Comparisons of two-, three-, and four-compartment models of body composition analysis in men and women

R. T. WITHERS,[1] J. LaFORGIA,[1] R. K. PILLANS,[1] N. J. SHIPP,[1]
B. E. CHATTERTON,[2] C. G. SCHULTZ,[2] AND F. LEANEY[3]
[1]*Exercise Physiology Laboratory, School of Education, The Flinders University of South Australia,
Adelaide, South Australia 5001;* [2]*Department of Nuclear Medicine, Royal Adelaide Hospital,
Adelaide, South Australia 5000; and* [3]*Commonwealth Scientific and Industrial Research
Organization, Division of Water Resources, Glen Osmond, South Australia 5064, Australia*

Withers, R. T., J. LaForgia, R. K. Pillans, N. J. Shipp, B. E. Chatterton, C. G. Schultz, and F. Leaney. Comparisons of two-, three-, and four-compartment models of body composition analysis in men and women. *J. Appl. Physiol.* 85(1): 238–245, 1998.—This study compared the traditional two-compartment (fat mass or FM; fat free mass or FFM) hydrodensitometric method of body composition measurement, which is based on body density, with three (FM, total body water or TBW, fat free dry mass)- and four (FM, TBW, bone mineral mass or BMM, residual)-compartment models in highly trained men ($n = 12$), sedentary men ($n = 12$), highly trained women ($n = 12$), and sedentary women ($n = 12$). The means and variances for the relative body fat (%BF) differences between the two- and three-compartment models [2.2 ± 1.6 (SD) % BF; $n = 48$] were significantly greater ($P \leq 0.02$) than those between the three- and four-compartment models (0.2 ± 0.3% BF; $n = 48$) for all four groups. The three-compartment model is more valid than the two-compartment hydrodensitometric model because it controls for biological variability in TBW, but additional control for interindividual variability in BMM via the four-compartment model achieves little extra accuracy. The combined group ($n = 48$) exhibited greater ($P < 0.001$) FFM densities (1.1075 ± 0.0049 g/cm^3) than the hydrodensitometric assumption of 1.1000 g/cm^3, which is based on analyses of three male cadavers aged 25, 35, and 46 yr. This was primarily because their FFM hydration (72.4 ± 1.1%; $n = 48$) was lower ($P \leq 0.001$) than the hydrodensitometric assumption of 73.72%.

hydrodensitometry; total body water; dual-energy X-ray absorptiometry; sedentary subjects; endurance athletes

---

THE MEASUREMENT OF BODY composition is of interest to medical personnel, nutritionists, and sports scientists. Two of the traditional methods involve the determination of body density (BD) by hydrodensitometry and total body water (TBW) via isotopic dilution. These methods are based on the premise that the body can be separated into two chemically distinct compartments, namely, the fat mass (FM) and fat-free mass (FFM). The FM, which is defined as chemically extractable fat, is assumed to have a density of 0.9007 g/cm^3 (5) and be anhydrous, whereas the FFM is regarded as having a density of 1.1000 g/cm^3 (3) and a water content of 73.72% (3). Most of the error associated with these two-compartment models lies not in the technical accuracy of the measurements but in the validity of the previously outlined assumptions, which are based on analyses of just three male cadavers (3). Siri (21) was aware of this limitation, and he accordingly proposed a three-compartment model [FM, TBW, fat-free dry mass

(FFDM)], which is based on measurements of both BD and TBW while a constant mineral-to-protein ratio of 0.35 is assumed. The three-compartment model, therefore, controls for interindividual variation in FFM hydration. The advent of recent body composition technology such as dual-energy X-ray absorptiometry (DEXA), which yields values for FM and bone mineral, has now facilitated the measurement in vivo of two (TBW, bone mineral) of the four (TBW, bone mineral, nonbone mineral, protein) FFM components. A four-compartment model (FM, water, bone mineral, residual) of body composition analysis has therefore emerged (2, 7, 8, 11, 27). This model is theoretically more valid than the three-compartment model because it controls for biological variability in both bone mineral and TBW.

Although two-, three-, and four-compartment body composition models have been compared previously (2, 7, 8, 11), the effect of physical training on differences among these models and the assumptions inherent in the two-compartment models have only been examined when male weight trainers are compared with physically active but non-weight-training controls (17). Also, the effect of aerobically orientated physical training on the relationship between DEXA estimates of relative body fat (%BF) and those via the four-compartment criterion model has not been fully explored. A final consideration is that the four-compartment model subtracts the masses and volumes of TBW and bone mineral from the mass and volume of the whole body that are measured during hydrodensitometry. This enables the remainder to be partitioned into fat and residual masses. However, the propagation of measurement error from the determinations of BD, TBW, and bone mineral may offset the increase in validity resultant from controlling for biological variability in the latter two variables. The aims of this study were therefore to *1*) examine differences between two-, three-, and four-compartment models of body composition analysis in highly trained and sedentary men and women; *2*) determine the effect of interindividual differences in TBW and bone mineral in the four preceding groups of subjects on the traditional two-compartment models that are based on hydrodensitometry and isotopic dilution; *3*) assess the validity in highly trained and sedentary men and women of FM determined via DEXA against the criterion of FM measured by using the four-compartment model; and *4*) calculate the extent to which measurement errors are propagated when body composition is estimated via the four-compartment model.

http://www.jap.org

METHODS

Subjects. Forty-eight young (18–36 yr) nonobese (Quetelet's index <29 kg/m²) Caucasian subjects volunteered for this study. They all reported weight stability within ±2.0 kg and good health for the preceding 2 yr. None was taking medications other than a contraceptive pill, and all the women were eumenorrheic. There were 12 subjects in each of the following 4 groups: sedentary men and women and highly trained men and women. The sedentary subjects reported abstinence from physical training for the previous 2 yr, whereas those who were highly trained had prepared for middle-distance running events or triathlons at a state or national level for at least the preceding 2 yr. This study was approved by the Flinders Medical Centre's Committee on Clinical Investigation, and informed consent was obtained in accordance with the established protocol for human subjects.

Protocol. All experiments were conducted when the subjects were 12 h postprandial, euhydrated, and had not exercised for 24 h. The effect of fluid retention by the women was minimized by not testing them either during the 7 days preceding menstruation or during menstruation. The BD and TBW tests were administered on the same morning to minimize within-subject biological variability. The DEXA measurements for 34 of the subjects were also conducted on the same morning as were the two other tests; however, it was not possible to schedule the DEXA test for 13 subjects until the following morning, and a combination of scheduling and menstrual cycle timing prevented 1 woman from being tested until 13 days later.

Hydrodensitometry. BD was measured by underwater weighing at residual volume. Corrections were made for water density and the ventilated residual volume that was determined by helium dilution with the subjects immersed in water to neck level and in the same posture as during the underwater mass determinations. Our methodology has been fully described elsewhere (28). The %BF was then estimated by the equation of Brozek et al. (3), and the FFM was obtained by subtraction

$$\%BF = (497.1/BD) - 451.9$$

Our latest precision data for two trials in six subjects yielded an intraclass correlation coefficient of 0.998 and a technical error of measurement (TEM) (4) of 0.3% BF.

TBW. This was measured by ^2H_2O dilution. A saliva sample was collected from subjects on their arrival at the laboratory to determine the background ^2H_2O concentration. A 40-mg ^2H_2O/kg dose, which was adjusted to ~100 ml with distilled water, was then drunk through a straw. The container was furthermore rinsed three times with ~30 ml of distilled water, which was also ingested by the subject. An equilibrium saliva sample was taken 3.5 h later. Precautions were taken to minimize isotopic fractionation during the collection of all saliva samples. The subjects were not allowed to eat, drink, or exercise during the intervening period.

The ^2H_2O concentrations in the doses and saliva samples were determined on a V. G. Micromass 602 D (Micromass, Manchester, UK) isotope ratio mass spectrometer that was calibrated against Vienna Standard Mean Ocean Water (V-SMOW) and International Atomic Energy Agency (IAEA) enriched standards 302A and 302B. The SDs for repeated trials on the background and two enriched standards were ≤1.0 and ~3.0‰, respectively. The isotope dilution space was calculated in accordance with the recommendations of Schoeller et al. (18), who advocate a 4% correction factor for the exchange of ^2H_2O with labile hydrogen of protein and other body constituents. Our latest reliability data for two TBW

trials on consecutive days ($n = 5$) produced an intraclass correlation coefficient of 0.999 and a TEM of 0.25 kg. Finally, if we assume that 72% of the FFM is water in the normally hydrated subject, then

$$FFM\ (kg) = TBW\ (kg)/72 \times 100$$

Finally, the FM was calculated by subtraction and represented as a percentage of the body mass.

DEXA. Measurements were conducted at the Royal Adelaide Hospital's Department of Nuclear Medicine with a Lunar DPX-L total body scanner (Lunar, Madison, WI) (14) by using version 1.3Z software in the medium scan mode (~20 min). The machine was calibrated daily by using the phantom supplied by the manufacturer. The men and women were measured while wearing one- and two-piece bathing suits, respectively. Duplicate trials in six subjects yielded intraclass correlation coefficients of 1.0, 0.996, and 1.0 and TEM of 16, 300, and 252 g for bone mineral content (BMC), fat, and lean tissue mass (FFM − BMC), respectively.

The BMC reported in the DEXA printout represents ashed bone (23). One gram of bone mineral yields 0.9582 g of ash (15) because labile components such as bound H_2O and CO_2 are lost during heating at a temperature of over 500°C (3, 15). The BMC or bone ash was therefore converted to bone mineral mass (BMM) by multiplying it by 1.0436 (11, 12).

Derivation of two-, three-, and four-compartment models of body composition analysis. The classic two-compartment model of body composition analysis via hydrodensitometry partitions the body into the FM and FFM, which are assumed to have respective densities of 0.9007 (5) and 1.1000 g/cm³ (3) at 36°C. If the body mass is equal to unity and FM + FFM = 1.0, then substitution of the preceding information into the following formula

$$\frac{1}{BD} = \frac{FM}{FM\ density} + \frac{FFM}{FFM\ density}$$

yields

$$\%BF = \frac{497.1}{BD} - 451.9$$

The three-compartment model builds on the two-compartment model by measuring TBW in addition to BD. Hence we have three compartments, namely, the FM, TBW, and FFDM, the densities of which at 36°C are assumed to be 0.9007, 0.9937 (13), and 1.569 g/cm³ (26), respectively. Substitution of these values into the following formula

$$\frac{1}{BD} = \frac{FM}{FM\ density} + \frac{TBW}{H_2O\ density} + \frac{FFDM}{FFDM\ density}$$

produces

$$\%BF = \frac{211.5}{BD} - 78.0\left(\frac{TBW}{body\ mass}\right) - 134.8$$

The four-compartment model (FM, TBW, bone mineral, residual), which incorporates the additional variable of bone mineral, can be represented as follows

$$\frac{1}{BD} = \frac{FM}{FM\ density} + \frac{TBW}{H_2O\ density} + \frac{BMM}{BM\ density} + \frac{RM}{R\ density}$$

where BM density is bone mineral density, RM is residual mass, and R density is residual density. If it is assumed that

Table 1. *Descriptive statistics for body composition variables*

Variable	Men		Women	
	Trained ($n=12$)	Sedentary ($n=12$)	Trained ($n=12$)	Sedentary ($n=12$)
Age, yr	22.3 ± 5.1	24.7 ± 4.5	23.8 ± 5.7	22.4 ± 2.8
Height, cm	175.2 ± 5.7	178.1 ± 8.5	169.4 ± 8.9	163.1 ± 5.6
Mass, kg	67.87 ± 5.30	73.33 ± 9.70	57.51 ± 5.93	55.39 ± 3.92
Quetelet's index, kg/m^2	22.1 ± 1.0	23.2 ± 3.4	20.0 ± 1.4	20.9 ± 1.7
Body density, g/cm^3	1.0767 ± 0.0083	1.0549 ± 0.0185	1.0679 ± 0.0052	1.0391 ± 0.0128
Total body water, kg	43.23 ± 3.59	41.08 ± 4.56	34.68 ± 3.48	28.64 ± 2.08
Total body bone mineral, kg	3.40 ± 0.33	3.22 ± 0.39	2.73 ± 0.38	2.39 ± 0.26
%Body fat				
2C Body density	9.8 ± 3.6	19.5 ± 8.3	13.6 ± 2.3	26.6 ± 5.9
2C Total body water	11.5 ± 3.0	21.6 ± 8.2	16.2 ± 2.8	28.1 ± 4.2
DEXA	8.6 ± 2.8	20.5 ± 9.3	15.1 ± 3.0	28.5 ± 4.9
3C	12.0 ± 2.8	21.7 ± 8.1	16.2 ± 2.3	28.4 ± 4.7
4C	12.1 ± 2.8	21.8 ± 8.2	16.4 ± 2.4	28.9 ± 4.7
FFM, kg				
2C Body density	61.17 ± 4.67	58.59 ± 6.39	49.67 ± 5.04	40.58 ± 3.31
2C Total body water	60.04 ± 4.98	57.06 ± 6.34	48.17 ± 4.83	39.78 ± 2.89
DEXA	62.05 ± 4.43	57.88 ± 6.77	48.91 ± 4.84	39.25 ± 2.58
3C	59.73 ± 4.66	56.96 ± 6.22	48.18 ± 4.80	39.60 ± 2.93
4C	59.65 ± 4.60	56.92 ± 6.24	48.04 ± 4.75	39.32 ± 2.92

Values are means $\pm$ SD. *n*, No. of subjects; 2C, 2-compartment model; DEXA, dual-energy X-ray absorptiometry; 3C, 3-compartment model; 4C, 4-compartment model; FFM, fat-free mass.

the densities of BMM and RM are 2.982 (15) and 1.404 g/cm^3 (1), respectively, then the equation for the four-compartment model is

$$\%BF = \frac{251.3}{BD} - 73.9 \left(\frac{TBW}{body\ mass}\right) + 94.7 \left(\frac{BMM}{body\ mass}\right) - 179.0$$

Statistical analyses. Relative body fat differences between the four groups (trained and sedentary men, trained and sedentary women) and three primary treatments (BD, TBW, DEXA) were examined by using a 4×3 factorial design ANOVA with repeated measures across treatments. The corrected degrees of freedom were used if the assumption of sphericity was violated. Tukey's post hoc tests were conducted in the event of statistically significant F-ratios ($P \le 0.05$) for the two main effects and their interaction. Interclass correlation coefficients were also computed among the %BF values for the three primary treatments.

Differences among the four groups for FFM density, FFM hydration, and %BMM in the FFM, which were calculated by using the four-compartment criterion model, were examined by using a 2 (activity level: sedentary and trained) $\times$ 2 (gender: men and women) factorial design ANOVA. In the absence of statistical significance ($P \le 0.05$), data were pooled for comparison with the two-compartment hydrodensitometric constants via single sample t-tests ($P \le 0.05$).

The effect of interindividual variability in FFM hydration was determined by plotting the %BF difference between the three- and two-compartment models against the %BF from the four-compartment criterion model. The %TBW in the FFM (4-compartment model) was also regressed on the FFM density (4-compartment model). The effect of biological variability of the %BMM in the FFM was elicited by plotting the %BF difference between the four- and three-compartment models against the %BF from the four-compartment criterion model. The %BMM in the FFM (4-compartment model) was furthermore regressed on the FFM density (4-compartment model). Finally, the means and variances for the %BF differences between the two- and three-compartment models were compared with those between the three- and four-compartment models by using dependent t-tests ($P \le 0.05$).

Differences between DEXA and the four-compartment criterion method for %BF were examined by using a 2 (methods) $\times$ 4 (groups) factorial design ANOVA ($P \le 0.05$) with repeated measures across methods.

The SE of estimate (SEE) and TEM from the reliability data for the measurement of BD, TBW, and BMM were used to calculate propagated errors for %BF (22).

RESULTS

The %BF data are contained in Table 1 and graphed in Fig. 1. The main effects for groups and treatments were both significant ($P < 0.001$). All pairwise comparisons among groups (trained men: 10.0% BF; sedentary men: 20.5% BF; trained women: 15.0% BF; sedentary women: 27.7% BF) were significant at $P \le 0.05$, except those between the trained men and trained women and between the trained women and untrained men, both of which were significant beyond the 0.10 level. All pair-
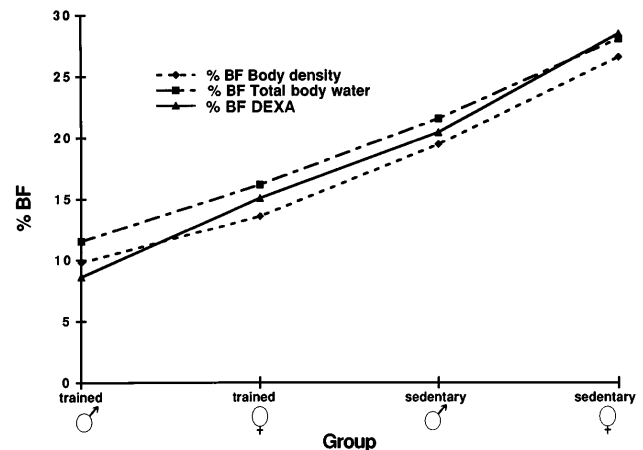

Fig. 1. Profile graph showing interaction between primary methods and groups for %body fat (BF). DEXA, dual-energy X-ray absorptiometry. ♀, women; ♂, men.

wise comparisons among methods (BD: 17.4% BF; TBW: 19.4% BF; DEXA: 18.2% BF) were significant at $P \leq 0.05$. Simple main effects analysis demonstrated that the interaction ($P < 0.025$) depicted in Fig. 1 was due to the DEXA %BF, which was significantly less ($P < 0.05$) than that via TBW for the trained men. Although interclass correlations among the three primary methods ranged from 0.946 to 0.983 (all $P < 0.001$), deviations from the lines of identity in Fig. 2 emphasize that some of the intraindividual differences are large.

The FFM density, FFM hydration, and BMM/FFM (%) data are presented in Table 2. The main effects (activity level: $P = 0.88$; gender: $P = 0.38$) and interaction ($P = 0.63$) for the 2×2 factorial design ANOVA on FFM density were not statistically significant. The data were therefore pooled, and the overall mean of 1.1075 g/cm³ was significantly greater ($P < 0.001$) than the two-compartment hydrodensitometric assumption of 1.1000 g/cm³. Individual FFM densities ranged from 1.0974 to 1.1177 g/cm³, and they resulted in hydrodensitometric over- and underestimates of 0.9 and 5.9% BF, respectively. The main effects (activity level: $P = 0.58$; gender: $P = 0.57$) and interaction ($P = 0.18$) for FFM hydration were also not statistically significant, and the pooled mean of 72.4% was significantly less ($P < 0.001$) than the two-compartment hydrodensitometric assumption of 73.72%. However, there was a significant gender effect ($P < 0.001$) for BMM/FFM (%), but the main effect for activity level ($P = 0.12$) and the interaction ($P = 0.08$) was not statistically significant; the two activity groups for each gender were therefore pooled. The mean of 6.13% for women was significantly greater ($P < 0.001$) than the two-compartment hydrodensitometric constant of 5.63%, but the value of 5.68% for men differed little ($P = 0.50$) from its hydrodensitometric counterpart.

In Fig. 3, the deviations from the horizontal dotted line at zero on the ordinate range from -1.5 to 5.6% BF and represent the large errors that occur when there is no control for biological variability in TBW. Figure 4 also demonstrates the inverse linear relationship ($r = -0.897$; $P < 0.001$) between FFM density and its water content. However, Fig. 5 shows that, despite the significant linear relationship ($r = 0.653$; $P < 0.001$) between FFM density and BMM/FFM (Fig. 6), controlling for interindividual differences in the BMM had little effect on the %BF values of our subjects. Individual differences between the two-compartment hydrodensitometric and three-compartment models for all five groups accordingly exhibited significantly ($P \leq 0.02$) greater means and variances than those between the three- and four-compartment models.

There was a significant interaction effect ($P < 0.001$) for the 4×2 factorial design ANOVA. The %BF values in Table 1 accordingly indicate that the differences between DEXA and the four-compartment model increase as the %BF decreases. Hence, although there was no difference ($P = 0.37$) between the two methods for the untrained women, the differences for the sedentary men ($P = 0.019$), trained women ($P = 0.002$), and trained men ($P < 0.001$) were all statistically significant.
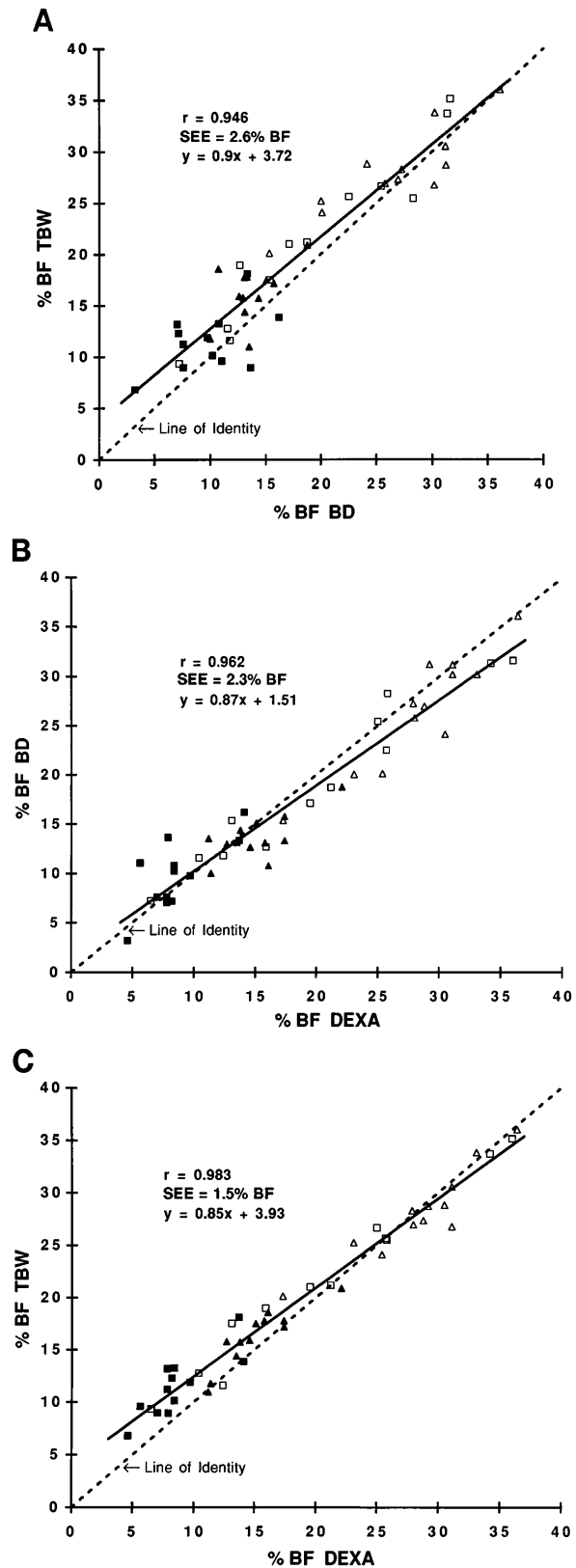

Fig. 2. Relationships between %BF via body density (BD) and total body water (TBW; A); BD and DEXA (B); and DEXA and TBW (C). □, Sedentary men; ■, trained men; △, sedentary women; ▲, trained women; solid line, regression line. TBW assumes 72% H_2O in fat-free mass (FFM). SEE, SE of estimate.

Table 2. *Descriptive statistics generated by using the 4-compartment model estimate of FFM*

Group	n	FFM Density, g/cm³	FFM Hydration, %	Bone Mineral in FFM, %
Combined	48	1.1075 ± 0.0049 (1.0974–1.1177)	72.4 ± 1.1 (70.4–75.1)	5.91 ± 0.49 (4.96–7.05)
Men				
Trained	12	1.1063 ± 0.005 (1.0974–1.1137)	72.5 ± 1.3 (71.0–75.1)	5.69 ± 0.29 (5.42–6.11)
Sedentary	12	1.1073 ± 0.0044 (1.0983–1.1142)	72.2 ± 1.0 (70.7–74.7)	5.67 ± 0.44 (4.96–6.51)
Women				
Trained	12	1.1084 ± 0.0046 (1.0989–1.1177)	72.2 ± 0.8 (70.4–73.8)	5.93 ± 0.44 (5.11–6.76)
Sedentary	12	1.1079 ± 0.0059 (1.0981–1.1152)	72.8 ± 1.3 (71.1–75.0)	6.34 ± 0.48 (5.31–7.05)

Values are means ± SD with range in parentheses. *n*, No. of subjects.

Our earlier reported reliability data for the measurement of BD, TBW, and BMM yielded SDs for propagated error of 1.0 and 0.6% BF for the SEE and TEM data, respectively.

DISCUSSION

The two-compartment BD or hydrodensitometric model assumes a FFM density of 1.1000 g/cm³, which is invariant of age, gender, genetic endowment, and training (3). This density is based on analyses of just three male cadavers, ages 25, 35, and 46 yr (3). Our data challenge this assumption because the overall FFM density mean of 1.1075 g/cm³ was significantly greater ($P < 0.001$) than 1.1000 g/cm³. This greater FFM density was primarily because the TBW, which at 0.9937 g/cm³ has by far the lowest density of any of the four FFM components, comprised less than the two-compartment hydrodensitometric assumption of 73.72% FFM. Hence, Table 1 demonstrates that conventional hydrodensitometry underestimated the group means by 2.3–2.8% BF compared with the four-compartment criterion model. Accordingly, extrapolation of the regression ($r = -0.982$; $P < 0.001$) of %BF via the four-compartment model on measured BD indicated densities of 0.8700 and 1.1064 g/cm³ for 100 and 0% BF, respectively; these results are somewhat different from the two hydrodensitometric assumptions of 0.9007 and 1.1000 g/cm³.

A further concern is the biological variability of the 48 FFM densities that were calculated via the four-compartment model. They ranged from 1.0974 to 1.1177 g/cm³, with a SD (0.0049 g/cm³) that was equivalent to ±1.6% BF; the differences between the four-compartment criterion model and conventional hydrodensitometry by using the equation of Brozek et al. (3) spanned −0.9 to 5.9% BF. Although there were no significant activity level and gender differences for FFM hydration, the BMM/FFM (%) for women was significantly greater ($P < 0.001$) than that for men. An attempt was therefore made to minimize differences between the four-compartment criterion model and the two-compartment hydrodensitometric model by developing gender-specific equations for the latter [men: %BF = (483.7/BD) − 437.0; women: %BF = (481.2/BD) − 434.3] by using the four-compartment FFM densities of 1.1068 and 1.1081 g/cm³ for the men and women, respectively. The range of the 48 differences between the four-compartment criterion model and our revised equations for the two-compartment model was less biased and decreased slightly (−3.3 to 3.1% BF). Although our TBW and BMM were not directly measured in cadavers, the small SDs for these variables may justify our generation of new gender-specific two-compartment equations. Siri (21) stated that the population SD, because of biological variability in FFM density, was 0.0084 g/cm³ (3.8% BF). He identified the variation in FFM hydration (Table 2: SD = 1.1%; range = 70.4–75.1%) as the largest source of error and accordingly proposed a three-compartment model (FM, TBW, FFDM) that is based on measurements of both BD and
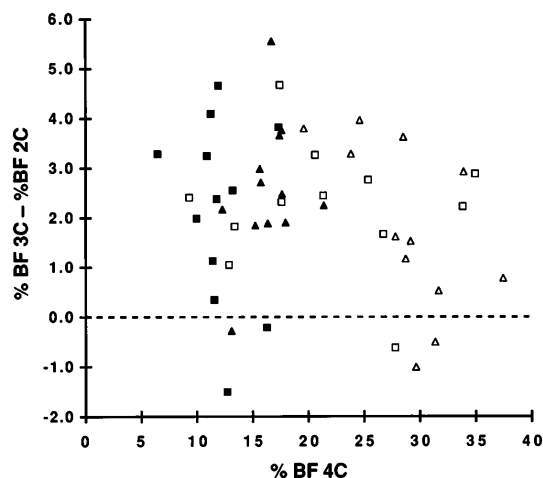

Fig. 3. %BF difference between 3- and 2-compartment (C) models graphed against %BF via 4C model. Symbols are defined as in Fig. 2.
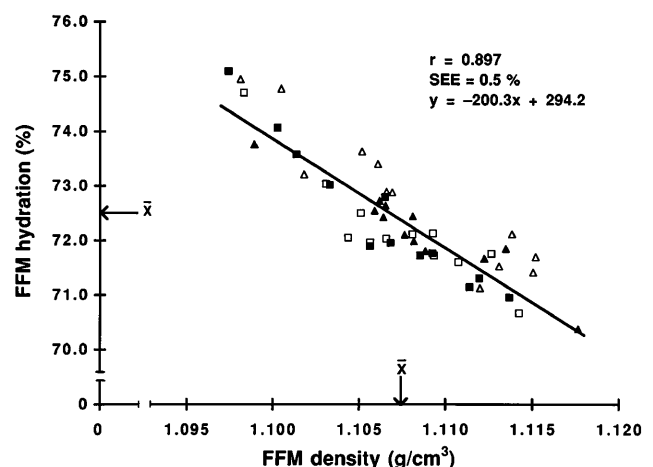

Fig. 4. Relationship between FFM hydration and density estimated via 4C model. x̄, Mean; other symbols are defined as in Fig. 2.
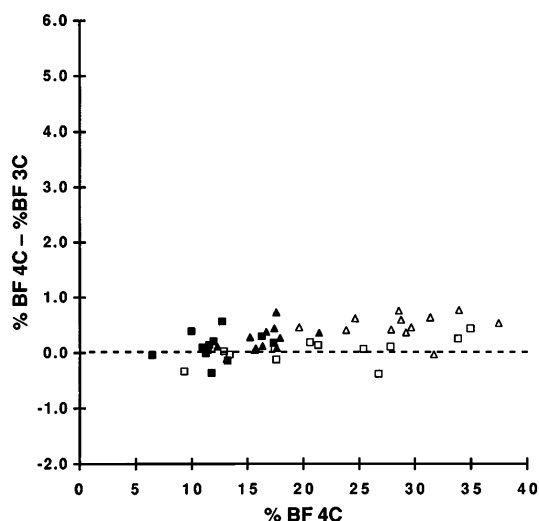
Fig. 5. %BF difference between 4C and 3C models graphed against %BF via 4C model. Symbols are defined as in Fig. 2.

TBW and assumes a constant mineral-to-protein ratio of 0.35. Siri suggested that the SD of the error would be reduced to 1.5% BF if the coefficient of variation for TBW measurement was reduced to 1% of body mass. Nevertheless, few investigators have used this model.

The literature contains the following data for the FFM hydration of five male cadavers: 67.4, 70.4 (6); 77.56 (16); 62.1 (19); and 72.62% (25). The mean of 72.0% is similar to those for our four groups, which ranged from 72.2 to 72.8% (overall mean = 72.4%). For this reason we used a constant of 72% when estimating the %BF from TBW as opposed to the two-compartment hydrodensitometric assumption of 73.72%, which is based on analyses of fewer cadavers. Hence the former constant produced %BFs (overall mean = 19.4% BF) that were closer to those for hydrodensitometry (overall mean = 17.4% BF) than the latter constant (overall mean = 21.2% BF) because it was more typical of the subjects' FFM hydration [overall mean = 72.4 ± 1.1 (SD) %]. Nevertheless, Fig. 2A indicates that five of the
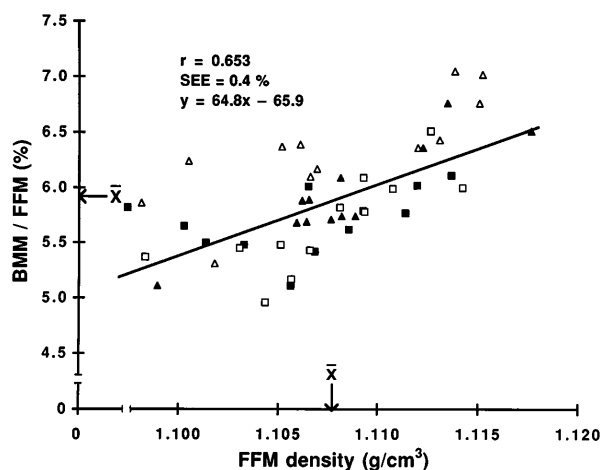

Fig. 6. Relationship between %bone mineral mass (BMM) in FFM and FFM density estimated via 4C model. Symbols are defined as in Fig. 2.

individual differences between the above-mentioned two-compartment models were ≥5.0% BF. This reflects the different assumptions of the two methods. Hence, if the FFM hydration is greater than the assumed constant, then isotopic dilution will underestimate the %BF, whereas the converse applies to hydrodensitometry because the increased water reduces FFM density.

Interestingly, the DEXA %BF for the trained men was 3.5% lower than that via the four-compartment model compared with 0.4 to 1.3% BF lower for the three other groups. The DEXA fat score is based on the inverse linear relationship between %BF and the ratio of the attenuations at 40 and 70 keV for each pixel that contains at least 3 g/cm^2 of soft tissue but <0.05 g/cm^2 of bone (14). A small change in the slope of this line would therefore have a greater impact on subjects with high or low %BF than on those whose levels are nearer the middle of the distribution. Perhaps another contributory factor for our lean subjects is that essential fat, which has a greater density (15) and hence higher attenuation than triglyceride, comprises a much larger percentage of the FM than in more overweight subjects. Nevertheless, DEXA has advantages in that it is atraumatic, expedient, does not rely on the assumptions of the two-compartment models, and yields data on BMC.

The deviations from the horizontal dotted line at zero on the ordinate in Fig. 3 represent the large errors (range from −1.5 to 5.6% BF) that occur when the experimenter does not control for interindividual variability in TBW but instead makes the two-compartment BD model assumption that 73.72% of the FFM is water. The variability of these errors was due to the wide range of FFM hydration that Fig. 4 indicates was from 70.4 to 75.1%. Forty-two of the forty-eight subjects had FFM hydrations that were below the two-compartment BD assumption of 73.72%. Figure 4 clearly demonstrates that the smaller the FFM hydration, the larger the tendency for the two-compartment BD model to yield lower %BF than the three-compartment model. This is because, all other things being equal, the FFM density increases as the degree of hydration decreases below the two-compartment BD assumed constant of 73.72%, because water has by far the lowest density (0.9937 g/cm^3 at 36°C) (13) of any of the four FFM components. Hence, as the FFM density increases above the assumed constant of 1.1000 g/cm^3, the greater the extent to which the two-compartment BD model will underestimate the %BF. Notwithstanding the lack of independence between the variables on both axes of Figs. 3 and 4, these data indicate that the three-compartment model is more valid than the two-compartment model because it controls for biological variability of the large and acutely variable TBW compartment.

A limitation of expressing the TBW as a percentage of the FFM derived via the four-compartment model is that the former variable is also embedded within the denominator (10). The FFM estimate is therefore not independent of the measured TBW. Although this approach is preferable to one using a less accurate estimate of the FFM, it does reduce the variability

within the sample for FFM hydration (10). This applies to a lesser extent to BMM because it comprises a much smaller percentage of the FFM.

Figure 6 shows that the greater the percentage of BMM in the FFM, the greater the density of the four-compartment FFM. This is logical because BMM has the relatively high density of 2.982 g/cm^3. However, when the %BF differences between the four- and three-compartment models are graphed against the %BF from the four-compartment criterion model, the individual deviations from zero on the ordinate are very small (Fig. 5: range from -0.4 to 0.8% BF). These differences from zero represent the error remaining after control for interindividual variability in TBW but not in BMM. Clearly, little extra accuracy is gained by measuring the latter. These contrasting effects for control of biological variability in TBW and BMM can be attributed to differences between our data and the classic cadaver constants (3) on which the two- and three-compartment models are based. The mean FFM hydration of 72.4% for our combined group was lower than that of 73.72% for the classic cadaver analyses and ranged from 70.4 to 75.1% with a SD of 1.1%. Conversely, the BMM/FFM (%) for our combined group of 5.91% was slightly greater than that of 5.63% for the classical cadaver analyses but only ranged from 4.96 to 7.05% with a lower SD of 0.49%. If the FFM hydration is less than that for the classical cadaver analyses (3), then the combined percentage of the remaining three FFM components must increase. However, the small differences between the four- and three-compartment models displayed in Fig. 5 support the contention that the three-compartment assumption of a total mineral-to-protein ratio of 0.354 (3), with an overall density of 1.569 g/cm^3 (26), remained essentially unchanged.

The residual mass in our four-compartment model comprises mainly protein, which is 19.41% FFM in the classic cadaver analyses (3), plus some nonbone mineral (1.24% FFM) (3) and glycogen ($<$1% body mass). Several investigators have estimated nonbone mineral from its ratio to BMM in the three cadaver analyses to yield a value for total body mineral (2, 8, 11, 20). This has enabled them to partition the remaining body mass into fat and protein. Such a procedure ignores the small glycogen component, which was presumably not considered in the cadaver analyses because of its relatively small amount and rapid postmortem autolysis, and uses a density of 1.34 g/cm^3 for hydrated protein (9). However, the glycogen stores have a density (glucose = 1.562 g/cm^3) (24) that is similar to 1.565 g/cm^3 for the much larger FFDM compartment (protein and mineral) identified by Siri (21) in his three-compartment model. Nevertheless, it was decided instead to use the density of 1.404 g/cm^3 at 37°C reported by Allen et al. (1) for the dry fat- and bone mineral-free mass (i.e., protein, nonbone mineral, and glycogen), which is based on measurements of 364 human and animal tissue samples.

Notwithstanding the methods used to calculate FFM density after removal of the TBW and BMM, the four-compartment equations of Baumgartner et al. (2),

Friedl et al. (7), Fuller et al. (8), Heymsfield et al. (11), and Siconolfi et al. (20) all produced means that differed by no more than 0.7% BF from the overall mean for our 48 subjects. This is not surprising because all investigators are relying on the same cadaver analyses and density values.

The preceding discussion demonstrates how the four-compartment model is an improvement over the two-compartment hydrodensitometric model because it controls for biological variability in TBW and BMM. However, although greater validity should be associated with the measurement of more compartments, there is some concern that this extra control may be offset somewhat by the propagation of measurement error associated with the determinations of BD, TBW, and BMM. A worst-case scenario for this propagation of error can be calculated by assuming that the squared errors or error variances (SEE^2 or TEM^2) are independent and additive (22)

SD of total error

$$= \left(\begin{array}{ccc} \text{SEE}^2 \text{ or TEM}^2 & \text{SEE}^2 \text{ or TEM}^2 & \text{SEE}^2 \text{ or TEM}^2 \\ \text{for \%BF} & + \text{ for \%BF} & + \text{ for effect of} \\ \text{via BD} & \text{via TBW} & \text{BMM on \%BF} \end{array} \right)^{0.5}$$

Test-retest reliability data collected in our laboratory on five to six subjects, who were representative of our study group, yielded SDs for total or propagated error of 1.0 and 0.6% BF for SEE and TEM, respectively. The former is slightly less than the $\sim$1.6% BF proposed by Heymsfield et al. (11). The SEE includes both between- and within-subject error variance, whereas the TEM, which is the SE of a single measurement (4), considers only the latter. The SD for the total error via the TEM is therefore less than that from the SEE. Nevertheless, both errors are much less than 3.8% BF (21) because of interindividual variability in FFM density when body composition is estimated via the two-compartment hydrodensitometric model. The theoretical extra accuracy of the four-compartment model would therefore not be offset by the propagation of measurement error. Our propagated errors may well represent the technical limits of precision for the measurement of %BF via the indirect four-compartment model. However, although great confidence can be placed in the numbers used for the densities of water and chemically extracted fat from adipose tissue, there will be some unaccounted error because of four-compartment model assumptions, such as those for the densities of bone and residual (dry, fat-, and bone mineral-free) masses and the use of a 4% correction factor for nonaqueous hydrogen exchange when the TBW is estimated via ^{2}H$_2$O dilution.

In summary, our limited data on 48 subjects suggest that conventional hydrodensitometry underestimates the %BF because the four-compartment body composition model indicates that the FFM density is $>$1.1000 g/cm^3. Furthermore, DEXA yields lower %BF values than the four-compartment model in very lean subjects. Finally, differences between the two-compartment hydrodensitometric and four-compartment models were significantly associated with biological variability in

FFM hydration. Differences between these two models were not significantly associated with interindividual variability in BMM, which provided only a marginal increase in accuracy.

REFERENCES

1. **Allen, T. H., H. J. Krzywicki, and J. E. Roberts.** Density, fat, water, and solids in freshly isolated tissues. *J. Appl. Physiol.* 14: 1005–1008, 1959.

2. **Baumgartner, R. N., S. B. Heymsfield, S. L. Lichtman, J. Wang, and R. N. Pierson, Jr.** Body composition in elderly people: effect of criterion estimates on predictive equations. *Am. J. Clin. Nutr.* 53: 1345–1353, 1991.

3. **Brozek, J., F. Grande, J. T. Anderson, and A. Keys.** Densitometric analysis of body composition: revision of some quantitative assumptions. *Ann. NY Acad. Sci.* 110: 113–140, 1963.

4. **Dahlberg, G.** *Statistical Methods for Medical and Biological Students.* London: Allen & Unwin, 1940, p. 122–132.

5. **Fidanza, F., A. Keys, and J. T. Anderson.** Density of body fat in man and other mammals. *J. Appl. Physiol.* 6: 252–256, 1953.

6. **Forbes, R. M., and A. M. Lewis.** Total sodium, potassium and chloride in adult man. *J. Clin. Invest.* 35: 596–600, 1956.

7. **Friedl, K. E., J. P. Deluca, L. J. Marchitelli, and J. A. Vogel.** Reliability of body fat estimations from a four-compartment model by using density, body water, and bone mineral measurements. *Am. J. Clin. Nutr.* 55: 764–770, 1992.

8. **Fuller, N. J., S. A. Jebb, M. A. Laskey, W. A. Coward, and M. Elia.** Four-component model for the assessment of body composition in humans: comparison with alternative methods, and evaluation of the density and hydration of fat-free mass. *Clin. Sci. (Colch.)* 82: 687–693, 1992.

9. **Haurowitz, F.** *The Chemistry and Function of Proteins* (2nd ed.). New York: Academic, 1963, p. 111–123.

10. **Hewitt, M. J., S. B. Going, D. P. Williams, and T. G. Lohman.** Hydration of the fat-free body mass in children and adults: implications for body composition assessment. *Am. J. Physiol.* 265 (*Endocrinol. Metab.* 28): E88–E95, 1993.

11. **Heymsfield, S. B., S. Lichtman, R. N. Baumgartner, J. Wang, Y. Kamen, A. Aliprantis, and R. N. Pierson, Jr.** Body composition of humans: comparison of two improved four-compartment models that differ in expense, technical complexity, and radiation exposure. *Am. J. Clin. Nutr.* 52: 52–58, 1990.

12. **Heymsfield, S. B., J. Wang, J. Kehayias, S. Heshka, S. Lichtman, and R. N. Pierson, Jr.** Chemical determination of human body density in vivo: relevance to hydrodensitometry. *Am. J. Clin. Nutr.* 50: 1282–1289, 1989.

13. **Lentner, C.** (Editor). Geigy scientific tables. In: *Units of Measurement: Body Fluids, Composition of the Body, Nutrition* (8th ed.). Basel: Ciba-Geigy, 1981, vol. 1, p. 50.

14. **Mazess, R. B., H. S. Barden, J. P. Bisek, and J. Hanson.** Dual energy x-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. *Am. J. Clin. Nutr.* 51: 1106–1112, 1990.

15. **Méndez, J., A. Keys, J. T. Anderson, and F. Grande.** Density of fat and bone mineral of the mammalian body. *Metabolism* 9: 472–477, 1960.

16. **Mitchell, H. H., T. S. Hamilton, F. R. Steggerda, and H. W. Bean.** The chemical composition of the adult human body and its bearing on the biochemistry of growth. *J. Biol. Chem.* 158: 625–637, 1945.

17. **Modlesky, C. M., K. J. Cureton, R. D. Lewis, B. M. Prior, M. A. Sloniger, and D. A. Rowe.** Density of the fat-free mass and estimates of body composition in male weight trainers. *J. Appl. Physiol.* 80: 2085–2096, 1996.

18. **Schoeller, D. A., E. Ravussin, Y. Shutz, K. J. Acheson, P. Baertschi, and E. Jéquier.** Energy expenditure by doubly labeled water: validation in humans and proposed calculation. *Am. J. Physiol.* 250 (*Regulatory Integrative Comp. Physiol.* 19): R823–R830, 1986.

19. **Shohl, A. T.** *Mineral Metabolism.* New York: Reinhold, 1939, p. 19.

20. **Siconolfi, S. F., R. J. Gretebeck, and W. W. Wong.** Assessing total body protein, mineral, and bone mineral content from total body water and body density. *J. Appl. Physiol.* 79: 1837–1843, 1995.

21. **Siri, W. E.** Body composition from fluid spaces and density: analysis of methods. In: *Techniques for Measuring Body Composition,* edited by J. Brozek and A. Henschel. Washington, DC: National Academy of Sciences National Research Council, 1961, p. 223–244.

22. **Taylor, J. R.** *Introduction to Error Analysis.* Mill Valley, CA: University Science Books, 1982, p. 52–56 and 124.

23. **Wahner, H. W., W. L. Dunn, R. B. Mazess, M. Towsley, R. Lindsay, L. Markhard, and D. Dempster.** Dual-photon Gd-153 absorptiometry of bone. *Radiology* 156: 203–206, 1985.

24. **Weast, R. C.** (Editor). *Handbook of Chemistry and Physics* (56th ed.). Cleveland, OH: CRC, 1975, p. C-311.

25. **Widdowson, E. M., R. A. McCance, and C. M. Spray.** The chemical composition of the human body. *Clin. Sci. (Colch.)* 10: 113–125, 1951.

26. **Withers, R. T., J. LaForgia, S. B. Heymsfield, Z.-M. Wang, and R. K. Pillans.** Two-, three- and four-compartment chemical models of body composition analysis. In: *Anthropometrica*, edited by K. I. Norton and T. S. Olds. Sydney, Australia: UNSW, 1996, p. 199–231.

27. **Withers, R. T., D. A. Smith, B. E. Chatterton, C. G. Schultz, and R. D. Gaffney.** A comparison of four methods of estimating the body composition of male endurance athletes. *Eur. J. Clin. Nutr.* 46: 773–784, 1992.

28. **Withers, R. T., N. O. Whittingham, K. I. Norton, J. La Forgia, M. W. Ellis, and A. Crockett.** Relative body fat and anthropometric prediction of body density of female athletes. *Eur. J. Appl. Physiol.* 56: 169–180, 1987.