Magnitude and variation of fat-free mass density: a cellular-level body composition modeling study

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Wang, ZiMian, Stanley Heshka, Jack Wang, Lucian Wielopolski, and Steven B. Heymsfield. Magnitude and variation of fat-free mass density: a cellular-level body composition modeling study. Am J Physiol Endocrinol Metab 284: E267–E273, 2003; 10.1152/ajpendo.00151.2002.—The mean density of fat-free mass (FFM) is remarkably stable at 1.10 g/cm3 in healthy adult humans, and this stability is a cornerstone of the widely applied densitometry-based two-compartment model for estimating total body fat. At present, the usual means of exploring FFM density is by in vitro or in vivo experimental studies. The purpose of the present investigation was to develop a cellular-level body composition model that includes seven factors that determine FFM density. The model, when applied with available empirical coefficients, predicted an FFM density similar to that observed in vivo. An analysis of the seven model components indicates that the ratio of extracellular solids to total body water is a major determinant of individual variation in FFM density. The difference in FFM density across sex, race, and age groups was examined with the developed model. The present study thus provides a conceptual framework for the systematic study of FFM density in humans.

body composition; body fat measurement; bone mineral; total body water

AN IMPORTANT AIM of body composition research is to identify stable component relationships such as the fraction of fat-free mass (FFM) as water (i.e., total body water/FFM = 0.73; see Ref. 27). The origin of these observed stable component relationships is of scientific interest, and some of these presumed stable associations are the basis of body composition methods (e.g., fat = body mass − total body water/0.73; see Refs. 17, 20, 26).

A classic body fat estimation method relies on the densitometry-based two-compartment model in which body mass (BM) is expressed as the sum of fat and FFM. Two simultaneous equations can be written

\[
BM = \text{fat} + \text{FFM} \tag{1}
\]

\[
BM/D_b = \text{fat}/D_{\text{fat}} + \text{FFM}/D_{\text{FFM}} \tag{2}
\]

where \(D_b\) is body density, \(D_{\text{fat}}\) is the density of fat, and \(D_{\text{FFM}}\) is the FFM density. Solving the two equations

\[
\text{fat}/BM = \frac{D_{\text{fat}} \times (D_{\text{FFM}} - D_b)}{D_b \times (D_{\text{FFM}} - D_{\text{fat}})} \tag{3}
\]

Inserting the values of \(D_{\text{fat}}\) at 0.900 g/cm\(^3\) (5) and the assumed constant \(D_{\text{FFM}}\), one can estimate the fraction of BM as fat based on the measurement of body density.

Up to the present time, the usual means of exploring FFM density in humans is by cadaver analysis or by in vivo experimental studies. On the basis of the analysis of several cadavers, Siri (21) proposed an FFM density value of 1.100 g/cm\(^3\), Brožek et al. (1) compiled data based on the body composition of Reference Man. The authors assumed that the FFM fractions as water, protein, bone mineral (Mo), and soft tissue minerals (Ms) are 0.737, 0.194, 0.056, and 0.012, respectively. With the combination of the known densities of water (0.9937 g/cm\(^3\) at 36°C; see Ref. 3), protein (1.34 g/cm\(^3\)), Mo (2.982 g/cm\(^3\)), and Ms (3.317 g/cm\(^3\)), Brožek et al. calculated the density of FFM as 1.100 g/cm\(^3\) for Reference Man. On the basis of the results obtained from nine in vitro cadaver studies from 1945 to 1986, we calculate an FFM density (mean ± SD) of 1.099 ± 0.015 g/cm\(^3\), with a range from 1.072 to 1.114 g/cm\(^3\) (Table 1).

With FFM density taken to be 1.100 g/cm\(^3\) and fat density at 0.900 g/cm\(^3\), Eq. 3 can be simplified to

\[
\text{fat}/BM = 4.95/D_b - 4.50
\]

or

\[
\text{fat} = 4.95 \times BM/D_b - 4.50 \times BM \tag{4}
\]

Body fat mass can thus be predicted from BM and body density measured by underwater weighing or air displacement plethysmography (9). The two-compartment method based on Eq. 4 is often applied as the criterion for body fat measurement. Other clinically applied and less-accurate body fat prediction methods, such as an-

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throppometric estimations and bioelectrical impedance analysis, have been calibrated and cross-validated against fat estimates derived using Eq. 4.

Although the literature on FFM density has expanded greatly over the past five decades and a value of 1.100 g/cm$^3$ is the cornerstone of the densitometry-based two-compartment model, there remain fundamental questions related to the density of FFM. Specifically, we still lack a body composition model that can provide insights into the magnitude and constancy of FFM density in humans.

The purpose of this study was to develop a cellular-level body composition model that permits a systematic examination of the factors that lead to the magnitude and variation in FFM density. In our previous studies, we developed two cellular-level body composition models, one for FFM hydration and the other for the ratio of total body potassium to FFM (25, 28). The current study is the third investigation in our modeling series.

**FFM DENSITY MODEL**

The strategy of the present study, which differs from earlier empirical approaches, was to separate FFM into several components on the cellular body composition level and then to construct FFM density from interconnected ratios. BM is composed of the following three components on the cellular level: cells, extracellular fluid (ECF), and extracellular solids (ECS; see Ref. 27). The cellular component can be further divided into fat and body cell mass (BCM). According to Moore et al. (14), BCM includes intracellular water (ICW), protein, and Ms but does not include stored fat. On the basis of this definition, FFM can be expressed as (Fig. 1)

$$FFM = BCM + ECF + ECS$$

Similarly, the volume of FFM can be expressed as the sum of the volumes of the three components

$$FFM_{\text{volume}} = BCM/D_{BCM} + ECF/D_{ECF} + ECS/D_{ECS}$$

where $D_{BCM}$, $D_{ECF}$, and $D_{ECS}$ are the densities of BCM, ECF, and ECS, respectively.

An FFM density model is derived based on Eqs. 5 and 6

$$D_{FFM} = \frac{BCM + ECF + ECS}{BCM/D_{BCM} + ECF/D_{ECF} + ECS/D_{ECS}} \quad (7)$$

In the next stage, our aim was to resolve the model into relevant component ratios.

Both BCM and ECF contain aqueous compartments as ICW and extracellular water (ECW). Components BCM and ECF can thus be expressed, respectively, as $BCM = ICW/a$ and $ECF = ECW/b$, where $a$ is the ratio of ICW to BCM and $b$ is the ratio of ECW to ECF. In addition, ECS is expressed as a function of total body water (TBW, the sum of ICW and ECW), ECS = $c \times TBW = c \times (ICW + ECW)$, where $c$ is the ratio of ECS to TBW (25). Equation 7 can be converted to

$$D_{FFM} = \frac{ICW/a + ECW/b + c \times (ICW + ECW)}{ICW(a \times D_{BCM}) + ECW(b \times D_{ECF}) + c \times (ICW + ECW)/D_{ECS}} \quad (8)$$

ECW can be expressed as a function of ICW, $ECW = (E/I) \times ICW$, where $E/I$ is the ratio of ECW to ICW.

*Table 1. In vitro measurement of FFM density in 8 adult cadavers*
Density of FFM

The mean density of FFM can thus be calculated for healthy adults

\[
D_{FFM} = \frac{1}{(0.70 + 0.95/0.98 + 0.135 + 0.135 \times 0.95)}
\]

\[
= \frac{1}{(0.70 \times 1.078) + 0.95/(0.98 \times 1.01)}
\]

\[
+ 0.135/1.96 + 0.95 \times 0.135/1.96
\]

\[
= 1.10 \text{ g/cm}^3
\]

Table 2. Body composition and in vivo measurement of \(D_{FFM}\) in group 1 subjects

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>D_{FFM}, g/cm^3</td>
<td>1.102 ± 0.007</td>
<td>1.084–1.135</td>
</tr>
</tbody>
</table>

MODEL FEATURES

Many authors have studied the magnitude and variation in FFM density and its relation to sex, race, and age (2, 16, 24). In the present study, we evaluated two groups of healthy subjects (Appendix) to demonstrate how the proposed cellular-level model can provide new insights into factors responsible for the density of FFM.

Can the Model Reproduce the Mean and Range of FFM Density Observed in Adults?

Mean values of the seven model determinants are described above, i.e., \(a = 0.70\), \(b = 0.98\), \(c = 0.135\), \(E/I = 0.95\), \(D_{BCM} = 1.078 \text{ g/cm}^3\), \(D_{ECF} = 1.010 \text{ g/cm}^3\), and \(D_{ECS} = 1.96 \text{ g/cm}^3\). The mean density of FFM can thus be calculated for healthy adults

\[
D_{FFM} = \frac{1}{(0.70 + 0.95/0.98 + 0.135 + 0.135 \times 0.95)}
\]

\[
= \frac{1}{(0.70 \times 1.078) + 0.95/(0.98 \times 1.01)}
\]

\[
+ 0.135/1.96 + 0.95 \times 0.135/1.96
\]

\[
= 1.10 \text{ g/cm}^3
\]
neutron activation analysis, dual-energy X-ray absorptiometry (DEXA), and whole body counting (Eq. A1).

In previous studies, the reported FFM densities vary within a narrow range in adults (Table 1 and Ref. 1). As indicated above, each of the seven determinants may vary within a range: a from 0.69 to 0.71, b from 0.97 to 0.99, c from 0.12 to 0.16, E/I from 0.58 to 1.36, DBCM from 1.067 to 1.089 g/cm³, D_ECF from 1.008 to 1.012 g/cm³, and D_ECS from 1.94 to 1.98 g/cm³. Of the seven determinants, only E/I is in inverse proportion to FFM density, whereas the other six determinants are in direct proportion to FFM density. We can thus predict the variation range of FFM density if the seven determinants take their extreme values. When predicting the variation range of FFM density if the seven determinants, only E/I is in inverse proportion to FFM density. We can thus predict the variation range of FFM density if the seven determinants take their extreme values. When predicting the variation range of FFM density if the seven determinants take their extreme values. When predicting the variation range of FFM density if the seven determinants take their extreme values. When predicting the variation range of FFM density if the seven determinants take their extreme values.

When a = 0.69, b = 0.97, c = 0.12, E/I = 1.36, D BCM = 1.067 g/cm³, D ECF = 1.008 g/cm³, and D ECS = 1.94 g/cm³, FFM density reaches its low value

\[
D_{FFM} = \frac{1/0.69 + 1.36/0.97 + 0.12 + 0.12 \times 1.36}{1/(0.69 \times 1.067) + 1.36/(0.97 \times 1.008)} + 0.12/1.94 + 1.36 \times 0.12/1.94
= 1.083 g/cm³
\]

When a = 0.71, b = 0.99, c = 0.16, E/I = 0.58, D BCM = 1.089 g/cm³, D ECF = 1.012 g/cm³, and D ECS = 1.98 g/cm³, FFM density reaches its high value

\[
D_{FFM} = \frac{1/0.71 + 0.58/0.99 + 0.16 + 0.16 \times 0.58}{1/(0.71 \times 1.089) + 0.58/(0.99 \times 1.012)} + 0.16/1.98 + 0.58 \times 0.16/1.98
= 1.124 g/cm³
\]

The model-predicted range of FFM densities for healthy adults is thus approximately from 1.083 to 1.124 g/cm³. This variation range is similar to the results of cadaver studies (1.072–1.114 g/cm³; Table 1) and our in vivo study (1.084–1.115 g/cm³ for group 1; Table 2). The proposed model thus indicates that the observed FFM density range can be accounted for by variation in the seven determinants.

Does FFM Density Vary with Growth?

Previous studies indicate that FFM density varies with growth (18). Moulton (15), in his classic investigation, summarized the chemical analysis results of nine mammals, including mice, rats, guinea pigs, rabbits, cats, dogs, pigs, cattle, and humans. At birth, all mammals show a high FFM hydration (e.g., 0.82 for mammals) and low FFM fractions as protein and minerals (e.g., respectively, 0.14 and 0.03 for humans). During growth in mammals, FFM hydration rapidly declines, and protein and mineral concentrations increase. The density of FFM is thus low at birth (1.064 g/cm³) and high in adults (1.100 g/cm³).

Can the Proposed Model be Applied in Exploring the Relationship Between FFM Density and Growth?

Of the seven determinants, a = 0.70, b = 0.98, D BCM = 1.078 g/cm³, D ECF = 1.010 g/cm³, and D ECS = 1.96 g/cm³ are assumed stable throughout life for modeling purposes. The FFM density model (i.e., Eq. 9) can thus be simplified to

\[
D_{FFM} = 1.429 + 1.020 \times (E/I) + c + c \times (E/I) \\
1.325 + 1.010 \times (E/I) + 0.510 \\
\times c + 0.510 \times c \times (E/I)
\]

Determinant c changes directly, and the E/I ratio changes inversely with FFM density. Based on Reference Child data (5), factor c is very low at birth (i.e., 0.07) and increases rapidly to adolescence (i.e., 0.135). In contrast, E/I is high at birth (i.e., 1.7) and decreases rapidly to 1.0 in adults.

We are thus able to predict the change in FFM density during growth. At birth, c = 0.07 and E/I = 1.7; according to Eq. 10, the calculated FFM density is 1.07 g/cm³. The FFM density then increases to 1.10 g/cm³ for adults when c = 0.135 and E/I = 1.0. This trend is the same as measured FFM densities, 1.064 g/cm³ at birth and 1.100 g/cm³ in adults (15). As indicated by Eq. 10, both an increase in determinant c and a decrease in E/I cause an increase in FFM density during growth.

Are There Major Model Determinants of FFM Density?

In the present study, we evaluated a second large group of healthy adult subjects (group 2, n = 267; APPENDIX and Table 3) to examine the major model determinants of FFM density. The FFM density in this group was calculated from measured body density, body fat, and BM (Eq. A4).

Among the seven model determinants, a, b, D BCM, D ECF, and D ECS can be assumed for practical purpose to be stable in adults. Factor c and water distribution (E/I) are the only two determinants that vary substantially among healthy adults and possibly affect the density of FFM (i.e., Eq. 10).

Water distribution in group 2 was measured with total body potassium and TBW as described in the APPENDIX (Eq. A5). There was no significant correlation between the E/I ratio and FFM density (r = 0.01, P > 0.05). The calculated FFM density is 1.07 g/cm³ for adults when c = 0.135 and E/I = 1.0. This trend is the same as measured FFM densities, 1.064 g/cm³ at birth and 1.100 g/cm³ in adults (15). As indicated by Eq. 10, both an increase in determinant c and a decrease in E/I cause an increase in FFM density during growth.

Table 3. Body composition and in vivo measurement of D_{FFM} in group 2 subjects

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Range</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>267</td>
<td>127M, 140F</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>41.8 ± 15.4</td>
<td>18–86</td>
<td></td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>75.3 ± 15.5</td>
<td>40.8–114</td>
<td></td>
</tr>
<tr>
<td>Height, m</td>
<td>1.70 ± 0.10</td>
<td>1.44–1.92</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.1 ± 4.8</td>
<td>16.9–41.3</td>
<td></td>
</tr>
<tr>
<td>Fat, kg</td>
<td>21.8 ± 11.0</td>
<td>2.6–56.2 DEXA</td>
<td></td>
</tr>
<tr>
<td>FFM, kg</td>
<td>53.5 ± 11.8</td>
<td>31.5–82.9 Body mass-fat</td>
<td></td>
</tr>
<tr>
<td>TBW, kg</td>
<td>39.4 ± 8.9</td>
<td>22.0–70.0 H₂O dilution</td>
<td></td>
</tr>
<tr>
<td>Mo, kg</td>
<td>2.85 ± 0.60</td>
<td>1.59–4.55 DEXA</td>
<td></td>
</tr>
<tr>
<td>TBK, mmol</td>
<td>3203 ± 874</td>
<td>1610–5270 Whole body 40K counting</td>
<td></td>
</tr>
<tr>
<td>E/I</td>
<td>1.00 ± 0.20</td>
<td>0.54–1.50 Equation A5 calculation</td>
<td></td>
</tr>
<tr>
<td>Mo/TBW</td>
<td>0.073 ± 0.008</td>
<td>0.048–0.103</td>
<td></td>
</tr>
<tr>
<td>D_{FFM}, g/cm³</td>
<td>1.102 ± 0.015</td>
<td>1.062–1.150 Equation A4 calculation</td>
<td></td>
</tr>
</tbody>
</table>

E/I, ratio of extracellular water to intracellular water; Mo/TBW, ratio of bone minerals to TBW.
Determinant \( c \) is the ratio of ECS to TBW. A direct method of measuring ECS is unavailable at present, although Mo account for \( \sim 60\% \) of ECS and can be measured by DEXA. We thus substitute the Mo-to-TBW ratio for the closely related ECS-to-TBW ratio. The correlation between the Mo-to-TBW ratio and FFM density was significant (\( r = 0.34, P < 0.001 \)) for the group 2 subjects. The Mo-to-TBW ratio, a measure of bone mass relative to soft tissue hydration, is thus a major model determinant of FFM density.

As described above, the ECS fraction as Mo was assumed to be 0.577 (i.e., Mo/ECS = 0.577 or ECS = 1.73 \( \times \) Mo; see Ref. 22). Equation 11 can be further simplified for review purposes to

\[
D_{\text{FFM}} = \frac{2.398 + 3.374 \times (\text{Mo/TBW})}{2.285 + 1.720 \times (\text{Mo/TBW})} \tag{11}
\]

Equation 11 indicates that the relationship between Mo/TBW and FFM density is nonlinear, although this function is almost linear within the Mo/TBW biological range (Fig. 2). The Mo/TBW ratio is 0.0729 \( \pm \) 0.0082 with a variation range 0.048–0.103 for the 267 group 2 subjects of the present study (Table 3). When the Mo/TBW ratio increases from 0.05 to 0.10, according to Eq. 11, FFM density increases from 1.083 to 1.113 g/cm\(^3\), showing that the Mo/TBW ratio strongly influences the magnitude of FFM density.

**Does FFM Density Vary with Sex, Race, and Age?**

On the basis of the group 2 database, we explored the possible affects of sex, race, and age on the FFM density. The densities of FFM were predicted from the Mo/TBW ratio, as described by Eq. 11. The predicted FFM densities are close to the measured FFM densities for different sex, race, and age groups (Table 4).

**Sex.** Women have a higher Mo/TBW ratio than men (Table 4). According to Eq. 11, the predicted FFM densities are 1.099 and 1.097 g/cm\(^3\) for black women and men <60 yr and 1.099 and 1.095 g/cm\(^3\) for white women and men <60 yr, respectively, indicating that the sex difference in FFM density (0.002–0.004 g/cm\(^3\)) might be detected by in vivo measurements (\( P < 0.01 \) for women vs. men; Table 4). Women thus appear to have a slightly higher Mo/TBW ratio and FFM density than men.

**Race.** No significant difference in FFM density was detected in an earlier investigation between black and white adults (24). However, this experimental study did not provide insights into why black and white subjects had nonsignificant differences in FFM density. In the present study, black women <60 yr had a slightly lower Mo/TBW ratio (difference: 0.0009) than white women, whereas black men <60 yr had a slightly higher Mo/TBW ratio (difference: 0.0036) than white men (Table 4). According to Eq. 11, the differences in predicted FFM densities between black and white groups are very small, although the trend is for slightly higher FFM densities in black subjects. This small difference in FFM density may not be detected by in vivo studies (\( P > 0.05 \) for black vs. white women and black vs. white men; Table 4).

**Age.** Elderly subjects (\( \geq 60 \) yr) in group 2 have a smaller Mo/TBW ratio than young adults (Table 4). According to Eq. 11, the predicted FFM densities are 1.099 and 1.096 g/cm\(^3\) for black young and old subjects and 1.099 and 1.094 g/cm\(^3\) for white young and old subjects, respectively. These differences in FFM density (0.003–0.005 g/cm\(^3\)) were detected by in vivo measurements (\( P < 0.05 \) for young and old subjects; Table 4).

**DISCUSSION**

The constancy of FFM density in healthy adults has been recognized for over five decades and led to the classic densitometry-based method of estimating fatness in humans. Deviations from “constant” FFM density in earlier reports were often recognized as aberrations or methodological errors. The present study, to our knowledge, is the first effort aimed at providing an

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**Table 4. Effects of sex, race, and age on FFM density in group 2 subjects**

<table>
<thead>
<tr>
<th>Race</th>
<th>Women, yr</th>
<th>Men (20–59)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20–59</td>
<td>60+</td>
</tr>
<tr>
<td>Black</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mo/TBW, kg/kg</td>
<td>0.0757</td>
<td>0.0717</td>
</tr>
<tr>
<td>D_{\text{FFM}} (predicted), g/cm(^3)</td>
<td>1.099</td>
<td>1.096</td>
</tr>
<tr>
<td>D_{\text{FFM}} (measured), g/cm(^3)</td>
<td>1.000 ( \pm ) 0.016</td>
<td>1.096 ( \pm ) 0.017</td>
</tr>
<tr>
<td>White</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mo/TBW, kg/kg</td>
<td>0.0766</td>
<td>0.0686</td>
</tr>
<tr>
<td>D_{\text{FFM}} (predicted), g/cm(^3)</td>
<td>1.099</td>
<td>1.094</td>
</tr>
<tr>
<td>D_{\text{FFM}} (measured), g/cm(^3)</td>
<td>1.105 ( \pm ) 0.014</td>
<td>1.085 ( \pm ) 0.014</td>
</tr>
</tbody>
</table>

Means \( \pm \) SD shown for measured \( D_{\text{FFM}} \).

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**Fig. 2. Density of FFM by the simplified model (Eq. 11) on the ordinate and the ratio of Mo to total body water (Mo/TBW) on the abscissa. The simplified model, FFM density = \( \frac{2.398 + 3.374 \times (\text{Mo/TBW})}{2.285 + 1.720 \times (\text{Mo/TBW})} \), is a nonlinear function. However, this function is almost linear within the range of Mo/TBW from 0.05 to 0.11.**
explanation for the observed variation in FFM density with a cellular-level body composition model. The derived model, when combined with available data, accounts for the magnitude and variation range of FFM density and explores the effects of sex, race, and age on FFM density. Moreover, although there are seven determinants in the model, the ratio of ECS to TBW (i.e., ECS/TBW or related Mo/TBW) is a major factor leading to the variability in FFM density. This study thus provides new insights into our understanding and application of FFM density for quantifying total body fat mass.

APPENDIX

Two subject groups were evaluated in the present study. Each subject completed a medical history, physical examination, and blood studies to exclude the presence of underlying diseases. We applied two different approaches in calculating the density of FFM.

There were 233 healthy adults, 26 males and 207 females, in group 1 (Table 2). At the molecular body composition level, FFM is composed of the following four components: TBW, protein, Mo, and Ms (27). The density of FFM can thus be calculated as

$$D_{\text{FFM}} = \frac{\text{FFM mass/FFM volume}}{(\text{TBW} + \text{protein})}$$

$$+ \frac{\text{Mo} + \text{Ms})(\text{TBW}/0.9937 + \text{protein}/1.34)}{(\text{TBW} + \text{Mo} + \text{Ms}/3.317) \text{Mo}/2.982 \text{Ms}/3.317}$$

where TBW was measured by the $^3$H or $^2$H dilution method (19); protein is calculated from total body nitrogen (TBN) by prompt-γ in vivo neutron activation analysis (4)

$$\text{protein} = 6.25 \times \text{TBN}$$

Total body Mo was measured by DEXA; and total body Ms was calculated from total body potassium (TBK), sodium (TBNa), chlorine (TBCl), and calcium (TBCa, all in kg; Ref. 10)

$$\text{Ms} = 2.76 \times \text{TBK} + \text{TBNa} + 1.43$$

$$\times \text{TBCl} - 0.038 \times \text{TBCa}$$

TBK was measured by whole body $^{40}$K counting, and TBNa, chlorine (TBCl), and calcium (TBCa, all in kg; Ref. 10)

$$\text{TBK} = \frac{\text{0.900} \times \text{D}_{\text{I}} \times (\text{BM} - \text{fat})}{0.900 \times \text{BM} - \text{fat} - \text{D}_{\text{I}}}$$

where $D_{\text{I}}$ is measured by underwater weighing (9), and fat is body fat mass estimated by DEXA. Fat estimates by DEXA are not measurably influenced by hydration fluctuations in healthy adults (23).

Water distribution (i.e., the ratio of ECW to ICW or E/I) was measured in both groups based on TBK and TBW (6, 25)

$$E/I = (155 \times \text{TBW} - \text{TBK})/\text{TBK} - 5 \times \text{TBW}$$

where TBK is expressed in millimoles and TBW is in kilograms. TBK was measured by whole body $^{40}$K counting.

Water volume, estimated by $^3$H or $^2$H dilution, was assumed to overestimate TBW by 4% (19). This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-42618.

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