Metabolically Active Component of Fat-Free Body Mass: Influences of Age, Adiposity, and Gender

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Fat-free body mass (FFM) is often considered the metabolically active compartment and is widely used to adjust between-subject differences in resting energy expenditure for body composition. The use of FFM as the metabolically active portion of body weight makes the assumption that the body cell mass (BCM) component which is more difficult to measure, maintains a relatively constant relationship to FFM within and between subjects. The aim of this study was to test the hypothesis that BCM and FFM are associated independently of age, adiposity (as represented by body density), and gender in healthy white women and men. BCM and FFM were estimated by whole-body ^{40}K-counting and dual-energy x-ray absorptiometry (DXA), respectively. Multiple regression analysis was used to model the relationships between BCM as the dependent variable and FFM, age, body density, and gender as potential independent variables. FFM alone explained 51% and 63% of between-individual BCM differences in women (n = 269) and men (n = 204) (both $P = .0001$), respectively. Age contributed significantly ($P = .0001$) to BCM prediction after adjusting first for FFM in both women and men. Body density also added significantly ($P = .004$ and $P = .0001$) to FFM and age prediction of BCM in women and men, respectively. Lastly, gender contributed significantly to the composite model, with 91% of between-individual differences in BCM explained by FFM, age, body density, and gender. Hence, BCM does not maintain a fixed relationship to FFM, as often assumed, but varies significantly and independently of FFM with age, adiposity, and gender. These findings have implications for the study of metabolic indices such as resting energy expenditure.

FAT-FREE BODY MASS (FFM) is the compartment traditionally used to adjust resting energy expenditure measurements for between-subject differences in body composition.[1,2] FFM consists of chemical components, protein, water, and minerals,[3] which by themselves do not expend energy.[4] When organized into cellular-level components, the chemical constituents of FFM are distributed between body cell mass (BCM), extracellular fluid (ECF), and extracellular solids (ECS). Almost all resting thermogenesis occurs in the BCM component of FFM.[5]

The use of FFM as a means of adjusting resting energy expenditure measurements makes the fundamental assumption that BCM maintains a constant association with the larger FFM compartment. However, this hypothesis has not previously been critically evaluated. Moreover, a growing number of studies suggest that the quantitative relationship between BCM and FFM is influenced by at least three independent factors: age,[6-7] adiposity,[8] and gender.[9-11]

The study by Cohn et al,[12] performed in 133 healthy white adult women and men, examined the interrelationships between body composition components as a function of age. BCM was calculated from total body potassium (TBK), and FFM was calculated as the sum of total body water, protein, and mineral estimated from tritium dilution volume, total body nitrogen, and total body calcium, respectively. When the women were grouped by age in 30-year intervals, both BCM and FFM decreased as a percentage of body weight and the proportion of FFM as BCM also declined with increasing age. However, no attempt was made in this study to adjust body composition for between-group differences in body weight and stature, even though the older women were both shorter and heavier than their younger counterparts.

The investigation reported by Mazariegos et al,[13] which extended the study by Cohn et al,[12] compared body composition in 19 young white women and weight- and height-matched older women. The proportion of FFM as BCM was significantly lower in the older women, suggesting that older women have a smaller fraction of metabolically active tissue compared with their younger counterparts of similar weight and stature. This study was in a relatively small cohort, included only women at two age extremes, and controlled for between-group differences in body mass index (BMI) by matching young and old subjects for body weight and height.

The influence of adiposity on ECF and intracellular fluid compartments was examined in 65 lean and obese women by Waki et al.[8] With increasing adiposity, there was an increase in ECF relative to intracellular fluid. Since intracellular fluid and BCM components are closely related, this study suggests that ECF may increase to a greater extent than BCM with increasing fatness, and that there may be a corresponding reduction in BCM relative to FFM. The relationships between adiposity, BCM, and FFM have not previously been studied in men.

The association between BCM and FFM may also differ between women and men. Several studies suggest a lower TBK/FFM ratio in women compared with men,[9,14-15] and TBK is a measure of BCM.[5] The proportion of FFM as BCM thus appears smaller in women than in men. Earlier

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studies that explored gender differences in the TBK/FFM ratio were generally conducted on small samples,[14] failed to control adequately for age and body weight,[9,14-15] or used rough measures of FFM such as skinfold thicknesses.[9]

No study has yet fully explored the relationships between BCM and FFM in a large cohort of subjects with appropriate mathematical modeling. The aim of this study was to test the hypothesis that BCM and FFM are associated independently of age, adiposity, and gender in healthy white adults.

SUBJECTS AND METHODS

Experimental Protocol

The relationship between BCM and FFM was investigated using multiple regression analysis with BCM as the dependent variable and FFM, age, body density, and gender as independent variables. Body density was used as a proxy measure of adiposity based on two considerations: (1) there are good reported correlations between body density and total body fat[16]; and (2) body density determined by hydrodensitometry is measured independently of the two other main study components, BCM by whole-body ^{40}K-counting and FFM by dual-energy x-ray absorptiometry (DXA). Although it is possible to calculate total body fat or adipose tissue from body density, we chose to base our analyses directly on measured body density. There are no fully agreed-upon equations for converting body density to adiposity-related components that are applicable across gender and age groups.

Subjects

Healthy white women and men were recruited through advertisement in newspapers and flyers posted in the local community. These individuals were participants in a larger multiethnic body composition study.[16] Subjects with chronic medical conditions, taking prescription medications, or with severe obesity (BMI $\geq$ 35 kg/m^2) were excluded from study.

Subjects reported to the body composition laboratories at St. Luke's/Roosevelt Hospital after an overnight fast. A physical examination and routine blood studies were performed to ascertain good health. Ethnicity was determined by subject self-report. All subjects signed an informed-consent form approved by St. Luke's/Roosevelt Hospital Institutional Review Board. Results from these subjects were reported in earlier unrelated publications.[16-17]

Body Composition Measurements

Body composition measurements were performed on the same day. Body weight was measured to the nearest 0.1 kg using a digital scale (WI-102; Weight-Tronix, New York, NY), and height was measured to the nearest millimeter using a stadiometer (Holtain, Crosswell, Wales, UK). BCM was calculated from TBK measurements as described by Moore et al.[5] FFM was determined using DXA.[18-20]

Body density. Body density was derived from hydrodensitometry using a four-transducer platform scale system (Precision Biomedical Systems, University Park, PA) as previously described.[21] Residual lung volume was determined before submersion using a closed-circuit dilution method.[22] Within-subject error for body density measurements in our laboratory is 0.0035 g/cm^3.[23]

BCM. The St. Luke's 4-pi whole-body counter was used to measure ^{40}K levels.[9] ^{40}K net counts accumulated over 9 minutes were corrected for self-absorption based on an earlier ^{40}K calibration study.[24] The within-subject coefficient of variation in our laboratory for ^{40}K-counting is 4.7%.[25] TBK was calculated from measured ^{40}K.[9]

BCM was calculated from TBK using the equation, BCM (kilograms) = 0.00833 $\times$ TBK (millimoles)). This equation is based on two assumptions: (1) the average intracellular potassium content is approximately 3 mmol/g nitrogen, and (2) the nitrogen content is 0.04 g/g wet tissue.[5] There is good support for these assumptions in weight-stable healthy women and men across a wide age spectrum.[10,26] For example, Cohn et al[10] examined intracellular potassium concentration using a combination of whole-body counting and isotope dilution methods. Intracellular potassium concentration was similar in women and men and was not influenced by age.

FFM. FFM was measured using whole-body DXA (model DPX, version 3.4 software; Lunar, Madison, WI) as previously reported from our laboratory.[19-20,27] The within-subject coefficient of variation for DXA FFM in our laboratory is 1.2%.[20] Due to certain technical limitations associated with the use of dual-photon methods in markedly overweight subjects,[28] persons with a BMI greater than 35 kg/m^2 were excluded from the current analyses.

Statistical Methods

Group results are expressed as the mean $\pm$ SD. Differences in age, body weight, height, BMI, body density, and body composition between women and men were tested using Student's t test. P less than .05 was considered statistically significant.

Pearson correlation coefficients were used to investigate associations between BCM and FFM. Multiple regression analyses were used with BCM as the dependent variable and FFM, age, body density, and gender as independent variables. Gender was entered as a dummy variable (0 = women and 1 = men). The possible contribution of interaction terms by independent variables to the models were tested.

RESULTS

Baseline Characteristics

Baseline characteristics of the subjects are shown in Table 1. The subject pool consisted of 269 women and 204 men (N = 473). Subjects ranged in age from 20 to 89 years for women and 20 to 94 years for men. The mean ages of women and men were similar (50.5 *v* 47.7 years). Men were heavier, taller, and had a significantly greater BMI ($P < .001$) and body density ($P < .005$) than women. BCM and FFM were significantly larger in men compared with women (both $P = .0001$).

BCM Regression Models

FFM. BCM was strongly associated with FFM in women ($r = .71$) and men ($r = .79$, both $P = .0001$; Fig 1). FFM

Table 1. Subject Characteristics

	Women (n = 269)	Men (n = 204)	*P*
Age (yr)	50.5 ± 18.8	47.7 ± 18.7	.06
Weight (kg)	70.0 ± 10.4	77.7 ± 12.0	.001
Height (cm)	162.8 ± 6.6	175.5 ± 6.7	.001
BMI (kg/m^2)	23.4 ± 3.8	25.2 ± 3.4	.001
Body density (g/cm^3)	1.029 ± 0.018	1.044 ± 0.017	.005
TBK (mmol)	2,395 ± 370	3,789 ± 569	.0001
BCM (kg)	19.9 ± 3.0	31.6 ± 4.7	.0001
FFM (kg)	41.2 ± 4.7	61.7 ± 8.0	.0001
BCM/FFM ratio	0.48 ± 0.05	0.51 ± 0.05	.0001

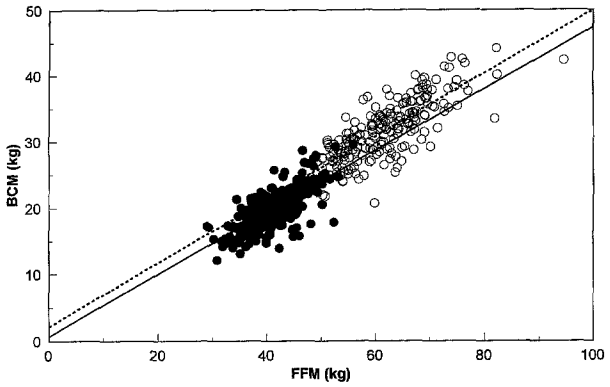

Fig 1. BCM versus FFM in women (●) and men (○). Linear regression lines for women (—) and men (---). Women: slope = 0.47 kg, intercept = 0.93 kg, r = .71, P = .0001, n = 272. Men: slope = 0.48 kg, intercept = 2.72 kg, r = .79, P = .0001, n = 206.

explained 51% and 63% of the variance in BCM in women and men, respectively (Table 2).

FFM and age. Age contributed significantly to the regression model containing FFM in women and men (both P = .0001; Table 2), thereby suggesting that the relation between BCM and FFM is age-dependent. Addition of age to the multiple regression model increased the explained variance from 51% to 61% in women and from 63% to 75% in men. The model suggests that when FFM is kept constant, there is a smaller BCM with increasing age.

The influence of age on the BCM-FFM relationship is shown in Fig 2A for a hypothetical woman and man. The regression models presented in Table 2 were solved for BCM by assuming a constant FFM of 50 kg and variable age in 10-year increments. The curves generated by the model indicate a decline in BCM with increasing age, despite a fixed FFM, in both women and men.

Body density. Body density contributed significantly to the model in women (P = .004) and men (P = .0001; Table 3). In both women and men, the relationship between BCM and FFM was influenced by body density. Body density correlated significantly with age in women (r = −.42, P = .0001) and men (r = −.22, P = .0001), suggesting that in women and men the influence of body density on the relationship between BCM and FFM may be, in part, explained by age.

In women and men, age and body density independently influenced the relationship between BCM and FFM. The explained variance in BCM-FFM relationships increased from 51% to 62% and from 63% to 77% in women and men, respectively, after controlling for the additional covariates, age and body density. The model was also developed in which FFM and body density were entered followed by age. Body density and age were statistically significant (both P < .001) in this model for women and men.

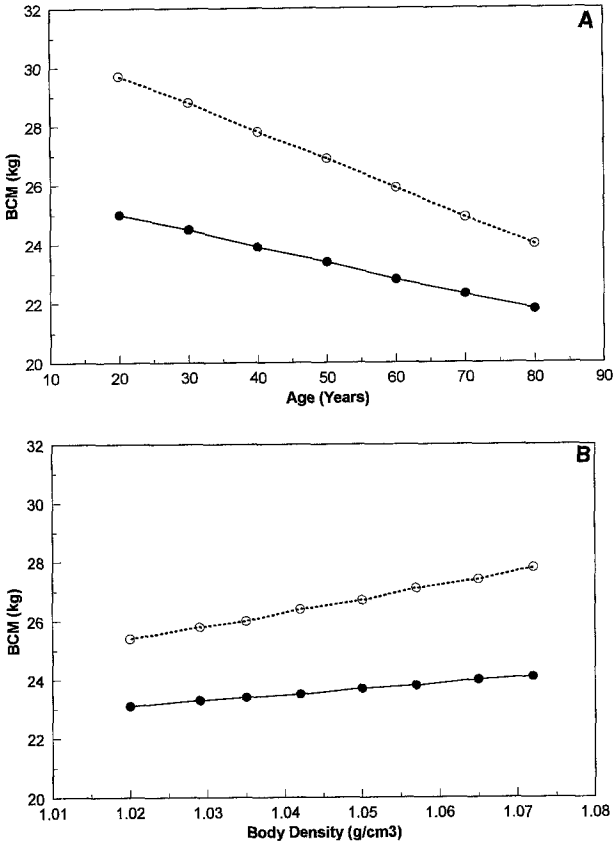

Fig 2. (A) Influence of age on the BCM-FFM relationship in a hypothetical woman (●) and man (○) with FFM of 50 kg. Curves were generated from regression models presented in Table 2. (B) Influence of body density on the BCM-FFM relationship in a hypothetical woman (●) and man (○) with FFM of 50 kg and aged 49 years. Curves were generated from regression models presented in Table 3 using body densities within the physiological range.

The influence of body density on the BCM-FFM relationship is shown in Fig 2B for a hypothetical woman and man. The regression models presented in Table 3 were solved for BCM by assuming a constant FFM of 50 kg, an age of 49 years, and variable body densities within the physiological range. The curves generated by the model indicate a

Table 2. Results of Regression Analyses With BCM as the Dependent Variable and FFM and Age as Independent Variables

Group	Regression Coefficients (mean ± SE)			R^2	SEE	P
	FFM	Age	Intercept			
Women	0.458 ± 0.028*	—	1.047 ± 1.153	.51	2.13	.0001
	0.388 ± 0.026*	−0.054 ± 0.007*	6.669 ± 1.228*	.61	1.91	.0001
Men	0.496 ± 0.027*	—	1.097 ± 1.665	.63	2.86	.0001
	0.390 ± 0.022*	−0.096 ± 0.009*	12.152 ± 1.581*	.75	2.36	.0001

Abbreviation: SEE, model standard error of estimate.
*P = .0001.

Table 3. Results of Regression Analyses With BCM as the Dependent Variable and FFM, Age, and Body Density as Independent Variables

Group	Regression Coefficients (mean ± SE)				R^2	SEE
	FFM	Age	Body Density	Intercept		
Women	0.436 ± 0.027†	—	38.480 ± 6.879†	−37.749 ± 7.021†	.55	2.02
	0.379 ± 0.026†	−0.048 ± 0.007†	19.907 ± 6.911*	−13.832 ± 7.369	.62	1.86
Men	0.471 ± 0.02†	—	82.034 ± 10.843†	−83.452 ± 11.272†	.71	2.53
	0.413 ± 0.023†	−0.074 ± 0.010†	46.309 ± 10.861†	−38.911 ± 11.792*	.77	2.25

*$P < .05$.
†$P < .0001$.

decline in BCM with decreasing body density (ie, increasing adiposity), despite a fixed FFM and age.

Gender. The relationship between BCM, FFM, and age was significantly influenced by gender. Gender ($P = .0001$) and the interaction term, age · gender ($P = .0001$), added significantly to the model containing FFM and age as independent variables. This observation has two implications: (1) the relationship between BCM and FFM differs between women and men such that women have relatively less BCM compared with men of the same FFM (Fig 1); and (2) after controlling for FFM, women appear to have a smaller relative decrease in BCM with age than men. The smaller reduction in BCM with increasing age in women versus men is evident in Fig 2A. In the total study population, 91% of the variance in BCM was explained by FFM, age, body density, and gender.

DISCUSSION

The main finding of the current study is that age, adiposity, and gender all contributed significantly to between-individual differences in BCM after controlling first for FFM. These observations indicate that the two main metabolically active body composition components, BCM and FFM, do not maintain a constant relationship to each other across heterogeneous populations.

Our results indicate that approximately half of the between-individual variation in BCM observed in our subjects could be accounted for by individual variation in FFM. The explained variance increased to greater than 90% with the addition of three covariates, age, adiposity, and gender. The question therefore arises as to what underlying factors these variables are controlling for beyond that explained by FFM alone.

One possible explanation is based on the relationship between FFM, a "molecular-level" component, and BCM, a "cellular-level" component.[3]

$$\text{FFM} = \text{BCM} + \text{ECF} + \text{ECS}, \quad (1)$$

$$\text{and BCM} = \text{FFM} - (\text{ECF} + \text{ECS}). \quad (2)$$

Equation 2 suggests additional biological sources of variation in the BCM-FFM relationship, namely ECF and ECS. Since we observed age, adiposity, and gender as additional statistically significant covariates, it is likely that these three factors somehow impact ECF and/or ECS.

In the discussion that follows, we review the available pertinent but limited literature that considers the effects of age, adiposity, and gender on ECF, ECS, and the FFM-BCM relationship.

Age as a Covariate

The present cross-sectional study results indicate that with increasing age there is a reduction in BCM relative to FFM. After controlling for FFM, old subjects have a smaller BCM compared with young subjects. In addition, we found that the influence of age on the BCM-FFM relationship differed significantly between women and men. The decrease of BCM relative to FFM with increasing age was smaller in women than in men.

Although the specific mechanism(s) associated with a relative reduction in BCM with increasing age is unclear, there appears to be a small but consistent increase in ECF with advancing age in adults. Pierson et al[6] examined ECF and total-body fluid volumes as a function of age using dilution of ^{24}Na and $^{3}H_2O$, respectively. The ratio of ^{24}Na to $^{3}H_2O$ was approximately 20% greater in subjects over age 80 years compared with those less than age 30 years, suggesting an age-related relative expansion in ECF. Borkan and Norris[7] made a similar observation, indicating a relative expansion in ECF compared with intracellular fluid with increasing age. Mazariegos et al[13] examined ECF and FFM in young and old women using ^{24}Na measurements for ECF and a four-compartment model for FFM estimations. The ECF/FFM ratio was significantly higher ($P < .001$) in old women (0.38 ± 0.03) compared with young women (0.34 ± 0.03).

The ECS component may also change with age. Studies that have explored this area are limited and contradictory. ECS are an important component of both the skeleton and bone minerals.[3] The skeleton mass decreases with age in both women and men,[29] and this change is in a direction opposite to that of ECF. Cohn et al[12] examined neutron-activation–derived total-body calcium in women and men of varying ages. Estimates of ECS and FFM were made by the investigators from total-body calcium and a model based on multicomponent neutron-activation analysis, respectively. The ratio of ECS to FFM was similar between young and old women (mean, 0.12 *v* 0.11) and young and old men (0.11 *v* 0.11). Mazariegos et al[13] investigated the proportion of FFM as mineral in a study of weight- and height-matched young and old women. The old women had significantly less ($P < .005$) mineral relative to FFM (0.063 ± 0.010) than the young women (0.072 ± 0.007). Hence, some reduction in ECS may occur relative to FFM with increasing age, although the limited available literature suggests that the magnitude of this change is small.

An important observation in our cross-sectional study is that as age increases, women appear to lose BCM relative

to FFM at a slower rate than men (Table 2 and Fig 2). Examination of previous studies lends support to this observation. Cohn et al[12] reported a linear decrease in the BCM (ie, TBK) to FFM ratio with a slope of 0.00332 in women and 0.00623 in men aged 20 to 79 years. Forbes[14] created a composite analysis of TBK as a function of age based on several different sources. A similar analysis was completed for 24-hour urinary creatinine excretion, a measure of skeletal muscle mass. Both analyses, based on cross-sectional data, strongly suggest a lower peak and slower loss of BCM and skeletal muscle with increasing age in women compared with men. These earlier observations, the mechanisms of which are not known, are supported by results from the current study. Further mechanistic and longitudinal investigations are needed to expand on these descriptive cross-sectional observations.

These collective observations suggest that age influences relationships between BCM, ECF, ECS, and FFM. Our findings in this cross-sectional cohort also suggest that gender moderates the effects of aging on body composition proportions.

Adiposity as a Covariate

The present study findings indicate that adiposity, as represented by body density, independently influences the relationship between BCM and FFM after controlling first for age. The influence of body density on the BCM-FFM relationship was observed in both women and men, although the magnitude of the effect was small after adjusting for age (1% to 2% increase in explained variance).

The mechanism that explains why body density moderates the relationship between BCM and FFM is unknown. Adipose tissue has a large ECF and small fat-free cell mass per unit weight, and therefore, large amounts of adipose tissue may cause a relative expansion of total-body ECF. The large relative ECF content of adipose tissue is shown in Fig 3, which presents the ratio of BCM to FFM for representative organs and tissues based on direct chemical analysis.[30-31] This hypothesis is supported by studies in adults[8] and children[32] that demonstrate an expansion of ECF relative to intracellular fluid, a component closely related to BCM, in obese subjects compared with lean counterparts.

We were unable to find any published studies that examine ECS in relation to BCM, FFM, and body density.

Gender as a Covariate

Women appear to have a smaller BCM after controlling first for FFM than men, and this observation supports the earlier studies of Pierson et al,[9] Cohn et al,[10] and Novak.[11]

Previous studies suggest that women have relatively more adipose tissue and less skeletal muscle mass than men.[14] Adipose tissue has a proportionately small fat-free cell mass, whereas skeletal muscle has a high fat-free cell mass[30-31] (Fig 3), and this may account for some of the observed difference in the BCM-FFM relationship between women and men.

The study by Cohn et al[10] suggests that women and men have similar amounts of ECS relative to FFM.[10] This suggests that the gender effect observed in our regression models was mainly due to variation in ECF between women and men.

Body Composition and Energy Expenditure

Taken collectively, the current study results suggest that a complex relationship exists between BCM, FFM, and other body composition components. The majority of energy expenditure studies use FFM as a means of adjusting and therefore compensating for between-subject differences in metabolically active tissue mass. As an example, studies using FFM as an energy-producing component suggest that resting energy expenditure is influenced by fat mass, age, and gender.[33-34] After controlling first for FFM, a greater fat mass and older age are associated with a higher and lower resting energy expenditure, respectively. Women are reported to have a lower sedentary metabolic rate than men after controlling first for FFM, fat mass, and age.[34] A hypothesis advanced by Ferraro et al[34] is that hormonal mechanisms may, in part, account for the observed gender differences in sedentary energy expenditure. However, our results suggest that an equally plausible hypothesis is that the metabolically active portion of FFM in women is smaller than in men, and that gender differences in energy expenditure may no longer be statistically significant when BCM is used to adjust for between-individual body composition differences. Similar interpretative concerns may apply when small energy expenditure changes are examined in relation to weight loss or gain.[35]

Therefore, our study results emphasize the need to cautiously interpret metabolic data in light of the selected body composition reference component. Moreover, even BCM is a composite of the cell mass of multiple tissues[30-31] (Fig 3) and, like FFM, is not a homogeneous compartment. Adjusting metabolic data for BCM would therefore require an equally cautious interpretation of results, as with FFM.

Conclusion

The present study explored the relationships between two metabolically active body composition components, FFM and BCM. Our results indicate that FFM and BCM

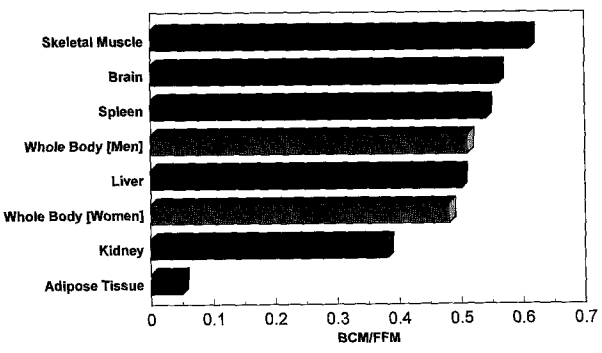

Fig 3. Ratio of BCM to FFM for representative organs and tissues based on direct chemical analysis.[30-31] BCM was calculated as potassium content of organ or tissue × 0.0083.[5] Whole-body BCM/FFM ratios are shown for the women and men in the current study.

are not equivalent measures of body composition, but instead their relationship is moderated by age, adiposity, and gender. Further studies should be directed at examining resting energy expenditure in relation to FFM, BCM, and when feasible additional components such as skeletal muscle and visceral organs (eg, liver). These potential future observations would have important implications for the expression and interpretation of metabolic data.

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