each group, whereas paired-sample t tests were performed to compare both methods. The main focus was to identify the potential variables associated with between-method differences in ECW measurements between and within each subgroup. For each group of children, obese, and nonobese adults, multiple regression analysis was applied to investigate the association of ECW differences with potential influencing factors, including sex, race, age, amount of ECW (the mean of the ECW methods), weight, FM, and 2-factor interactions (weight by race, FM by race, age by race, weight by sex, FM by sex, and age by sex). Variables or interactions making no significant contribution were eliminated from the final model. During model development, homogeneity of variance and normality of residuals were tested. In all cases, the models were in compliance with the assumptions of multiple linear regression.

Adjusted (for significant covariates) ECW differences were plotted separately against potential explanatory variables within each group. To obtain these adjusted differences we first obtained residuals from the regression of the simple differences on all significant covariates except the explanatory variable that would be on the x-axis in the plot. Adjusted values were then calculated by adding the mean value of the group to the residuals after completing the regression analysis. Using this procedure we were able to look at the effect of each significant predictor variable adjusted for covariates.

RESULTS

Subject characteristics

The characteristics of the subjects are summarized in Table 1.

ECW methods comparison

Comparisons of mean ECW amounts and differences are shown in Table 2 and Table 3. Significantly higher values of ECW_{TBK-TBW} than of ECW_{NaBr} were found for the nonobese African American females, whereas no significant differences were found for nonobese African American males or white males or females. The pooled pediatric group showed significantly higher ECW_{NaBr} than ECW_{TBK-TBW} values. In the obese adult group, higher mean ECW_{TBK-TBW} than ECW_{NaBr} values were found in females, but not in males, likely because of the small sample size of obese males.

Associations of subjects’ characteristics with ECW agreement in nonobese adults

Age

Age per se did not show a significant association with the difference between the 2 ECW methods but an age-by-race interaction was found to be significantly associated. Regression models showed that ECW differences were larger by 0.033 kg for each year of age in African American (P = 0.004), but there was no significant relation in whites (β = −0.006, P = 0.500). This finding indicated that ECW_{TBK-TBW} values increase more rapidly than do ECW_{NaBr} values with age in African Americans. Also, race was related to the differences between ECW methods as a main effect, with whites presenting a positive coefficient (β = 4.948, P = 0.022).

ECW amount

The amount of ECW obtained from the mean of both ECW methods was significantly associated with the difference between methods in estimating ECW, which indicated that ECW_{TBK-TBW} values increase more rapidly than ECW_{NaBr} values. The interaction with sex showed that ECW differences increased less rapidly with each kilogram of ECW in males (β = 0.479, P < 0.001) than in females (β = 0.387, P < 0.001).

Weight

Weight alone was not significantly related to ECW differences, but a weight-by-race interaction was found. Regression analysis showed that, after control for other variables, ECW differences were larger by −0.081 units for each kilogram of body weight in whites (P = 0.001), whereas the weight coefficient for African Americans was not significant (β = −0.028, P = 0.250). Thus ECW_{TBK-TBW} values increased less rapidly than ECW_{NaBr} values in whites than in African Americans.

The differences between ECW methods in healthy white males and females and in African American males and females at the observed sample means of age, weight and ECW, are illustrated in Figure 1. The association of ECW between-methods differences with the amount of ECW in nonobese males and females, after adjustment for the effects of race and interactions, is shown in Figure 2.

Associations of subjects’ characteristics with ECW agreement in obese females

Our sample included 6 obese males (3 whites, 3 African Americans). Initial analyses included the males, which found no main effects or interactions with sex and other variables on between-method differences. However, because the power to detect such effects would be low given the small number of males, we present the results and interpretation of the associations of subject characteristics using only obese females.
TABLE 1

<table>
<thead>
<tr>
<th>Gender</th>
<th>Subjects</th>
<th>Age (y)</th>
<th>Weight (kg)</th>
<th>Height (m)</th>
<th>BMI kg/m²</th>
<th>Fat mass (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>Children (n = 43)</td>
<td>11.2 ± 3.4</td>
<td>45.9 ± 18.9</td>
<td>1.50 ± 0.21</td>
<td>19.4 ± 3.4</td>
<td>9.1 ± 6.6</td>
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<td></td>
<td>White (n = 18)</td>
<td>10.0 ± 3.0</td>
<td>40.9 ± 15.7</td>
<td>1.46 ± 0.19</td>
<td>18.7 ± 2.8</td>
<td>9.7 ± 6.7</td>
</tr>
<tr>
<td></td>
<td>AA (n = 25)</td>
<td>12.0 ± 3.5</td>
<td>49.5 ± 20.4</td>
<td>1.54 ± 0.21</td>
<td>19.9 ± 3.7</td>
<td>8.7 ± 6.6</td>
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<tr>
<td></td>
<td>Nonobese adults (n = 63)</td>
<td>47.0 ± 21.7</td>
<td>77.9 ± 9.5</td>
<td>1.77 ± 0.07</td>
<td>24.8 ± 2.5</td>
<td>14.6 ± 6.0</td>
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<td>White (n = 45)</td>
<td>43.8 ± 20.2</td>
<td>77.0 ± 9.0</td>
<td>1.77 ± 0.07</td>
<td>24.4 ± 2.3</td>
<td>13.9 ± 6.3</td>
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<tr>
<td></td>
<td>AA (n = 18)</td>
<td>55.0 ± 24.0</td>
<td>80.0 ± 10.6</td>
<td>1.76 ± 0.09</td>
<td>25.7 ± 2.8</td>
<td>16.4 ± 5.2</td>
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<td></td>
<td>Obese adults (n = 6)</td>
<td>54.8 ± 22.5</td>
<td>93.4 ± 9.5</td>
<td>1.72 ± 0.06</td>
<td>31.7 ± 1.1</td>
<td>28.1 ± 8.9</td>
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<tr>
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<td>White (n = 3)</td>
<td>52.3 ± 18.9</td>
<td>99.1 ± 10.5</td>
<td>1.74 ± 0.07</td>
<td>32.5 ± 0.9</td>
<td>32.2 ± 11.3</td>
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<tr>
<td></td>
<td>AA (n = 3)</td>
<td>57.3 ± 29.8</td>
<td>87.8 ± 4.5</td>
<td>1.69 ± 0.04</td>
<td>30.8 ± 0.4</td>
<td>23.9 ± 4.6</td>
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<tr>
<td>Females</td>
<td>Children (n = 43)</td>
<td>10.4 ± 3.1</td>
<td>41.6 ± 14.5</td>
<td>1.46 ± 0.15</td>
<td>18.9 ± 3.4</td>
<td>11.8 ± 7.4</td>
</tr>
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<td>White (n = 14)</td>
<td>10.4 ± 3.3</td>
<td>39.3 ± 13.8</td>
<td>1.43 ± 0.14</td>
<td>18.6 ± 3.2</td>
<td>11.4 ± 6.1</td>
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<td>AA (n = 29)</td>
<td>10.4 ± 3.0</td>
<td>42.7 ± 14.9</td>
<td>1.47 ± 0.16</td>
<td>19.1 ± 3.5</td>
<td>12.1 ± 8.1</td>
</tr>
<tr>
<td></td>
<td>Nonobese adults (n = 130)</td>
<td>52.3 ± 22.3</td>
<td>63.8 ± 9.7</td>
<td>1.61 ± 0.07</td>
<td>24.5 ± 3.4</td>
<td>21.5 ± 7.9</td>
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<td>White (n = 70)</td>
<td>47.8 ± 21.9</td>
<td>62.8 ± 8.6</td>
<td>1.61 ± 0.06</td>
<td>24.2 ± 3.5</td>
<td>21.2 ± 7.8</td>
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<td>AA (n = 60)</td>
<td>57.5 ± 21.9</td>
<td>65.0 ± 10.8</td>
<td>1.62 ± 0.07</td>
<td>24.8 ± 3.3</td>
<td>21.9 ± 8.1</td>
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<tr>
<td></td>
<td>Obese adults (n = 241)</td>
<td>48.2 ± 13.9</td>
<td>92.8 ± 10.6</td>
<td>1.63 ± 0.06</td>
<td>35.1 ± 3.7</td>
<td>42.6 ± 6.8</td>
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<tr>
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<td>White (n = 109)</td>
<td>49.0 ± 13.5</td>
<td>91.9 ± 10.4</td>
<td>1.62 ± 0.06</td>
<td>34.9 ± 3.9</td>
<td>43.1 ± 6.7</td>
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<td>AA (n = 132)</td>
<td>47.5 ± 14.2</td>
<td>93.5 ± 10.8</td>
<td>1.63 ± 0.06</td>
<td>35.2 ± 3.6</td>
<td>42.3 ± 6.8</td>
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<td>Total sample</td>
<td>Children (n = 86)</td>
<td>10.8 ± 3.2</td>
<td>43.7 ± 16.9</td>
<td>1.48 ± 0.18</td>
<td>19.2 ± 3.4</td>
<td>10.5 ± 7.1</td>
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<td>Nonobese adults (n = 193)</td>
<td>50.6 ± 22.2</td>
<td>68.4 ± 11.7</td>
<td>1.67 ± 0.10</td>
<td>24.6 ± 3.1</td>
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<tr>
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<td>Obese adults (n = 247)</td>
<td>48.3 ± 14.2</td>
<td>92.8 ± 10.6</td>
<td>1.63 ± 0.06</td>
<td>35.0 ± 3.7</td>
<td>42.3 ± 7.2</td>
</tr>
<tr>
<td></td>
<td>Males (n = 112)</td>
<td>33.7 ± 24.8</td>
<td>66.4 ± 21.6</td>
<td>1.66 ± 0.19</td>
<td>23.1 ± 4.3</td>
<td>13.2 ± 7.7</td>
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<td>White (n = 66)</td>
<td>35.0 ± 23.0</td>
<td>68.2 ± 20.7</td>
<td>1.68 ± 0.19</td>
<td>23.3 ± 4.0</td>
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<td>AA (n = 46)</td>
<td>31.8 ± 27.2</td>
<td>64.0 ± 22.9</td>
<td>1.64 ± 0.20</td>
<td>22.9 ± 4.8</td>
<td>12.7 ± 7.5</td>
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<td></td>
<td>Females (n = 414)</td>
<td>45.5 ± 20.4</td>
<td>78.4 ± 21.1</td>
<td>1.61 ± 0.09</td>
<td>30.1 ± 7.1</td>
<td>32.8 ± 14.0</td>
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<tr>
<td></td>
<td>White (n = 193)</td>
<td>45.8 ± 19.3</td>
<td>77.5 ± 20.1</td>
<td>1.61 ± 0.09</td>
<td>29.9 ± 7.0</td>
<td>32.8 ± 13.9</td>
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<td>AA (n = 221)</td>
<td>45.3 ± 21.3</td>
<td>79.1 ± 22.0</td>
<td>1.61 ± 0.10</td>
<td>30.2 ± 7.1</td>
<td>32.8 ± 14.0</td>
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<td>Total (n = 526)</td>
<td>43.1 ± 21.9</td>
<td>75.8 ± 21.8</td>
<td>1.62 ± 0.12</td>
<td>28.6 ± 7.2</td>
<td>28.6 ± 15.2</td>
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<td>White (n = 259)</td>
<td>43.0 ± 20.8</td>
<td>75.2 ± 20.6</td>
<td>1.63 ± 0.12</td>
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<td>AA (n = 267)</td>
<td>43.0 ± 23.0</td>
<td>76.5 ± 22.8</td>
<td>1.61 ± 0.12</td>
<td>29.0 ± 7.3</td>
<td>29.3 ± 15.2</td>
</tr>
</tbody>
</table>

1 All values are x ± SD. AA, African American.

ECW amount

The amount of ECW obtained from the mean of both ECW methods was significantly associated with the difference between methods in estimating ECW. Regression analysis showed that ECW differences between methods were larger by 0.500 units for each kilogram of ECW (P < 0.001). These results are illustrated in the left panel of Figure 3. After control for weight, TBK-TBW values increased more rapidly than NaBr values with increasing ECW.

**Weight, age, fat mass, race, and sex**

Weight affected the degree of agreement between methods. Regression parameters showed that the ECW differences were larger by 0.082 units for each kilogram of body weight (P < 0.001). ECW_{NaBr} increased more rapidly with increasing weight than did ECW_{TBK-TBW}, and the results are illustrated in the right panel of Figure 3, after control for the effect of mean ECW. Age, FM, race, sex, and their interactions were not related to the differences between the 2 methods.

**Associations of the subjects’ characteristics with ECW agreement in children**

ECW amount

The amount of ECW obtained from the mean of both ECW methods affected the degree of agreement between methods. Regression showed that ECW differences between methods were larger by 0.323 units for each kilogram of ECW (P = 0.005). The results, after adjustment for the effects of body weight, are shown in the left panel of Figure 4.

**Weight, age, fat mass, race, and sex**

An association was found between body weight and the differences between ECW methods in the pediatric sample. Regression parameters showed that the differences between ECW methods were larger by −0.097 units for each kilogram of body weight (P = 0.011). After control for the effect of mean ECW, this relation is illustrated in the right panel of Figure 4. Age, FM, race, and sex or their interactions were not related to the differences between the 2 methods.
with the TBK-TBW method; ECWdiff, ECWTBK-TBW minus ECWNaBr. A nonsignificant sex-by-race interaction was found.

A preliminary regression analysis using the pooled sample of nonobese and obese adults showed a significant sex-by-race-by-obesity interaction, which explained the ECW differences (P < 0.01); therefore, a separate analysis was conducted.

The agreement between methods using the Bland-Altman method (ie, plotting the differences between ECWTBK-TBW and ECWNaBr values against the mean of ECW obtained from both methods) is illustrated in Figure 5.

DISCUSSION

This was the first large comprehensive study to address a comparison between ECW methods using a diverse sample of subjects, ranging widely in age, weight, race, and sex. The ECW estimates determined by using TBK-TBW were significantly lower than those determined by using sodium bromide in children, whereas these differences were higher for adult obese subjects and nonobese females. Nevertheless, the methods were highly correlated within each group, ranging from 0.6 (obese adults) to 0.8 (nonobese adults) to 0.9 (children).

Part of the difficulty in establishing between-method comparability based on previous studies is applied ECF definitions. Although ECF is often considered the sum of 2 fluid compartments, plasma and interstitial fluid, Cheek (8) and Edelman et al. (9) suggested that ECF also includes joint transcellular fluids and connective tissue, lymph, central nervous system, eye, gastrointestinal tract, pericardium, and pleural fluids. Bromide, chloride, thiocyanate, thiosulfate, sulfate, sucrose, and mannitol are used as ECW markers, but behave differently with respect to the underlying assumptions of ECW measurement (10). Previous reviews have indicated that the disaccharides fail to penetrate dense connective tissue and transcellular water and thiosulfate.

**TABLE 2**

<table>
<thead>
<tr>
<th>Adults</th>
<th>TBK</th>
<th>TBW</th>
<th>ECWNaBr</th>
<th>ECWTBK-TBW</th>
<th>ECWdiff</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
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</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonobese adults (n = 63)</td>
<td>3945.9 ± 674.7</td>
<td>45.0 ± 6.8</td>
<td>19.7 ± 2.8</td>
<td>19.6 ± 3.4</td>
<td>-0.15 ± 2.6</td>
</tr>
<tr>
<td>White (n = 45)</td>
<td>3984.7 ± 613.1</td>
<td>44.7 ± 6.1</td>
<td>19.6 ± 2.7</td>
<td>19.0 ± 3.1</td>
<td>-0.59 ± 2.45</td>
</tr>
<tr>
<td>AA (n = 18)</td>
<td>3848.9 ± 820.7</td>
<td>45.7 ± 8.5</td>
<td>19.8 ± 3.2</td>
<td>20.9 ± 3.9</td>
<td>1.01 ± 2.89</td>
</tr>
<tr>
<td>Obese adults (n = 6)</td>
<td>4106.1 ± 567.2</td>
<td>47.3 ± 4.6</td>
<td>20.3 ± 2.2</td>
<td>20.8 ± 1.9</td>
<td>0.53 ± 1.49</td>
</tr>
<tr>
<td>White (n = 3)</td>
<td>4071.8 ± 330.6</td>
<td>47.0 ± 0.8</td>
<td>19.8 ± 1.4</td>
<td>20.8 ± 2.3</td>
<td>1.02 ± 1.76</td>
</tr>
<tr>
<td>AA (n = 3)</td>
<td>4140.3 ± 831.6</td>
<td>47.5 ± 7.2</td>
<td>20.8 ± 2.9</td>
<td>20.8 ± 1.0</td>
<td>0.04 ± 1.31</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonobese adults (n = 130)</td>
<td>2432.6 ± 382.2</td>
<td>31.0 ± 3.8</td>
<td>14.8 ± 1.9</td>
<td>15.4 ± 2.3</td>
<td>0.55 ± 2.07</td>
</tr>
<tr>
<td>White (n = 70)</td>
<td>2411.1 ± 299.2</td>
<td>30.5 ± 3.3</td>
<td>14.8 ± 1.8</td>
<td>15.1 ± 2.1</td>
<td>0.22 ± 1.86</td>
</tr>
<tr>
<td>AA (n = 60)</td>
<td>2457.6 ± 462.1</td>
<td>31.5 ± 4.2</td>
<td>14.8 ± 2.0</td>
<td>15.7 ± 2.5</td>
<td>0.93 ± 2.24</td>
</tr>
<tr>
<td>Obese adults (n = 241)</td>
<td>2803.5 ± 391.6</td>
<td>37.3 ± 4.5</td>
<td>18.0 ± 2.5</td>
<td>19.3 ± 3.1</td>
<td>1.30 ± 2.56</td>
</tr>
<tr>
<td>White (n = 109)</td>
<td>2740.6 ± 346.5</td>
<td>36.2 ± 4.2</td>
<td>17.7 ± 2.5</td>
<td>18.6 ± 2.9</td>
<td>0.99 ± 2.62</td>
</tr>
<tr>
<td>AA (n = 132)</td>
<td>2855.5 ± 419.4</td>
<td>38.2 ± 4.6</td>
<td>18.4 ± 2.5</td>
<td>19.9 ± 3.2</td>
<td>1.55 ± 2.50</td>
</tr>
<tr>
<td>Total sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonobese adults (n = 193)</td>
<td>2926.6 ± 866.8</td>
<td>35.5 ± 8.3</td>
<td>16.4 ± 3.2</td>
<td>16.7 ± 3.4</td>
<td>0.32 ± 2.96</td>
</tr>
<tr>
<td>Obese adults (n = 247)</td>
<td>2835.2 ± 443.3</td>
<td>37.5 ± 4.8</td>
<td>18.1 ± 2.5</td>
<td>19.4 ± 3.1</td>
<td>1.28 ± 2.54</td>
</tr>
</tbody>
</table>

1 All values are x ± SD. AA, African American; ECWNaBr, ECW measured with the sodium bromide dilution method; ECWTBK-TBW, ECW measured with the TBK-TBW method; ECWdiff, ECWTBK-TBW minus ECWNaBr. A preliminary regression analysis using the pooled sample of nonobese and obese adults showed a significant sex-by-race-by-obesity interaction, which explained the ECW differences (P < 0.01); therefore, a separate analysis was conducted.

2 Significant difference between methods, P < 0.05 (paired-sample t test).

3 Whites differed from African Americans, P < 0.05 (independent-sample t test).

4 Significant difference between methods in African Americans, P < 0.01 (paired-sample t test).

5 Significant sex-by-race interaction in nonobese adults, P < 0.01.

6 Significant difference between methods in whites, P < 0.01 (paired-sample t test).

**TABLE 3**

<table>
<thead>
<tr>
<th>Children</th>
<th>TBK</th>
<th>TBW</th>
<th>ECWNaBr</th>
<th>ECWTBK-TBW</th>
<th>ECWdiff</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
</tr>
<tr>
<td>Males (n = 43)</td>
<td>2523.4 ± 971.8</td>
<td>27.0 ± 11.0</td>
<td>11.7 ± 4.4</td>
<td>10.7 ± 5.0</td>
<td>-0.99 ± 1.92</td>
</tr>
<tr>
<td>White (n = 18)</td>
<td>2204.9 ± 702.0</td>
<td>23.2 ± 8.3</td>
<td>10.1 ± 3.5</td>
<td>8.9 ± 4.0</td>
<td>-1.16 ± 1.52</td>
</tr>
<tr>
<td>AA (n = 25)</td>
<td>2752.8 ± 1082.8</td>
<td>29.8 ± 12.1</td>
<td>12.9 ± 4.7</td>
<td>12.0 ± 5.3</td>
<td>-0.86 ± 2.18</td>
</tr>
<tr>
<td>Females (n = 43)</td>
<td>1916.2 ± 552.0</td>
<td>22.1 ± 6.0</td>
<td>10.1 ± 2.8</td>
<td>9.7 ± 2.7</td>
<td>-0.43 ± 1.60</td>
</tr>
<tr>
<td>White (n = 14)</td>
<td>1838.1 ± 595.9</td>
<td>20.4 ± 5.9</td>
<td>9.7 ± 2.6</td>
<td>8.6 ± 2.2</td>
<td>-1.09 ± 1.15</td>
</tr>
<tr>
<td>AA (n = 29)</td>
<td>1954.0 ± 536.3</td>
<td>22.6 ± 6.0</td>
<td>10.4 ± 2.9</td>
<td>10.3 ± 2.8</td>
<td>-0.12 ± 1.71</td>
</tr>
<tr>
<td>Total sample (n = 86)</td>
<td>2219.8 ± 842.9</td>
<td>24.6 ± 9.2</td>
<td>10.9 ± 3.8</td>
<td>10.2 ± 4.0</td>
<td>-0.71 ± 1.78</td>
</tr>
</tbody>
</table>

1 All values are x ± SD. AA, African American; ECWNaBr, ECW measured with the sodium bromide dilution method; ECWTBK-TBW, ECW measured with the TBK-TBW method; ECWdiff, ECWTBK-TBW minus ECWNaBr. A nonsignificant sex-by-race interaction was found (P = 0.111).

2 Significant difference between methods, P < 0.05 (paired-sample t test).
and sulfate fails to penetrate transcellular water (25). Bromide and isotopic chloride dilution provides the closest approximation of the extracellular space (9), and, with the advent of improved analytic techniques, bromide has become the most commonly used tracer. Neither bromide nor any other tracer used to date provides an exact measure of ECW because the physiologic properties of the various compartments of ECW differ from one another. Chemical analysis and radiochlorine dilution studies indicate a bromide intracellular penetration that accounts for about half is attributable to erythrocytes (9). Assumptions about other than plasma are enlarged in obese subjects. This means that the ECW amount in an obese subject, as a result of the contribution of all the subcompartments, will be corrected with a plasma-related assumption of 10%, independently of the actual percentage of contribution of each subcompartment in the total amount of ECW.

Effect of body weight

Body weight in nonobese white adults, obese females, and children were negatively associated with the between-methods difference. The results indicate that ECWNaBr values tend to increase more rapidly than ECWTBK-TBW values in heavier nonobese whites, obese females, and children. For nonobese white adults, a potential explanation may be a related underestimation of (TBKICW) hydration due to an enlargement of the ECW compartment relative to the ICW compartment. It has been suggested (27) that an enlarged adipose tissue compartment that contains 14% of TBW would be a possible explanation. Therefore, a 10% correction for the penetration of bromide into the ICW may overestimate the actual percentage of bromide that is penetrating into ICW (particularly erythrocytes), because ECW subcompartments other than plasma are enlarged in obese subjects. This means that the ECW amount in an obese subject, as a result of the contribution of all the subcompartments, will be corrected with a plasma-related assumption of 10%, independently of the actual percentage of contribution of each subcompartment in the total amount of ECW.

Effect of amount of ECW

The amount of ECW, represented by the mean of both methods, was the most powerful predictive variable in explaining the variability of the observed ECW differences in nonobese children and adults and in the obese group. The pattern of association was similar across the 3 groups: ECWNaBr values increased more rapidly than the ECWTBK-TBW values with increasing amounts of ECW. A plausible mechanism to account for this is that the assumptions related to ECWNaBr may cause underestimates of ECW in subjects with larger amounts of ECW. For example, it is recognized that obese persons tend to have a higher fat-free mass (FFM) hydration due to an enlargement of the ECW compartment relative to the ICW compartment. It has been suggested (27) that an enlarged adipose tissue compartment that contains 14% of TBW would be a possible explanation. Therefore, a 10% correction for the penetration of bromide into the ICW may underestimate the actual percentage of bromide that is penetrating into ICW (particularly erythrocytes), because ECW subcompartments other than plasma are enlarged in obese subjects. This means that the ECW amount in an obese subject, as a result of the contribution of all the subcompartments, will be corrected with a plasma-related assumption of 10%, independently of the actual percentage of contribution of each subcompartment in the total amount of ECW.

Effect of body weight

Body weight in nonobese white adults, obese females, and children were negatively associated with the between-methods difference. The results indicate that ECWNaBr values tend to increase more rapidly than ECWTBK-TBW values in heavier nonobese whites, obese females, and children. For nonobese white adults, a potential explanation may be a related understimation of (TBKICW) relative to the assumed value. In heavier subjects,
who are likely to have higher FFM values, the ECW compartment is underestimated with the TBK-TBW approach. If this is correct, a similar effect may also occur in heavier obese subjects. The assumptions for correcting the distribution of the bromide tracer may also be one of the reasons for disagreement. For example, the 5% Gibbs-Donnan’s effect may underestimate the true percentage value in heavier subjects because of denser connective tissue, which is known to contain 8–14% more chloride per liter than plasma (28). For children, the finding that heavier subjects had a higher rate of increase in ECW when the sodium bromide method was used, as opposed to the TBK-TBW method, was likely related to the underestimation of the actual rate ($\text{TBK}_{\text{ECW}}$), because it is recognized that FM and FFM tend to increase after puberty (2), and, therefore, body weight increases.

**Effects of age**

In nonobese adults, the relation of age with between-methods differences was mediated by race. Older nonobese African Americans tend to have a higher rate of increase in ECW when the TBK-TBW method is used than when the sodium bromide method is used. This interaction may be due to the rapid decline in TBK reported in older African Americans (12, 29) than in other race groups. Therefore, the assumed ($\text{TBK}_{\text{ECW}}$) value may be overestimating the real value ($\text{TBK}_{\text{ECW}}$) in older African Americans, which thus increases the estimates of $\text{ECW}_{\text{TBK-TBW}}$ compared with the $\text{ECW}_{\text{NaBr}}$ estimation.

**Study limitations**

There are several limitations of the current study. Our convenience sample did not have adequate numbers of obese males and enough racial diversity (Hispanics and Asians) to investigate all the possible effects surrounding ECW methodologic differences, although our findings do reveal some potential influencing factors. Additionally, our investigation was cross-sectional and, ideally, age-related inferences should be based on longitudinal data. Another concern is the accuracy of DXA measurements for FM estimation in the obese sample because the attenuation coefficients for soft tissue depend on the subject’s anteroposterior thickness of the body, if $\geq 25$ cm (30, 31).

**Conclusions**

In the present study we applied multiple regression modeling methods to evaluate ECW variation obtained from 2 different methodologic approaches. Overall, $\text{ECW}_{\text{TBK-TBW}}$ yielded larger mean ECW values for obese subjects, whereas smaller amounts were found in nonobese children compared with $\text{ECW}_{\text{NaBr}}$. We propose age-related tissue-organ body-composition differences as one potential explanation for these observations. We also
observed significant age-related differences among race groups, with the largest age-related differences in the rate of ECW increase observed in nonobese African American subjects using TBK-TBW, whereas weight-related differences were observed in nonobese adults with the highest rate of ECW increase obtained from ECWNaBr in the heaviest whites. Our findings suggest that these 2 methods should not be considered interchangeable. Finally, the underlying assumptions of the methods used in this study to estimate ECW across the life span and disease state are worthy of future investigation.

The authors’ responsibilities were as follows—AMS: responsible for data pooling, screening, analysis, and manuscript writing; SH: responsible for data analysis and manuscript writing and provided advice and consultation; DG, JA, XFP-S, RNP, JW, ZW, and SBW: responsible for data collection; and SBW, LBS, and ZW: responsible for manuscript writing and provided administrative support, supervision, and advice. None of the authors had a conflict of interest in any company or organization sponsoring this study.

REFERENCES


