Evaluation of modified multicompartiment models to calculate body composition in healthy males1–3

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ABSTRACT The purpose of this study was to develop flexible and accurate multicompartment equations to calculate body composition and compare the results with methods using common two-compartment equations. Twenty-two healthy male volunteers 22–59 y of age were studied. Body volume was measured by underwater weighing (UWW) or with a skinfold caliper, bone mineral by dual-energy X-ray absorptiometry (DXA), and body water by bioelectrical impedance analysis (BIA). The percentage of water and bone mineral in fat-free mass (FFM) had a significant effect on the difference in percentage fat obtained by the two-compartment model compared with a four-compartment model. FFM density was negatively \( r = -0.76, P < 0.001 \) and percentage water in FFM was positively correlated with age \( r = 0.75, P < 0.001 \). The three-compartment model based on field-adapted methods (skinfold thickness + BIA) to calculate percentage body fat correlated significantly with the more complex four-compartment model (UWW + BIA + DXA; \( r = 0.95, P < 0.001 \)). The advantages of three- and four-compartment equations are that they compensate for differences in body content of bone mineral and water. Am J Clin Nutr 1996;63:856–62.

KEY WORDS Bioimpedance, body composition, body fat, body water, bone mineral, dual-energy X-ray absorptiometry, multicompartiment models, skinfold thickness, underwater weighing

INTRODUCTION

To measure body composition in an accurate way is of great importance in human metabolic and physiologic studies. Several different approaches have been used to assess body composition (1). The most common and frequently used methods are based on a two-compartment model comprising fat and fat-free mass (FFM) because the amount of fat in the body is of special nutritional interest.

The three most common methods used to calculate body composition based on the two-compartment model are underwater weighing (UWW), bioelectrical impedance analysis (BIA), and skinfold-thickness measurements.

1) UWW has been used as the reference method since the beginning of the 1950s (2) and is based on the assumptions that fat has a density of 0.9 g/cm³ and that FFM has a constant density of 1.1 g/cm³.

2) Skinfold-thickness measurement has been used as a simple and inexpensive method to estimate the percentage fat since Durnin and Womersley (3) found a relation between the skinfold thickness measured with a caliper and body density (BD) measured by UWW. To calculate percentage fat, Siri’s (4) two-compartment equation was used, based on the assumed fixed FFM density of 1.1 g/cm³.

3) BIA is an indirect way to measure total body water from total body resistance. BIA is also a relatively simple method and is suitable in field studies. From the estimation of body water, body fat content has been calculated on the assumption that FFM contains 73.2% water (5). Dual-energy X-ray absorptiometry (DXA) was originally developed to measure bone mineral content. DXA can also be used to calculate body fat content and has slowly become one of the most frequently used methods to measure body composition. However, DXA equipment is expensive and like the equipment needed for UWW, is available at only a limited number of laboratories.

The two-compartment models assume that there is a fixed proportion of water, protein, and mineral in FFM. However, bone mineral mass, water mass, and protein mass vary among individuals (6, 7) and are influenced by age (8, 9), sex (10), ethnic and genetic factors (11–13), as well as diet and exercise (11). This could lead to considerable individual deviations from the average in the percentage of water in FFM as well as bone mineral in FFM and will consequently influence the calculated body fat content. Thus, Baumgartner et al (14) suggested that the variability in FFM composition necessitated the use of multicompartiment models when anthropometric and body water measurements were used to estimate percentage fat.

In this report we compared data from UWW, skinfold-thickness, BIA, and DXA measurements collected during an earlier study (15). The aim of the present study was to develop new, modified multicompartiment equations that take into consideration individual differences in bone mineral, protein, and water content in the body; and to compare results obtained by using the multicompartiment equations with results

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obtained with common two-compartment equations; and 3) to evaluate the use of field-adapted methods (ie, skinfold-thickness measurements and BIA) in multicompartments.

SUBJECTS AND METHODS

Subjects

Twenty-two healthy male volunteers participated in the study. Descriptive data on the subjects are shown in Table 1. Six of the men were well-trained elite athletes engaged in modern pentathlons, triathlons, and athletics and were recruited from different sports clubs. The remaining 16 subjects were recruited from students and staff members of the Faculty of Medicine. The subjects were relatively physically active and fit as indicated by $\VO_2\text{max}$ (maximum oxygen consumption) estimates (Table 1). All subjects gave their informed consent and the study was approved by the Ethical Committee of the Faculty of Medicine at Uppsala University.

Body-composition measurements

UWW was performed in the morning after subjects consumed a light breakfast. The subjects were weighed in air on a normal scale with high precision (KC120-ID1 MultiRange; Mettler Instrumente, Greifensee, Switzerland) and in water (model GM 49-1; Mettler) with the body totally submerged after maximal exhilation, ie, only the residual volume remaining in the lungs. Ten measurements were performed, the highest value for body weight underwater was excluded and the mean of the two next highest values was used in the calculations. The residual lung volume was determined with conventional body plethysmography (Siemens Siregost FD40/FD91; Siemens, Erlangen, Germany) on the same day as UWW was done or the day after. No estimation of gastrointestinal air was made.

Skinfold thickness was measured on the right side of the body at four different locations (biceps, triceps, subscapula, and suprailiac folds) with a Harpenden caliper (John Bull; British Indicators, St Albans, United Kingdom) with the subjects standing in a relaxed position. All skinfold-thickness measurements were done by the same investigator (AF). Each site was measured three times, $\pm 2$ s after application of the caliper to the nearest 0.1 mm, and the mean value was used in the calculations (the CV was 2.4% between measurements). BD was calculated according to Durnin and Womersley (3).

BIA was measured with a single-frequency (50kHz, 500 $\mu$A) bioreistance body-composition analyzer (model 1990B; Valhalla Scientific, San Diego). The electrodes were placed on the right wrist and ankle. Body water volume was calculated by using the equation of Kushner and Schoeller (16).

DXA measurements were made by the same examiner (AGJ) using the same DXA equipment and with the same software (DPX-L version 1.1; Lunar, Madison, WI). For each total body scan, total body bone mineral mass was obtained.

Calculations of body composition

The weight fractions of two compartments with different densities can be calculated from total BD by using a hyperbolic equation:

\[
\text{Fraction fat} = \frac{a}{BD} - c, \text{ where}
\]

\[
a = \left(\frac{\text{df} \times \text{dx}}{\text{dx} - \text{df}}\right) \quad \text{and} \quad c = \left(\frac{\text{df}}{\text{dx} - \text{df}}\right)
\]

(1)

In this equation, df is the density of the fat compartment and dx is the density of the second (nonfat) compartment, ie, FFM or FFM minus water. The density used for the fat compartment was 0.9 g/cm$^3$ in all equations.

Body volume was calculated from skinfold thickness by dividing body weight by BD. In all equations in which skinfold thicknesses were used, BD was assessed by using Durnin and Womersley’s (3) age-specific equation, except in the two-compartment model in which the non-age-specific equation was also used.

Equations for the two-compartment model (fat and FFM)

BD (from UWW or skinfold thickness) and body water (from BIA) were calculated as follows:

\[
\% \text{ Fat} = \left(\frac{4.95}{\text{BD} - 4.5}\right) \times 100
\]

(2)

\[
\% \text{ Fat} = \left(\frac{\text{body mass} - \text{body water}}{0.732}\right) \times 100
\]

(3)

Equation 2 is used for UWW and skinfold thickness and is based on the assumption that FFM has a density of 1.1 g/cm$^3$ (4). Equation 3 is used for BIA and is based on the assumption that FFM contains 73.2% water (5).

Equations for the three-compartment model (water, fat, and protein + bone mineral)

Body volume (from UWW or skinfold thickness) and water volume (from BIA) were calculated as follows:

\[
\text{Water mass} = \text{water volume} \times \text{water density}
\]

(4)

\[
\text{BD}_{\text{water}} = \frac{\text{(body mass} - \text{water mass})}{\text{(body volume} - \text{water volume})}
\]

(5)
Equations for the three-compartment model (bone mineral, fat, and protein + water)

Body volume (from UWW) and bone mineral mass (from DXA) were calculated as follows:

\[
\text{Bone mineral volume} = \frac{\text{bone mineral mass}}{\text{bone mineral density}} \quad (8)
\]

\[
\text{BD}_{\text{bone}} = \frac{(\text{body mass} - \text{bone mineral mass})}{(\text{body volume} - \text{bone mineral volume})} \quad (9)
\]

\[
\text{Fraction fat} = \frac{5.962}{\text{BD}_{\text{bone}}} - 5.625 \quad (10)
\]

Fat (kg)

\[
= \text{fraction fat} \times (\text{body mass} - \text{bone mineral mass}) \quad (11)
\]

Bone mineral volume (Eq 8) was calculated by using a density of 2.982 g/cm³ (17). BD_{bone} (Eq 9) stands for the density of the body minus bone mineral (ie, fat and protein + water). The constants in equation 10 are calculated according to equation 1, based on the assumption that FFM minus bone mineral (ie, protein + water) has a density of 1.06 g/cm³ (17, 18).

Equations for the four-compartment model (water, bone mineral, fat, and protein)

Body volume (from UWW), water volume (from BIA), and bone mineral mass (from DXA) were calculated as follows:

\[
\text{BD}_{\text{water, bone}} = \frac{(\text{body mass} - \text{water mass} - \text{bone mineral mass})}{(\text{body volume} - \text{water volume} - \text{bone mineral volume})} \quad (12)
\]

\[
\text{Fraction fat} = \frac{2.553}{\text{BD}_{\text{water, bone}}} - 1.837 \quad (13)
\]

Fat (kg)

\[
= \text{fraction fat} \times (\text{body mass} - \text{water mass} - \text{bone mineral mass}) \quad (14)
\]

BD_{water, bone} (Eq 12) stands for the density of the body minus bone mineral and water (ie, fat and protein). The constants in equation 13 are calculated according to equation 1, based on the assumption that FFM minus bone and water (ie, protein plus nonosseous mineral plus glycogen) has a density of 1.39 g/cm³ (17, 19). Percentage fat in the body was calculated as follows:

\[
\% \text{ Fat} = \left(\frac{\text{fat mass}}{\text{body mass}}\right) \times 100 \quad (15)
\]

Figure 1 illustrates the body compartments that can be evaluated by combinations of the various methods with the two-, three-, and four-compartment models.

\[\text{VO}_{2\max}\] was determined by an incremental test until exhaustion with an ergospirometer (model 2900Z; Sensormedics Corporation, Anaheim, CA) and an electronic cycle ergometer (model 829E; Monark Bodyguard, Vansbro, Sweden).

Statistics

The software program STATISTICA (version 4.5; StatSoft, Tulsa, OK) was used for all statistical calculations. A t test for dependent samples was used to compare methods, and simple-regression analysis to calculate the correlations.

RESULTS

Body fat percentages calculated with two- and three-compartment models are shown in Table 2. The calculated FFM densities from three- and four-compartment models are shown in Table 3. The densities of FFM_{bone} and FFM_{water} were also calculated from the four-compartment model and compared with previously reported values (2, 4, 17, 18).

Calculated weights of water, bone mineral, fat, and protein are shown as percentages of FFM and total weight, respectively, in Table 4. Percentage body fat obtained by using field-adapted methods are compared with those based on more complex methods in Table 5. The correlation of body weight and BMI with percentage body fat is also included in the table. The best correlation with the four-compartment model was obtained by using the three-compartment model with BIA and...
skinfold-thickness measurements \((r = 0.95, P < 0.001); \text{ Figure } 2\).

Age correlated significantly with FFM density, percentage of water in FFM, and percentage of protein in FFM (Figure 3). No significant correlation was obtained between age and percentage body fat (Figure 3). There was a negative correlation between age and the percentage of protein in the body \((r = -0.74, P < 0.001)\). The athletes had a low percentage of water and a high percentage of protein in FFM.

The difference between the amount of fat calculated with the UWW two-compartment model equation and our four-compartment model equation correlated with the percentage of water in FFM \((r = 0.98, P < 0.001)\) and bone mineral in FFM \((r = -0.52, P = 0.01)\). There was also a significant correlation between the difference in the amount of fat calculated with the BIA two-compartment model and our four-compartment model and the percentage of water in FFM \((r = 0.999, P < 0.001)\).

To further evaluate whether two-compartment models (by using UWW or BIA) could significantly over- or underestimate the amount of fat in a group of subjects who differ from the assumed constant fraction of water and bone mineral in FFM, we divided the 22 subjects based on water and bone mineral percentage as follows. The 11 subjects with the highest percentage (ie, above the median value) were compared with the 11 subjects with the lowest percentage (ie, below the median value) with respect to water and bone mineral in FFM, respectively. Compared with our four-compartment model, percentage body fat was significantly overestimated by UWW for subjects with a high percentage of water in FFM or a low percentage of bone mineral in

<table>
<thead>
<tr>
<th>TABLE 4</th>
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<tbody>
<tr>
<td>Calculated percentage of components in fat-free mass (FFM) and body weight by using the four-compartment model in healthy male volunteers (^1)</td>
</tr>
<tr>
<td>%</td>
</tr>
<tr>
<td>FFM</td>
</tr>
<tr>
<td>Body water</td>
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<tr>
<td>Protein</td>
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<tr>
<td>Bone mineral</td>
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<tr>
<td>Body weight</td>
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<tr>
<td>Water</td>
</tr>
<tr>
<td>Protein</td>
</tr>
<tr>
<td>Bone mineral</td>
</tr>
<tr>
<td>Fat</td>
</tr>
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\(^1\) \(\bar{x} \pm SD\); range in parentheses. \(n = 22\). UWW, underwater weighing; BIA, bioimpedance analysis; DXA, dual-energy X-ray absorptiometry.

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<th>TABLE 5</th>
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<td>Correlation coefficients ((r)) between values of percentage body fat obtained by field-adapted methods compared with more complex methods in healthy male volunteers (^1)</td>
</tr>
<tr>
<td>2c</td>
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</tr>
<tr>
<td>2c</td>
</tr>
<tr>
<td>UWW</td>
</tr>
<tr>
<td>BMI</td>
</tr>
<tr>
<td>Skinfold thickness</td>
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<tr>
<td>Age specific</td>
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<tr>
<td>BIA</td>
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<tr>
<td>3c</td>
</tr>
<tr>
<td>Skinfold thickness</td>
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\(^1\) \(n = 22\). 2c, Two-compartment model; 3c, three-compartment model; 4c, four-compartment model. Skinfold thickness was calculated by using the age-specific and non-age-specific equation of Durnin and Womersley (3). Only the age-specific equation was used in the 3c and 4c models. All correlations were significant, \(P < 0.01\). UWW, underwater weighing; BIA, bioimpedance analysis; DXA, dual-energy X-ray absorptiometry.
FFM, and by BIA for those with a low percentage of water in FFM. Percentage body fat was also significantly underestimated by UWW in subjects with a low percentage of water in FFM, and by BIA in those with a high percentage of water in FFM (Table 6). When the subjects were divided according to age, with the 11 youngest in one group and the 11 oldest in another, BIA significantly underestimated the percentage fat ($P < 0.05$), and UWW significantly overestimated the percentage fat ($P < 0.05$) compared with the four-compartment model for the older group.

The variation in individual body fat percentages obtained by using the various models is illustrated in Figure 4. Thus, for the oldest subject (age 59 y), who had a high water content (78.5%) and a low bone mineral content (4.9%) in FFM, UWW overestimated (22.8%) and BIA underestimated (9.6%) the fat percentage in relation to the values obtained by the four-compartment model (15.7%). The opposite was seen in a 30-y-old man, one of the athletes, who had a low water content (68.9%) and a high bone mineral content (6.5%) in FFM. For a 39-y-old man, the body fat estimates obtained by different models were very similar;

![Figure 2](https://academic.oup.com/ajcn/article-abstract/63/6/856/4650688)

**FIGURE 2.** Correlation between percentage fat assessed with a three-compartment model (3c) based on field-adapted methods [skinfold-thickness measurement + bioimpedance analysis (BIA)] and a four-compartment model (4c) (underwater weighing + BIA + dual-energy X-ray absorptiometry) ($y = 2.489 + 0.833x, r = 0.95, P < 0.001$).

![Figure 3](https://academic.oup.com/ajcn/article-abstract/63/6/856/4650688)

**FIGURE 3.** Correlations between age and fat-free mass (FFM) density ($r = -0.76, P < 0.001$), percentage water in FFM ($r = 0.75, P < 0.001$), percentage protein in FFM ($r = -0.73, P < 0.001$), and percentage fat ($r = 0.38, P = 0.084$) calculated by using the four-compartment model in athletes and nonathletes.
TABLE 6
Comparison between percentage fat calculated by the two-compartment (2c) and four-compartment (4c) models for those with percentages of bone mineral and percentages of water in fat-free mass (FFM) below or above the median values.

<table>
<thead>
<tr>
<th>Bone mineral/FFM</th>
<th>2c UWW</th>
<th>2c BIA</th>
<th>4c UWW + BIA</th>
<th>4c BIA + DXA</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1 ± 0.2 (n = 11)</td>
<td>22.4 ± 7.0</td>
<td>—</td>
<td>20.1 ± 6.4</td>
<td>—</td>
</tr>
<tr>
<td>5.7 ± 0.3 (n = 11)</td>
<td>13.6 ± 5.0</td>
<td>—</td>
<td>15.0 ± 3.5</td>
<td>—</td>
</tr>
<tr>
<td>Water/FFM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>71.5 ± 1.1 (n = 11)</td>
<td>13.3 ± 6.5</td>
<td>17.5 ± 5.2</td>
<td>15.6 ± 5.6</td>
<td>—</td>
</tr>
<tr>
<td>75.9 ± 1.5 (n = 11)</td>
<td>22.8 ± 4.8</td>
<td>16.6 ± 6.3</td>
<td>19.6 ± 5.3</td>
<td>—</td>
</tr>
</tbody>
</table>

1 X ± SD.
2,3 Significantly different from 4c: 2 P < 0.05, 3 P < 0.001.

this person had water and bone mineral contents in FFM similar to assumed values.

DISCUSSION

All calculations of body composition are based on indirect methods and there is no absolute gold standard. In fact, the generally accepted assumptions that fat has a density of 0.9 g/cm³ and FFM has a density of 1.1 g/cm³ are based on the analysis of three males (17). However, Brozek et al (17) in the same study reported the density of the fat-free, bone mineral-free body to be 1.06 g/cm³ and the density of the fat-free, bone mineral-free, and water-free body (ie, protein + nonosseous mineral + glycogen) to be 1.39 g/cm³. Allen et al found the same densities as did Brozek et al (1.06 and 1.39, respectively) in an analysis of 30 healthy subjects (18) and 736 tissue specimens from humans and animals (19). Siri (4) estimated that the density for the fat-free, water-free body is 1.56 g/cm³. To reduce the number of assumptions we therefore used the above-mentioned values of 1.06, 1.56, and 1.39 g/cm³ in our calculations.

The average FFM density, protein + water density, and protein + mineral density calculated by our three- and four-compartment-model equations (Table 3) agree well with the densities reported from previous studies (2, 4, 17, 18). The average percentage of water in FFM (Table 4) obtained in the present study agrees with previously reported values (5). The amount of fat calculated from our four-compartment equation agrees well with that obtained with the four-compartment equation suggested by Friedl et al (6). Although multicompartiment models to calculate body composition have been suggested by other authors (6, 7, 20), they used UWW to measure BD and DXA to measure bone mineral mass. The advantage of our model is that its methods are also applicable in field studies.

Durnin and Womersley (3) showed a good correlation between skinfold-thickness measurements and BD measured by UWW. They then developed age- and sex-specific equations to calculate BD from skinfold-thickness measurements when they found that older age groups and women had a lower BD with the same skinfold thickness. The reason, according to the authors, was considered to be that those groups had a greater proportion of total body fat situated internally than subcutaneously but they also discussed variations in skinfold compressibility, density of the skeleton with aging, and as well as changes in FFM density with obesity.

The major disadvantage of the two-compartment model is that it assumes fixed proportions for protein, water, and bone mineral in FFM. Any changes in these assumed proportions will affect the calculations of the percentage fat, which we also showed. In our study the differences in water content explained 96% whereas
bone mineral content only explained 28% of the variation in FFM density. Significant correlations were also obtained between the percentage of water and protein in FFM, as well as of FFM density compared with age. This led to an overestimation of the percentage body fat with UWW and an underestimation with BIA in a two-compartment model in older subjects.

The finding that the water content has the greatest effect on calculations of percentage fat in the body agrees with the finding by Baumgartner et al (14) in a study of 98 men and women between the ages of 65 and 94 y in which tritium dilution was used to estimate total body water. However, Baumgartner et al did not show any correlation between FFM density and percentage of water in FFM with age. However, they stated that variation in FFM composition necessitated the use of multicompartiment models and that a combination of BIA and anthropometry holds considerable promise. Schoeller (21), in his review of changes in total body water with age, concluded that it is likely that the hydration of FFM increases throughout normal aging. He assumed that the increase would be small and have little effect on the estimation of body composition in healthy subjects. However, our findings indicate that this increase is significant and has an effect on the calculations.

We used DXA equipment to estimate bone mineral content in more complex multicompartiment models; however, DXA is not a field-adapted method for estimating bone mineral content. Interestingly, Johansson et al (22) showed that bone mineral content measured by DXA correlated significantly \((r = 0.72, P < 0.001)\) with the serum concentration of insulin-like growth factor binding protein 3 (IGFBP-3) [bone mineral mass (kg) = 2.0509 + 0.00035 \(\times\) IGFBP-3 (\(\mu\)g/L)]. IGFBP-3 thus may offer a simple field-adapted method to estimate bone mineral density and bone mineral mass. However, bone mineral content estimated by DXA and IGFBP-3 did not have any major influence on the calculations of body composition in our study.

This study indicates that in a moderate number of subjects, of various ages and body composition, differences in FFM composition will influence calculations of body composition based on two-compartment models. It further illustrates that the calculations are improved when three- and four-compartment models are used because they are based on a reduced number of assumptions and take into account differences in bone mineral content and water content in the body. We also showed that it is possible to improve body-composition calculations by using field-adapted methods, ie, skinfold-thickness measurement and BIA in combination, and using multicompartiment instead of two-compartment equations.

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