Human hydrometry: comparison of multifrequency bioelectrical impedance with $^{2}\text{H}_{2}\text{O}$ and bromine dilution

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Ellis, Kenneth J., and William W. Wong. Human hydrometry: comparison of multifrequency bioelectrical impedance with $^{2}\text{H}_{2}\text{O}$ and bromine dilution. J. Appl. Physiol. 85(3): 1056–1062, 1998.—The traditional method of assessing total body water (TBW), extracellular water (ECW), and intracellular water (ICW) has been the use of isotopes, on the basis of the dilution principle. Although the development of bioelectrical impedance techniques has eliminated many of the measurement constraints associated with the dilution methods, the degree of interchangeability between the two methods remains uncertain. We used multifrequency bioelectrical impedance spectroscopy (BIS), $^{2}\text{H}_{2}\text{O}$ dilution, and bromine dilution to assess TBW, ECW, and ICW in 469 healthy subjects (248 males, 221 females) aged 3–29 yr. We found that the TBW, ECW, and ICW estimates for the BIS and dilution methods were significantly correlated ($r^2 = 0.80–0.96$, $P < 0.0001$, SE of the estimate = 2.3–2.7 liters). On the basis of population, the constants used in the BIS analysis could be adjusted so that the mean differences with the dilution methods would become zero. The SD values for the mean differences between the dilution and BIS methods, however, remained significant for both males and females: TBW (+2.1–2.8 liters), ECW (+1.4–1.6 liters), and ICW (2.0–3.1 liters). To improve the accuracy of the BIS measurement for an individual within the age range we have examined, further refinement of the constants used in the BIS analysis is needed.

BODY WATER IS THE HIGHEST fractional content of body weight, except in cases of extreme obesity. Body water is also the most abundant component of the fat-free mass (FFM) and remains relatively constant once adulthood is reached. Changes in the hydration status of the FFM, however, are part of the basic physiology of growth and appear to be part of the aging process in later life (7, 9). Both acute and chronic changes in hydration also can occur during various diseases and their clinical management. A major challenge in the science of body composition research has been to accurately monitor these changes, whether in healthy or diseased subjects. The classic approach for the measurement of total body water (TBW) has been the use of radioactive or stable isotopes (e.g., tritium, $^{3}\text{H}_{2}\text{O}$, oxygen-18) of water, on the basis of the dilution principle (20, 21, 29).

The distribution of water in the FFM can be further divided into two major physiological or cellular components: intracellular water (ICW) and extracellular water (ECW). The volume of the ECW compartment also has been estimated by using the dilution technique with bromine (Br), chlorine, and sucrose tracers (18, 30, 31). When TBW and ECW are known, the ICW volume has been defined as their difference. In the healthy state, the body’s water distribution (e.g., relative ratios of ECW to TBW or ICW) appears to be tightly regulated. In an abnormal state, these ratios can be significantly altered and are often attributable to an elevated ECW, whereas the ICW volume can remain relatively normal or reduced.

Until recently, the routine assessments of TBW and ECW could only be determined by dilution techniques (20) or on the basis of a multicomponent body composition model (e.g., relative ratios of ECW to TBW or ICW) appears to be tightly regulated. In an abnormal state, these ratios can be significantly altered and are often attributable to an elevated ECW, whereas the ICW volume can remain relatively normal or reduced.

The objective of the present study was to compare the BIS-derived estimates for TBW, ECW, and ICW with those based on the dilution methods. We particularly wanted to determine whether substitution of BIS for the dilution methods would continue to provide accurate assessments of these three water compartments in an individual.

METHODS

Subjects. A group of 469 subjects, consisting of 387 children (172 boys, 215 girls, age 3–18 yr) and 82 young adults (49
men, 33 women, age 19–29 yr), participated in this study. Subjects were from three ethnic groups (white, black, Hispanic). Body weight was measured by using an electronic balance to ±0.2 kg; height was measured by using a stadiometer to ±0.3 cm. The study was approved by the Institutional Review Board for Human Research at Baylor College of Medicine, and written informed consent was obtained for each subject.

Dilution measurements. TBW was measured by using $^2\text{H}_2\text{O}$ dilution (29). After providing a baseline blood sample, the subject drank water containing $^2\text{H}_2\text{O}$ at a dose of 70 mg $^2\text{H}_2\text{O}$/kg body weight. Three to four hours after the oral dose, a second blood sample was obtained. Plasma was separated from the blood samples and frozen at –70°C for later analysis. $^2\text{H}_2\text{O}$ enrichment was determined in plasma by isotope-ratio mass spectroscopy (29). The baseline (0 h) value was used to correct the background $^2\text{H}_2\text{O}$ concentration value for the 3- to 4-h sample. All assays were performed in duplicate, and repeat assays indicated an analytical precision of ≤2%

The calculation of TBW can be described by the following equation

$$ \text{TBW} = \frac{[^2\text{H}_2\text{O}]_\text{b} - [^2\text{H}_2\text{O}]_\text{b0}}{1.04} \tag{1} $$

where $[^2\text{H}_2\text{O}]$ denotes plasma concentration, and the constant (1.04) was used to adjust for exchange of $^2\text{H}_2\text{O}$ with nonaqueous hydrogen in the body.

The measurement of ECW was determined by using Br dilution (30). NaBr was added to the oral $^2\text{H}_2\text{O}$ dose such that the subject received 30 mg Br/kg body weight. Serum samples were obtained from the baseline and 3- to 4-h blood samples, frozen, and later assayed by using an HPLC anion-exchange method, after serum ultrafiltration (30). ECW was calculated as

$$ \text{ECW} = \frac{[\text{Br}]_\text{d} - [\text{Br}]_\text{b0}}{0.90 \cdot 0.95} \cdot 0.90 \cdot 0.95 \tag{2} $$

where [Br] denotes the serum Br concentration, and the constants (0.90, 0.95) are used to adjust for the overexpansion of Br into nonextracellular sites and for the Donnan equilibrium effect. Duplicate samples within an assay were performed with an analytic precision of ±2–3%. Intra-assay precision was <3%. ICF volume was defined as

$$ \text{ICW} = \text{TBW} - \text{ECW} \tag{3} $$

BIS. Whole body BIS measurements were performed by using a commercial instrument (Xitron 4000B, Xitron Technologies, San Diego, CA). All measurements were performed in accordance with the manufacturer's instruction manual. One set of electrodes was placed at the wrist, and a second set was placed at the ankle. All measurements were performed on the left side of the body after the subject had been in a supine position for 10–15 min. Total body resistance, reactance, and impedance were computed by using the frequency range 1 kHz to 1.2 MHz. A detailed description of the electrical circuit model used to analyze the impedance data has recently been published by De Lorenzo et al. (8). On the basis of the resistance values calculated for the theoretical limits at zero and infinite frequency, the resistance values representing the intracellular (R_i) and extracellular (R_e) components of the electrical circuit were obtained. Repeat measurements in six subjects indicated a precision <2% for the R_i and R_e estimates for an individual.

By using a series of cylinders to describe the volume of the arms, legs, and trunk, coupled with the mixture theory proposed by Hanai (12), the following equation was used to calculate the extracellular fluid volume

$$ \text{V}_{\text{ECF}} = \frac{\text{k}_{\text{ECF}} (\text{Ht}^2 \cdot \text{Wt}^{0.5} \cdot \text{R}_{\text{ECF}}^{0.3})}{3} \tag{4} $$

where Ht is height (cm), and Wt is body weight (kg). The $\text{k}_{\text{ECF}}$ term is defined as $10^{-3} \cdot \text{Kg}^2 \cdot \text{PECF}^2 / \text{D}_0$ (10³, where $\text{Kg}$ is a geometry factor that relates the relative volumes of the legs, arms, and trunk, $\text{PECF}$ is the resistivity of extracellular fluid, and $\text{D}_0$ is total body density. The values supplied with the BIS instrument were 4.3 for $\text{Kg}$, 1.05 kg/l for $\text{D}_0$, and 214 (males) and 206 (females) for $\text{PECF}$. When these values are used, the resultant values for the $\text{k}_{\text{ECF}}$ constants were 0.306 for males and 0.316 for females.

From the mixture theory model, the equation used for the calculation of the intracellular fluid volume ($\text{V}_{\text{ICF}}$) was

$$ (1 + \text{V}_{\text{ICF}}/\text{V}_{\text{ECF}})^{0.5} = (\text{R}_{\text{i}} + \text{R}_{\text{e}})/\text{R}_{\text{i}} \cdot (1 + \text{k}_{\text{ICF}}/\text{PECF}) \tag{5} $$

where the resistance values ($\text{R}_i$, $\text{R}_e$) were derived, as noted above, from the electrical circuit model, and $\text{k}_{\text{ICF}}$ is the resistivity ratio, defined as $(\text{ICF}/\text{PECF})$. The values supplied with the BIS instrument for $\text{PECF}$ were 824 (males) and 797 (females). The corresponding $\text{k}_{\text{ICF}}$ constants were 3.82 for males and 3.40 for females, respectively. The BIS value for TBW ($\text{V}_{\text{TBW}}$) was defined as

$$ \text{V}_{\text{TBW}} = \text{V}_{\text{ECF}} + \text{V}_{\text{ICF}} \tag{6} $$

Statistical analysis. Tabulated data in the tables are reported as means ± SD. Coefficient of variation (CV) was defined as 100 × (SD/mean). ANOVA was used to examine effects due to gender and ethnicity, with age, weight, and height as covariates. A paired t-test was used for comparison between the dilution and BIS methods for each water compartment; the correlation coefficient ($r$), probability value (P), and standard error of the estimate (SEE) are reported. The degree of interchangeability between the two methods was based on the approach proposed by Bland and Altman (5). Bias between the BIS and dilution methods for each water compartment was based on the mean difference. The limit of agreement between the two methods was defined as ±2 SD of the mean difference. For all statistical analyses, significance was defined as a P ≤ 0.05.

RESULTS

Table 1 provides the anthropometric characteristics of the study population, subdivided by gender and ethnicity. The results for the TBW, ECW, and ICF compartments, obtained by the $^2\text{H}_2\text{O}$- and Br-dilution methods, are included in Table 1. ANOVA indicated that, for the body water compartments, there were no differences among ethnic groups within a gender group. There were significant differences (P < 0.0001) for TBW and ICF, but not for ECW, between males and females.

Table 2 provides the mean and SD values for $\text{V}_{\text{TBW}}$, $\text{V}_{\text{ECF}}$, and $\text{V}_{\text{ICF}}$ for each gender and ethnic subgroup when the BIS method was used. ANOVA detected no differences for $\text{V}_{\text{TBW}}$, $\text{V}_{\text{ECF}}$, and $\text{V}_{\text{ICF}}$ among ethnic subgroups within a gender classification. The mean
Table 1. Anthropometric characteristics and body water volumes obtained by dilution methods

<table>
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<tr>
<th></th>
<th>n</th>
<th>Age, yr</th>
<th>Wt, kg</th>
<th>Ht, cm</th>
<th>TBW, liters</th>
<th>ECW, liters</th>
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<td>Males</td>
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<tr>
<td>White</td>
<td>72</td>
<td>12.4 ± 6.2</td>
<td>47.6 ± 25.4</td>
<td>147.2 ± 28.9</td>
<td>33.9 ± 13.2</td>
<td>12.9 ± 5.8</td>
<td>18.6 ± 9.1</td>
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<tr>
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<td>69</td>
<td>11.7 ± 5.8</td>
<td>48.3 ± 26.5</td>
<td>145.6 ± 26.7</td>
<td>31.6 ± 17.4</td>
<td>13.9 ± 6.8</td>
<td>17.6 ± 10.7</td>
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<tr>
<td>Hispanic</td>
<td>107</td>
<td>11.3 ± 5.6</td>
<td>45.6 ± 22.2</td>
<td>143.0 ± 28.6</td>
<td>29.0 ± 13.3</td>
<td>12.9 ± 6.0</td>
<td>16.5 ± 8.5</td>
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<td>Total</td>
<td>248</td>
<td>11.7 ± 5.8</td>
<td>47.0 ± 24.3</td>
<td>145.0 ± 28.1</td>
<td>31.3 ± 14.5</td>
<td>13.2 ± 6.1</td>
<td>17.5 ± 9.3</td>
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<td>Females</td>
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<tr>
<td>White</td>
<td>57</td>
<td>14.4 ± 6.9</td>
<td>46.4 ± 21.4</td>
<td>145.9 ± 27.8</td>
<td>26.3 ± 8.9</td>
<td>11.8 ± 4.6</td>
<td>13.0 ± 5.0</td>
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<tr>
<td>Black</td>
<td>67</td>
<td>13.1 ± 6.7</td>
<td>46.9 ± 22.0</td>
<td>141.7 ± 25.9</td>
<td>25.2 ± 10.2</td>
<td>10.6 ± 4.7</td>
<td>12.8 ± 5.0</td>
</tr>
<tr>
<td>Hispanic</td>
<td>97</td>
<td>13.9 ± 6.1</td>
<td>48.5 ± 20.0</td>
<td>144.7 ± 20.4</td>
<td>22.5 ± 7.9</td>
<td>11.2 ± 4.2</td>
<td>10.8 ± 4.1</td>
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<tr>
<td>Total</td>
<td>221</td>
<td>13.9 ± 6.4*</td>
<td>47.7 ± 20.6</td>
<td>144.6 ± 23.7</td>
<td>24.3 ± 8.8*</td>
<td>11.3 ± 4.4</td>
<td>12.0 ± 4.6*</td>
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</tbody>
</table>

Values are means ± SD; n, no. of subjects. Wt, weight; Ht, height; TBW, total body water (H2O dilution); ECW, extracellular water (bromine dilution); ICW, intracellular water (TBW − ECW). *Comparison between males and females (P < 0.0001; t-test).

values for VTBW, VECF, and VICF were significantly different between males and females.

The correlations between ECW and VECF were significant for males (r² = 0.84, P < 0.0005, SEE = 2.3 liters) and females (r² = 0.84, P < 0.0005, SEE = 2.3 liters). Figure 1 provides a plot of the difference (ECWdiff = VECF − ECW) vs. the average values for the two methods. For males, the distribution of ECWdiff values was independent of the average values (r² = 0.01, P > 0.2). For females, the individual ECWdiff values became more negative with increasing average values (r² = 0.06, P < 0.01). It is also evident in Fig. 1 that a number of subjects had ECWdiff values that were substantially displaced (outside ± 2 SD) from the mean differences for the total population. Repeat assays of stored serum samples and a reexamination of the goodness-of-fit parameters provided by the BIS analysis did not identify any clear technical reasons to eliminate these data. The value for ECWdiff for males was 0.25 ± 1.36 (SD) liters and was significantly different (P < 0.001) from the value of −0.63 ± 1.59 liters for females. Similar differences were obtained for each of the ethnic subgroups and are provided in Table 2. When the difference values were expressed as a percentage of the average value [ECWdiff/average ECW], the ECWdiff values for both males and females were independent of the average values. The values for ECWdiff were 0.1 ± 19.1 (SD)% for males and −6.5 ± 16.3% for females.

For the ICW compartment, the results for the dilution methods and BIS measurements were also significantly correlated for males (r² = 0.85, P < 0.0005, SEE = 2.5 liters) and females (r² = 0.85, P < 0.0005, SEE = 2.5 liters). The slope and intercept values, however, were statistically different (P < 0.001) different from the line of identity. Figure 2 provides a plot of the difference values (ICWdiff = VICF − ICW) vs. the average values for the two methods for males and females. It is clearly evident that the ICWdiff values are not independent of the average values but significantly decrease as the average values increase (males: r² = 0.35, P < 0.0001; females: r² = 0.13, P < 0.001). The value for ICWdiff for males was −2.79 ± 3.05 (SD) liters and was significantly different (P < 0.001) from the value of −1.80 ± 2.01 liters for females. When the ICWdiff values were expressed as a percentage of the average value [ICWdiff/average ICW], the ICWdiff values were independent of the average values. The values for ICWdiff were −18.1 ± 21.0 (SD)% for males and −15.3 ± 19.5% for females.

The relationship between TBW and VTBW was also highly correlated (r² = 0.95, P < 0.0005, SEE = 2.7

Table 2. Body water volumes obtained by using BIS and differences between the BIS and dilution methods

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>VTBW, liters</th>
<th>VECF, liters</th>
<th>VICF, liters</th>
<th>TBWdiff</th>
<th>ECWdiff</th>
<th>ICWdiff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
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<tr>
<td>White</td>
<td>72</td>
<td>25.9 ± 13.4</td>
<td>13.6 ± 6.8</td>
<td>11.5 ± 4.8</td>
<td>−3.35 ± 3.01</td>
<td>0.21 ± 1.46</td>
<td>−3.31 ± 3.01</td>
</tr>
<tr>
<td>Black</td>
<td>69</td>
<td>26.6 ± 14.2</td>
<td>13.4 ± 6.8</td>
<td>11.0 ± 4.4</td>
<td>−2.33 ± 2.94</td>
<td>0.12 ± 1.43</td>
<td>−2.56 ± 3.70</td>
</tr>
<tr>
<td>Hispanic</td>
<td>107</td>
<td>23.5 ± 11.7</td>
<td>12.1 ± 5.6</td>
<td>11.2 ± 3.8</td>
<td>−2.44 ± 2.51</td>
<td>0.36 ± 1.24</td>
<td>−2.52 ± 2.63</td>
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<tr>
<td>Total</td>
<td>248</td>
<td>25.1 ± 12.9</td>
<td>12.9 ± 6.3</td>
<td>11.3 ± 4.2</td>
<td>−2.70 ± 2.80</td>
<td>0.25 ± 1.36</td>
<td>−2.79 ± 3.05</td>
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<tr>
<td>Females</td>
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<tr>
<td>White</td>
<td>57</td>
<td>22.1 ± 9.4</td>
<td>11.5 ± 4.8</td>
<td>10.4 ± 4.7</td>
<td>−1.99 ± 2.11</td>
<td>−0.81 ± 1.66</td>
<td>−1.29 ± 1.90</td>
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<td>67</td>
<td>21.1 ± 8.9</td>
<td>11.0 ± 4.4</td>
<td>10.2 ± 4.5</td>
<td>−2.79 ± 2.25</td>
<td>−0.56 ± 1.44</td>
<td>−2.11 ± 2.09</td>
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<tr>
<td>Hispanic</td>
<td>97</td>
<td>21.2 ± 7.4</td>
<td>11.2 ± 3.8</td>
<td>10.0 ± 3.9</td>
<td>−2.31 ± 2.13</td>
<td>−0.64 ± 1.67</td>
<td>−1.78 ± 1.99</td>
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<tr>
<td>Total</td>
<td>221</td>
<td>21.5 ± 8.2*</td>
<td>13.3 ± 4.2*</td>
<td>10.2 ± 4.2*</td>
<td>−2.39 ± 2.13*</td>
<td>−0.63 ± 1.59*e</td>
<td>−1.80 ± 2.01*e</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of subjects. BIS, biopimpeance spectroscopy; VTBW, VECF, and VICF: volume of TBW, extracellular fluid, and intracellular fluid, respectively; TBWdiff, ECWdiff, and ICWdiff: difference values for TBW, ECW, and ICW, respectively. Tissue resistivity values (µ) were provided with Xitron 4000B instrument: µECF (215 for males, 206 for females); µECF (824 for males, 797 for females). For each individual, difference values were calculated as: TBWdiff = VTBW − TBW; ECWdiff = VECF − ECW; and ICWdiff = VICF − ICW. Four cases were extreme outliers > 3.5 SD and were removed from analysis (4). If these 4 outliers were included, the mean ECWdiff value would increase by −0.2 liter, whereas SD would increase by −1 liter. Comparison between males and females (t-test): *P < 0.03; **P < 0.01; ***P < 0.001; ****P < 0.0001.
liters). However, the BIS-derived V<sub>TBW</sub> values were consistently lower than those obtained by the 2H<sub>2</sub>O-dilution method. Figure 3 provides a plot of the difference values (TBW<sub>diff</sub> = V<sub>TBW</sub> - TBW) vs. the average values for the two methods for males and females. Regression analyses indicate that the TBW<sub>diff</sub> values significantly decreased with increasing average values for males (r<sup>2</sup> = 0.21, P < 0.0001) but not for females (r<sup>2</sup> = 0.01, P > 0.2). The values for TBW<sub>diff</sub> were $-2.70 \pm 2.80$ (SD) liters for males and $-2.39 \pm 2.13$ liters for females. When the difference values were expressed as a percentage of the average value (TBW<sub>ndiff</sub> = 100 × (TBW<sub>ndiff</sub>/average TBW)), the TBW<sub>ndiff</sub> values were independent of the average values. In this case, the value was $-9.2 \pm 9.2$ (SD) % for ICW<sub>ndiff</sub> for both males and females.

A summary of the mean differences ± SD between the dilution and BIS methods for each gender and the three ethnic subgroups is provided in Table 2. The mean values for TBW<sub>diff</sub> and ICW<sub>diff</sub> were significantly different from zero (P < 0.0005) for each gender and ethnic subgroup. The TBW<sub>diff</sub> values for males were significantly greater (P < 0.02) than were the corresponding values for females. The ICW<sub>diff</sub> values for males were also significantly greater (P < 0.001) than were the corresponding values for females. Although the mean ECW<sub>diff</sub> values for males and females were not statistically different from zero, the mean values were statistically different (P < 0.001) between the gender groups. The scatter in the individual values for ICW<sub>diff</sub> (see Fig. 2) and TBW<sub>diff</sub> (see Fig. 3) was not independent of the average values, whereas the individual data for ECW<sub>diff</sub> (see Fig. 1) were unrelated to the corresponding average values, although the scatter was large. The ±2 SD lines in each of the figures correspond to the limits of agreement as defined by Bland and Altman (5).

The population of children and young adults in the present study represented a wide range of weights, heights, and body sizes. This is reflected by the large SD values for each of the variables reported in Table 1. Likewise, the large SD values for the V<sub>ECF</sub>, V<sub>ICF</sub>, and V<sub>TBW</sub> values can be attributable to the population selection. Some of the spread in the TBW<sub>diff</sub>, ECW<sub>diff</sub>, and ICW<sub>diff</sub> values may also be due, in part, to this same reason. Therefore, to provide a more meaningful comparison with the findings recently reported by De Lorenzo et al. (8) for healthy young men, we have selected two subgroups from the male population in the present study. The first subgroup (group A) consisted of all men above 16 yr of age, with height in the same range (165–185 cm) as that reported by De Lorenzo et al. The second subgroup (group B) consisted of 14 individuals from within group A who were also weight matched on the basis of their body mass index (Wt/Ht<sup>2</sup>) with individuals in the De Lorenzo study. The mean ± SD values for the De Lorenzo et al. group and for groups A and B are given in Table 3. The mean TBW values among the three groups of men were not statistically different. The mean ECW values, however, were statistically different (P < 0.01), with those for the present study being higher, on average, by $-1.1$ liter. This, in turn, forced the calculated values for ICW for groups A and B to be lower by similar volumes. The mean values...
Table 3. Comparison with findings reported by De Lorenzo et al. (8)

<table>
<thead>
<tr>
<th></th>
<th>Wt, kg</th>
<th>Ht, cm</th>
<th>BMI, kg/m²</th>
<th>TBW, liters</th>
<th>ECW, liters</th>
<th>ICW, liters</th>
<th>R_ECF, Ω</th>
<th>R_ICF, Ω</th>
<th>V_TBW, liters</th>
<th>V_ECW, liters</th>
<th>V_ICW, liters</th>
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<td>De Lorenzo et al. (Ref. 8; n = 14 men)*</td>
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<td>%CV</td>
<td>12.2%</td>
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<td>8.8%</td>
<td>8.8%</td>
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<td>8.1%</td>
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<td>Group A in present study (n = 73 men)‡</td>
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<td>15.1%</td>
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<td>Group B in present study (n = 14 men)‡</td>
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<tr>
<td>%CV</td>
<td>10.3%</td>
<td>3.5%</td>
<td>7.7%</td>
<td>8.5%</td>
<td>17.4%</td>
<td>19.2%</td>
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<td>6.4%</td>
<td>16.6%</td>
<td>10.0%</td>
<td>8.7%</td>
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</table>

Values are means ± SD; n, no. of subjects. BMI, body mass index. Dilution measurements were made of TBW, ECW, and ICW. BIS measurements were made of resistance values for extracellular fluid and intracellular fluid (R_ECF and R_ICF, respectively), V_TBW, V_ECW, and V_ICF. *Men only, age 21–57 yr, height 167–185 cm [De Lorenzo et al. (8)]. ‡Men matched for height and BMI (kg/m²) with subjects examined by De Lorenzo et al. (8).

for ECF and ICF resistance values (R_ECF and R_ICF) were higher in groups A and B than were those observed by De Lorenzo et al., whereas the values for V_TBW, V_ECW, and V_ICF were not appreciably different. It is noteworthy that the range of values for ECW in height-matched (group A) subjects was about twice that reported by De Lorenzo et al. When the subjects were also matched for body mass index (group B), the range of ECW values was reduced when compared with group A, but it was still considerably greater than that reported by De Lorenzo et al.

The mathematical relationships given in Eqs. 4 and 5 provide the associations among the measured anthropometric parameters (height, weight), the calculated electrical parameters (R_ECF and R_ICF), and the theoretical values for V_ECW and V_ICF. We can rearrange the terms in Eqs. 4 and 5 to solve for the constants, denoted by k_ECF and k_ICF, with the dilution values for ECW and ICW substituted for the BIS estimates. On the basis of this approach and the data of De Lorenzo et al. (8), we calculated that the mean values for the k_ECF and k_ICF constants were 0.307 and 3.515, respectively. For the men in our group A, we obtained mean values of 0.339 for k_ECF and 3.234 for k_ICF. For the men in group B, the constants were very similar: 0.348 for k_ECF and 3.264 for k_ICF. In the total population in the present study, we obtained mean values of 0.370 for males and 0.358 for females for k_ECF. The corresponding mean values for k_ICF were 3.032 for males and 2.694 for females.

**DISCUSSION**

The BIS measurement has many practical advantages when compared with the dilution method, especially for the individual being examined. It does not require the subject to drink an extremely salty solution, to incur the discomfort or risk associated with the collection of several blood samples, or to remain fasting and available for 3–4 h during the equilibration period. In addition, the BIS instrumentation is relatively inexpensive and requires low maintenance and minimal operator training, and the measurements can be repeated as frequently as needed. A further benefit for the clinical setting is that the results are immediately available. These general characteristics clearly support BIS as the better choice in terms of the practical aspects of the measurement of body fluid volumes, especially when these measurements are to be obtained in children. Although a number of studies have reported the use of the single-frequency BIA measurement in children (13, 24), relatively few studies have used the BIS technique in this population (2, 3, 22). The present study, therefore, may provide the first direct comparison of BIS with the classic dilution methods for a large population of healthy children, adolescents, and young adults of both genders and of varying ethnicity. The age range examined in this study was chosen to ensure a wide variation in body size, shape, and composition. This variation was selected to adequately test the basic assumptions associated with the Hanai model (12) used in the BIS methodology.

In the present study, all three body water compartment estimates obtained by using BIS were highly correlated with the corresponding values for the dilution techniques. However, the regression lines for all three relationships did not match the line of identity (slope = 1, intercept = 0) when the values for the constants k_ECF and k_ICF, provided with the BIS instrument, were used. Because our study provides a comparison of two methods (dilution vs. BIS), it would not be appropriate to attribute all of the differences solely to one technique. For the dilution technique, questions may be raised with regard to the choice of tracer, the most appropriate body fluid to assay, and the point at which equilibration of the tracer is reached (20, 26). However, for the measurement of TBW, numerous studies have clearly shown that equilibration is reached in the plasma by at least 2 h after an orally administered dose of labeled water (20). Thus in our study it was very reasonable to assume that the oral 3H2O dose had reached equilibration by the time the second plasma sample was collected. Also, plasma was assayed, eliminating any questions related to the selection of body fluid. Furthermore, because our subjects restricted their fluid intake and refrained from voiding during this period, it is unlikely that there was any significant over- or underexpansion of the TBW compart-
ment. Although there may be some uncertainty as to what value to use for the constant to account for the incorporation of the tracer into the nonaqueous tissues, this choice has been shown to alter the TBW estimates by <0.5% (20). For the BIS measurement, total body water (Vr_TBW) was obtained as the sum of the extracellular (Vr_ECF) and intracellular (Vr_ICF) values. Thus the differences seen in Fig. 3 may reflect a bias associated with either or both of these BIS estimates.

For the ECW compartment, the Br-dilution and BIS methods produced comparable mean results (see Fig. 1 and Table 2) for males and females. The second criterion for interchangeability between methods (8), however, was not met for females because the individual ECWdiff values were not independent of the average values. Furthermore, a considerable number of males and females had ECWdiff values outside the ±2 SD limits. As we have already noted, when two methods are compared, it is usually not statistically appropriate to attribute all of these differences solely to one method. For the Br-dilution measurement of ECW, several different radioactive and stable tracers have been used, different body fluids have been sampled, and time to allow for equilibration of the tracer has varied among investigators (6, 20, 22, 26). However, ECW estimates based on Br dilution at 3–4 h have been shown to be in good agreement with those obtained by the direct measurement of total body chlorine (31). Furthermore, although no official consensus has been reached for standardization of the Br-dilution method, the most commonly reported procedure (as used in this study) has been to assay a plasma sample collected at 3–4 h after an oral Br dose (7, 9, 20, 30, 31).

For the BIS estimates for Vr_ECF, based on the Hanai model (12), to be successful, the term k_v in Eq. 4 must be constant. For this model to be applicable to a pediatric population, the value for k_ECF must be relatively invariant to changes in body composition with age and during growth. In the initial studies of adults by Van Loan et al. (25), the values for k_ECF and k_r were reported as 0.306 and 3.82 for males and 0.316 and 3.40 for females, respectively. For the 14 young adult men examined by De Lorenzo et al. (8), values used for the k_ECF and k_r constants were 0.307 and 3.498, respectively. Armstrong et al. (1) used values of 0.337 and 2.905 for the k_ECF and k_r constants, respectively, when they examined the relationship between the BIS and dilution methods in 13 healthy young men. Gudivaka et al. (11) used similar values (k_ECF = 0.338, and k_r = 2.968) when they examined the effects of skin temperature on multifrequency measurements in six healthy adults. Van Marken Lichtenbelt et al. (27) examined 10 healthy adults, and, on the basis of the specific resistivity values reported, we calculated the mean k_ECF values to be 0.245 for males and 0.238 for females. The corresponding values for k_r appear to be 6.408 for males and 6.469 for females. Only Smye et al. (22) have reported BIS measurements for Vr_ECF in children. When they compared the body’s clearance of 99mTc-labeled diethylene triamine pentaacetate with the results for the BIS measurement, the mean value calculated for k_ECF was 0.335. In the present study, we obtained mean values for k_ECF of 0.370 for the total male population and 0.358 for the female population. Unfortunately, we did not find either of these values to be constants because their %CVs were 19.1%. Similarly, when we calculated the mean values for k_r as 3.032 for males and 2.694 for females, the corresponding %CVs were 25–28%. To be considered as constants in terms of body composition parameters, one would expect the %CVs to be <5% (28). Although substitution of our recalculated gender-specific mean values for k_ECF and k_r produced new values for Vr_ECF, Vr_ICF, and Vr_TBW, such that the mean biases (ECWdiff, ICWdiff, and TBWdiff) relative to the dilution volumes were virtually zero, the wide range in individual differences was not significantly altered.

There are three parameters (K_B, D_p, p_ECF) that are used to derive the value for K_ECF. Although any one of these three parameters need not be constant over the age range examined in the present study, their product as defined by the equation for K_ECF must remain relatively constant for use with the Hanai model (8). Furthermore, the nature of the mathematical relationship among these three parameters within the equation for K_ECF shows that any one of the three can serve as a scalar for the determination of the Vr_ECF values. This is, with two of the values held constant, the third value can be adjusted such that the average bias (ECWdiff) for the Vr_ECF will become zero. It is important, however, to appreciate that although the average bias can be forced to zero, this will not appreciably reduce the range for the individual ECWdiff values. Therefore, to reduce the differences between the dilution and BIS estimates, it appears that the K_ECF term may not be constant among individuals for the full age range examined in this study. One possibility is that the tissue resistivity values (p_ECF, p_ICF) used to calculate k_ECF and k_r are not constant for all ages. Alternatively, K_B may need to be adjusted for age and gender (19, 23), especially during periods of rapid growth. Although the third possibility is that D_p for children needs to be changed with age (10), its relative impact on the K_ECF value is much less than that for either K_B or p_ECF. Also, because K_B is not used in the Vr_ICF equation, the most logical choice is to alter the p_ECF value. It appears that agreement between the BIS and dilution methods for adults has been best achieved when tissue resistivity values have been recalculated for each specific population (1, 2, 8, 14, 22, 27). That is, the BIS instrument can easily be recalibrated on a group basis to achieve approximately zero mean differences between the Vr_ECF and Vr_ICF or Vr_TBW estimates when compared with dilution-based values. This, however, does not necessarily ensure that the BIS estimates are accurate for any subsequent studies in a different population or even for individuals within the original calibration population. For multifrequency bioelectrical impedance methods to be universally applicable, the models and basic assumptions used to describe the body’s water distributions, including any inferred constants, should be independent of the population from which they were derived. In the present study, the %CVs for the recalculated K_ECF
and k, terms were too large (19–29%) to consider these parameters as constants within the context of body composition analysis (28). It remains unknown how much of this lack of agreement between the dilution methods and the BIS measurement can be attribute solely to the latter technique.

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