



# Six-compartment body composition model: Inter-method comparisons of total body fat measurement

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**OBJECTIVE:** To compare 16 currently used total body fat methods to a six-compartment criterion model based on *in vivo* neutron activation analysis.

**DESIGN:** Observational, inter-method comparison study.

**SUBJECTS:** Twenty-three healthy subjects (17 male and 6 female).

**MEASUREMENTS:** Total body water (TBW) was measured by tritium dilution; body volume by underwater weighing (UWW); total body fat and bone mineral by dual-energy X-ray absorptiometry (DXA), total body potassium (TBK) by whole-body <sup>40</sup>K counting; total body carbon, nitrogen, calcium, phosphorus, sodium and chlorine by *in vivo* neutron activation analysis; skinfolds/circumferences by anthropometry (Anth); and resistance by single-frequency bioimpedance analysis (BIA).

**RESULTS:** The average of total body fat mass measurements by the six-compartment neutron activation model was  $19.7 \pm 10.2$  kg (mean  $\pm$  s.d.) and comparable estimates by other methods ranged from 17.4–24.3 kg. Although all 16 methods were highly correlated with the six-compartment criterion model, three groups emerged based on their comparative characteristics (technical error, coefficient of reliability, Bland-Altman analysis) relative to criterion fat estimates, in decreasing order of agreement: 1. multi-compartment model methods of Baumgartner ( $19.5 \pm 9.9$  kg), Heymsfield ( $19.6 \pm 9.9$  kg), Selinger ( $19.7 \pm 10.2$  kg) and Siri-3C ( $19.6 \pm 9.9$  kg); 2. DXA ( $20.0 \pm 10.8$  kg), Pace-TBW ( $18.8 \pm 10.1$  kg), Siri-2C ( $20.0 \pm 9.9$  kg), and Brozek-UWW ( $19.4 \pm 9.2$  kg) methods; and 3. Segal-BIA ( $17.4 \pm 7.2$  kg), Forbes-TBN ( $21.8 \pm 10.5$  kg), Durnin-Anth ( $22.1 \pm 9.5$  kg), Forbes-TBK ( $22.9 \pm 11.9$  kg), and Steinkamp-Anth ( $24.3 \pm 9.5$  kg) methods.

**CONCLUSION:** Relative to criterion fat estimates, body composition methods can be organized into three groups based on inter-method comparisons including technical error, coefficient of reliability and Bland-Altman analysis. These initial groupings may prove useful in establishing the clinical and research role of the many available fat estimation methods.

**Keywords:** total body fat mass; multi-compartment model; body composition

## Introduction

At the present time there are more than ten methods of estimating total body fat *in vivo*. These methods can be generally organized into two groups.<sup>1</sup> The first group, statistically-derived or 'descriptive' fat estimation methods such as anthropometry (Anth) and bioimpedance analysis (BIA), share two features in common: they depend on a reference method and subsequent statistical data analysis for the development of a prediction formula. Methods of the second group are model-dependent or 'mechanistic' and are derived from well-known stable body composition component relationships. Examples of model dependent methods include the total body potassium (TBK)

method of Forbes,<sup>2,3</sup> the total body water (TBW) method of Pace and Rathbun,<sup>4</sup> the densitometry methods introduced and extended by Siri<sup>5</sup> and Brozek *et al.*,<sup>6</sup> the newly developed dual energy X-ray absorptiometry (DXA) approach,<sup>7</sup> and multi-compartment models.<sup>8</sup>

Although numerous statistically-derived and model-based methods are available, fat estimates in individuals often differ, sometimes substantially, from each other. An example is that mean total body fat predicted by the Segal-BIA method provided fat results far lower than that provided by the Forbes-TBK method (24.5% vs 38.0% of body weight) in the same subjects.<sup>9</sup> These discrepancies between methods demonstrate the need for an inter-method comparison study of total body fat measurement methods. Although these issues have been previously examined, two major problems exist in earlier reports.

The first problem with earlier studies involves the criterion for total body fat measurement. As the 'true' value of total body fat mass is unmeasurable in living

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humans, a criterion with high accuracy is necessary to evaluate other methods. Although several traditional methods (for example, the Forbes-TBK and Pace-TBW methods) are often used as criteria, they are not optimum for evaluating other methods because they are formulated on assumptions of uncertain validity.<sup>8</sup> The Cohn-neutron activation and DXA methods are also applied as criteria,<sup>7,10,11</sup> although these methods have inherent error sources that could bias or cause large within-individual errors in fat estimates. For example, the Cohn-neutron activation model fails to consider the glycogen and soft tissue mineral components. There are also several unresolved issues related to the DXA method such as hydration effects and underlying geometric models.<sup>12</sup> A more accurate criterion is thus needed for evaluation of total body fat.

Another problem with early studies is that up to the present a complete inter-method comparison of currently used total body fat estimation methods is lacking. Previous studies usually compared two or three methods and in a few studies seven or eight methods were compared.<sup>9,13,14</sup> Such incomplete inter-method comparisons cannot provide a comprehensive picture of total body fat estimation methods. Ideally, total body fat should be estimated in the same subject group using all currently available methods. However, this is not practical for most body composition laboratories, due to the limited number of available techniques at any one center.

The purpose of the present investigation was to carry out an inter-method comparison between a newly derived criterion based on *in vivo* neutron activation analysis and other currently used total body fat estimation methods. Thirteen model-based methods and three statistically-derived methods were compared to the six-compartment *in vivo* neutron activation analysis model.

## Methods

### Experimental approach

All subjects completed tritium dilution, underwater weighing (UWW), Anth, DXA, BIA, whole-body <sup>40</sup>K counting and *in vivo* neutron activation analysis studies. The completed evaluations were then used to estimate total body fat, according to the criterion method and additional 16 selected methods.

The criterion for estimating total body fat is based on an evolution of multi-compartment methods which rely on elemental analyses by neutron activation analysis. Cohn *et al*<sup>10</sup> first suggested a model which treats the whole body as a four compartment system composed of fat, water, protein and bone minerals (Mo). Total body fat mass (BF) was then determined as the difference between body mass (BM) and the sum of the other three components,  $BF = BM - (\text{water} + \text{protein} + \text{Mo})$ . Two components

in fat-free body mass (FFM), soft tissue mineral (Ms) and glycogen (G), are ignored in the Cohn model. Although Ms and G are much smaller than the other components, this simplification inevitably introduces a model error and leads to an over-estimation of BF.

A six-compartment model was proposed by Heymsfield *et al*,<sup>15</sup>

$$BF = BM - (\text{water} + \text{protein} + \text{Mo} + \text{Ms} + \text{G}) \quad (1)$$

where water is measured by tritium dilution; protein measurement is based on total body nitrogen (TBN),  $\text{protein} = 6.25 \times \text{TBN}$ ; Mo is calculated from total body calcium (TBCa),  $\text{Mo} = \text{TBCa}/0.364$ ; Ms is calculated from TBK, total body sodium (TBNa) and chlorine (TBCl), and TBCa,  $\text{Ms} = 2.76 \times \text{TBK} + \text{TBNa} + 1.43 \times \text{TBCl} - 0.038 \times \text{TBCa}$ ;<sup>16</sup> and G is estimated based on an assumption,  $\text{G} = 0.044 \times \text{protein} = 0.275 \times \text{TBN}$ .<sup>15</sup> In the present study, a corresponding model is applied as the criterion to compare with the 16 other total body fat estimation methods,

$$BF = BM - (\text{water} + 6.525 \times \text{TBN} + 2.709 \times \text{TBCa} + 2.76 \times \text{TBK} + \text{TBNa} + 1.43 \times \text{TBCl}) \quad (2)$$

This new equation was developed by combining the equations for protein, bone mineral, soft tissue mineral and G along with water, to solve for fat mass. The model error in equation 2 is small, although there still exists measurement errors for the various components.

### Subjects

The subject group consisted of 23 healthy adults (17 male and six female; 12 White, three African American and eight Puerto Ricans) who ranged in age from 19–69 y and in body weight from 54–106 kg. The subjects in the present investigation participated in other large studies of body composition. Healthy subjects were recruited from hospital staff and local residents. All subjects signed an informed consent that was approved by the Hospital's Institutional Review Board. Each subject completed a medical history, physical examination and routine blood studies, to exclude the presence of underlying diseases.

### Body composition measurements

The following evaluations were used to estimate total body fat according to the criterion method and 16 additional descriptive and model-based methods. Consenting subjects were studied after an overnight fast. BM was measured to the nearest 0.1 kg and height to the nearest 0.5 cm.

*Model-based methods.* Table 1 shows the calculation formulas of the 13 model-based methods as well as their main assumptions.

**Table 1** Thirteen model-based methods for estimating body fat mass

Method (Ref)	Prediction equation	Main assumption
Baumgartner (17)	$BF = 2.75 \times BV - 0.714 \times TBW + 1.148 \times M - 2.05 \times BM$	$M = 1.235 \times Mo$
Siri-2C (5)	$BF = 4.95 \times BV - 4.50 \times BM$	Densities of fat and FFM are 0.90 and 1.10 g/cm <sup>3</sup>
Brozek (6)	$BF = 4.570 \times BV - 4.142 \times BM$	Constant proportions of water, protein, and mineral in FFM
Cohn (10)	$BF = BM - TBW - 6.25 \times TBN - 2.747 \times TBCa$	1. Ignore Mo and G; 2. protein = 6.25 × TBN; 3. Mo = TBCa/0.364
DXA (7, 18)		constant hydration in fat-free soft tissues.
Forbes-TBK (2, 3)	$BF = BM - 375.6 \times TBK$ (males); $BF = BM - 398.4 \times TBK$ (females)	TBK/FFM = 0.00266 (males) and 0.00251 (females)
Forbes-TBN (19)	$BF = BM - 30.30 \times TBN$	TBN/FFM = 0.033
Heymsfield (8)	$BF = 2.513 \times BV - 0.739 \times TBW + 0.947 \times Mo - 1.79 \times BM$	density of mixture (protein+Ms+G) is 1.404
Kehayias (20)	$BF = 1.30 \times TBC - 4.45 \times TBN - 0.065 \times TBCa$	1. protein = 6.25 × TBN; 2. G = 0.044 × protein; 3. C/Ca = 0.05 g/g in bone.
Lohman (21)	$BF = 6.386 \times BV + 3.961 \times M - 6.09 \times BM$	1. TBW/protein = 3.80; 2. M = 1.235 × Mo
Pace (4)	$BF = BM - 1.3661 \times TBW$	TBW/FFM = 0.732.
Selinger (22)	$BF = 2.75 \times BV - 0.714 \times TBW + 1.129 \times Mo - 2.037 \times BM$	Ms = 0.0105 × BM
Siri-3C (5)	$BF = 2.118 \times BV - 0.78 \times TBW - 1.351 \times BM$	mineral/protein = 0.351

BF, total body fat mass (kg); BV, body volume (in liter); TBW, total body water (kg); M, mineral (kg); BM = body mass (kg); Mo, bone mineral (kg); FFM, fat-free body mass (kg); TBN = total body nitrogen (kg); TBCa = total body calcium (kg); G, glycogen (kg); DXA = dual-energy X-ray absorptiometry; TBK = total body potassium (kg); Ms, soft tissue mineral (kg); c/ca = ratio of carbon to calcium.

Tritium space (<sup>3</sup>H<sub>2</sub>O, in L) was measured at the Body Composition Unit of St. Luke's-Roosevelt Hospital with a precision (CV) of 1.5%.<sup>23</sup> The tritium space was then converted into TBW (in kg) by multiplying with the correction factor for non-aqueous hydrogen exchange and water density at 36°C ( $TBW = {}^3H_2O \times 0.96 \times 0.994$ ).

Body density was measured by UWW in a stainless steel water tank, using a standard method with a technical error of 0.0020 g/cm<sup>3</sup>.<sup>24</sup> Residual lung volume was estimated after immersion in a sitting position by means of the closed-circuit O<sub>2</sub> dilution method.<sup>25</sup>

A DXA scanner (Lunar DPX with software version 3.6; Madison, WI) was used, which has a precision of 1.28% for bone mineral<sup>15</sup> and in the range of 3–4% for body fat.<sup>26</sup> The method uses X-rays of two distinct energy levels that are differently attenuated by bone mineral, fat and fat-free soft tissues. The relative attenuation of the two X-ray energies is used to estimate the proportion of soft tissue as fat. The bone mineral content (BMC) measured by DXA represents ashed bone.<sup>27</sup> One gram of bone mineral yields 0.9582 g of ash, because labile components such as bound water and CO<sub>2</sub> are lost during heating.<sup>28</sup> The BMC therefore needs to be converted to bone mineral as  $Mo = BMC \times 1.0436$  (that is, 1/0.9582).

TBK was determined using the St. Luke's 4 π whole body counter with a precision of 3.2%.<sup>23</sup> Total body content of seven elements was quantified using the *in vivo* neutron activation facilities at Brookhaven National Laboratory.<sup>29</sup> TBN was determined by prompt-γ neutron activation with a precision of 1.8%; TBCa, total body phosphorus, TBNa and TBCl were measured using delayed-γ neutron activa-

tion with precisions of 1.6%, 4.5%, 1.8% and 1.2%, respectively, and TBC was measured using inelastic neutron scattering with a precision of 11.0%.

**Descriptive methods.** There were three descriptive methods, two based on Anth and one based on BIA.

The anthropometric equations of Durnin and Womersley<sup>30</sup> were used to predict body density with logarithmic transformation of the sum of four skinfold thicknesses, triceps, biceps, subscapula and iliac crest. The equations are age and gender specific. Body fat was then calculated from body density. The precision of this method is about 3.5% for percentage body fat.

Steinkamp's anthropometric method was based on assessment of seven circumferences, two limb lengths, five diameters and four skinfold thicknesses. Different prediction equations were applied to estimate body fat according to age, gender and race. The standard error of estimate (SEE) of total body fat was 2.0–3.8 kg.<sup>31</sup>

BIA was used to estimate total body fat using a standard protocol and an RJL instrument (model 101, RJL Systems, Mt Clemens, MI). An equation developed by Segal *et al*<sup>32</sup> was used in the present study to predict body density from resistance values. The Siri-2C equation was then applied to calculate percentage fat from body density.

#### Statistical analysis

Results are expressed as group mean and standard deviation (mean ± s.d.). Simple linear regression analysis was applied to describe the relationship between total body fat measured by the six-compartment model and that estimated by the 16 other methods. The difference in total body fat estimates between the six-compartment model and methods under examina-

tion was related to the six-compartment model, as described by Bland and Altman.<sup>33</sup>

Inter-method differences were compared using statistical methods described by Fleiss.<sup>34</sup> These analyses include calculation of mean absolute difference, technical error (TE) and intraclass coefficient of reliability (CR) to account for the degree of partitioned variance between the participants and the methods.<sup>34</sup> The values for TE were calculated as

$$TE = \sqrt{\frac{\sum_{i=1}^n (y_{i1} - y_{i2})^2}{2n}}$$

where  $y_{i1}$  and  $y_{i2}$  are total body fat, and  $n$  is the total number of subjects. The CR was computed as

$$CR\% = \frac{\sigma_s^2}{\sigma_s^2 + \sigma^2} \%$$

where  $\sigma_s^2 + \sigma^2$  is total variation and  $\sigma_s^2$  is between subject variation.

## Results

Physical characteristics and body composition analysis of the subjects are presented in Table 2. The mean total body fat measured by the six-compartment model was  $19.7 \pm 10.2$  kg or  $25.4 \pm 11.6\%$  of BM for the subject group (Table 3). Values estimated by the 16 methods varied from 17.4 kg (the Segal-BIA method<sup>32</sup>) to 24.3 kg (the Steinkamp anthropometric method<sup>31</sup>). There were no significant differences ( $< \pm 0.3$  kg, paired  $t$  test, all  $P > 0.05$ ) between total body fat measured by the six-compartment model and the values estimated by the Selinger, Baumgartner, Heymsfield, Siri-3C, Brozek, Siri-2C,

and DXA methods. In contrast, the Pace, Lohman, and Kehayias methods underestimated total body fat by 0.9, 1.1 and 2.2 kg (all  $P < 0.05$ ), and Forbes-TBN and TBK methods over-estimated total body fat by 2.1 and 3.2 kg (all  $P < 0.001$ ), respectively. All three statistically-derived methods (the Segal, Durnin, and Steinkamp) substantially under- or over-estimated total body fat ( $P < 0.01$ ).

There were strong correlations ( $r^2 > 0.80$ ) between the six-compartment model and all 16 methods. Very high correlations ( $r^2 > 0.97$ ) also existed between the six-compartment model and the Selinger,<sup>22</sup> Baumgartner,<sup>17</sup> Heymsfield,<sup>8</sup> Cohn,<sup>10</sup> Siri-3C,<sup>5</sup> Pace,<sup>4</sup> and DXA<sup>7,18</sup> methods. Regression equations were developed that relate total body fat estimated by the 16 methods to total body fat derived by the six-compartment model (Table 3).

The results of inter-method difference analysis comparing each method with the six-compartment model, are summarized in Table 4 and Figure 1. The methods of Siri-3C, Heymsfield, Selinger, Baumgartner, and Cohn gave results consistent with the six-compartment model. The technical errors were all less than 0.75 kg with coefficients of reliability above 99.5%. Bland-Altman analysis indicated that the standard deviations of difference between the six-compartment model and these methods were less than 1.1 kg.

## Discussion

### Total body fat reference method

As the 'true' value of total body fat is unmeasurable, a reference with high accuracy is necessary to evaluate other less accurate methods. The reference should meet two main criteria: it should avoid major assumptions and have maximal precision.

Several methods over the span of many years were applied as criteria for total body fat estimation, and these include Forbes-TBK, Pace, Brozek, and Siri-2C methods. Each of these methods is dependent on a major assumption such as potassium or water constancy in FFM or a constant proportion of main FFM components. These assumptions have been challenged over the years and the aforementioned methods may therefore not be ideal as criteria.

The DXA method was recently used as the criterion in some body fat comparison studies.<sup>7,11,28</sup> DXA is based on the differential X-ray attenuation of fat, bone mineral and fat-free soft tissues. This method is methodologically orthogonal to other fat estimation methods. However, the DXA method is dependent on geometric models and the assumption of constant hydration in fat-free soft tissues.<sup>12</sup> There are also now many animal studies which show small but statistically significant discrepancies between DXA fat estimates and carcass lipid content. The DXA

**Table 2** Characteristics and body composition analysis of subject group ( $n = 23$ )

	Mean	s.d.	Range
Age (y)	44.5	16.3	19–69
Body mass (kg)	76.7	12.7	53.9–105.9
Height (m)	1.70	0.11	1.51–1.89
BMI (kg/m <sup>2</sup> )	26.6	4.0	19.3–33.7
Body density (g/cm <sup>3</sup> )	1.0406	0.0244	1.0006–1.0830
Atomic Level			
TBC (kg)	19.7	6.9	9.3–33.9
TBN (kg)	1.81	0.37	1.19–2.53
TBCa (kg)	0.834	0.188	0.552–1.289
TBP (kg)	0.554	0.119	0.367–0.800
TBK (kg)	0.142	0.041	0.085–0.219
TBNa (kg)	0.077	0.013	0.055–0.110
TBCI (kg)	0.062	0.011	0.046–0.093
Molecular Level			
TBW (kg)	42.39	9.24	28.50–59.09
Mo (kg)	2.860	0.673	1.960–4.515

BMI = body mass index; Mo, bone mineral; TB = total body; TBW = total body water.

**Table 3** Total body fat assessed by the six-compartment model and 16 methods

Method (Ref)	Total body fat (kg)			Regression equation			
	Mean	s.d.	Range	a	b	r <sup>2</sup>	SEE (kg)
Segal (32)	17.4	7.2	6.8–32.0	1.36	– 3.94	0.923	2.88
Kehayias (20)	17.5	9.7	2.6–37.8	0.94	1.012	0.938	2.59
Lohman (21)	18.6	9.8	5.4–36.8	1.02	0.77	0.952	2.27
Pace (4)	18.8	10.1	5.2–38.7	0.99	1.006	0.992	0.95
Brozek (6)	19.4	9.2	7.1–39.1	1.08	– 1.25	0.956	2.18
Baumgartner (17)	19.5	9.9	7.0–39.2	1.02	– 0.40	0.989	1.07
Heymsfield (8)	19.6	9.9	6.9–39.5	1.03	– 0.41	0.990	1.02
Siri-3C (5)	19.6	9.9	6.7–40.0	1.02	– 0.24	0.996	0.97
6-C criterion model	19.7	10.2	6.0–40.0	–	–	–	–
Selinger (22)	19.7	10.2	6.9–39.9	1.01	– 0.22	0.989	1.08
DXA (7, 18)	20.0	10.8	4.9–39.4	0.93	1.17	0.972	1.73
Siri-2C (5)	20.0	9.9	6.5–41.2	1.00	– 0.35	0.955	2.21
Cohn (10)	20.7	10.1	7.2–40.9	1.01	– 1.20	0.999	0.22
Forbes-TBN (19)	21.8	10.5	7.2–42.8	0.94	– 0.75	0.937	2.60
Durnin (30)	22.1	9.5	9.7–44.0	0.98	– 1.84	0.837	4.19
Forbes-TBK (2, 3)	22.9	11.9	6.2–44.5	0.82	0.85	0.926	2.82
Steinkamp (31)	24.3	9.5	10.5–44.8	1.02	– 5.07	0.908	3.14

6-C criterion model = the six-compartment neutron activation model; *a* and *b*, the slope and intercept of linear regression equation body mass (BF<sub>6-C model</sub> = *b* + *a* × BF by other method); *r* = correlation coefficient; s.d. = standard deviation; SEE, standard error of estimate.

**Table 4** Inter-method comparison between the six-compartment model and 16 methods

Method (Ref)	Absolute difference		CV	TE	CR %	Bland-Altman analysis		
	mean	s.d.				mean Δ	s.d.	r
Siri-3C (5)	0.78	0.56	0.0343	0.67	99.6	0.12	0.96	0.28
Heymsfield (8)	0.84	0.56	0.0362	0.71	99.5	0.09	1.02	0.34
Selinger (22)	0.86	0.58	0.0372	0.73	99.5	– 0.06	1.06	0.18
Baumgartner (17)	0.88	0.60	0.0380	0.75	99.5	0.08	1.07	0.32
Cohn (10)	1.02	0.23	0.0367	0.74	99.5	– 1.02	0.23	0.39
Pace (4)	1.04	0.75	0.0466	0.90	99.2	0.89	0.92	0.15
DXA (7, 18)	1.51	1.11	0.0661	1.31	98.4	– 0.30	1.87	– 0.27
Siri-2C (5)	1.75	1.25	0.0760	1.51	97.7	– 0.34	2.15	0.22
Lohman (21)	1.78	1.69	0.0897	1.72	97.0	1.08	2.22	0.29
Brozek (6)	1.81	1.30	0.0799	1.56	97.4	0.24	2.24	0.51
Forbes-TBN (19)	2.76	1.94	0.1143	2.37	94.8	– 2.20	2.68	– 0.04
Kehayias (20)	2.87	1.66	0.1256	2.33	94.6	2.18	2.53	0.29
Segal (32)	3.30	2.95	0.1671	3.10	87.8	2.29	3.82	0.85
Forbes-TBK (2, 3)	4.08	2.42	0.1565	3.33	91.1	– 3.26	3.48	– 0.37
Durnin (30)	4.13	2.25	0.1584	3.31	88.9	– 2.40	4.11	0.35
Steinkamp (31)	4.75	2.82	0.1768	3.89	85.2	– 4.60	3.07	0.36

CV = coefficient of variation; CR = coefficient of reliability; TE = technical error (kg); mean Δ = mean difference between the standard 6-C model and the other methods (kg); *r* = correlation coefficient; s.d. standard deviation; DXA = dual-energy X-ray absorptiometry

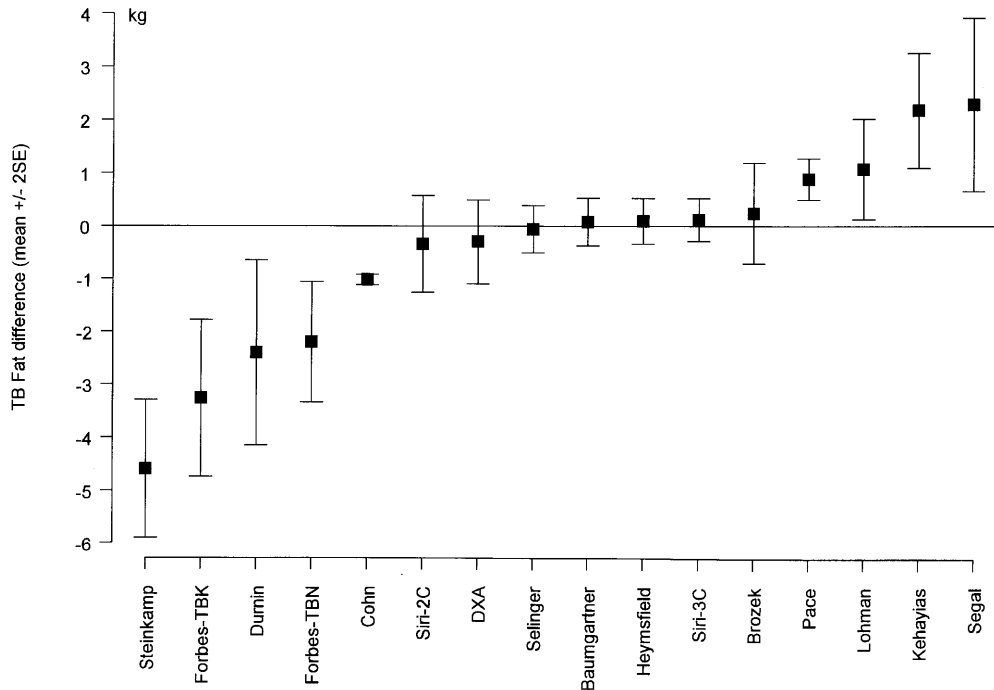
method may, therefore, not be an ideal fat measurement criterion.

The Cohn neutron activation model was also applied as a criterion in earlier studies.<sup>9</sup> In this model, FFM is estimated by summing the major chemical components including water, protein and bone mineral. However, Cohn’s model ignored two components, soft tissue mineral and G. This results in a small, but systematic, over-estimation of total body fat.

A six-compartment body composition model was applied in the present study that extends the earlier approach suggested by Cohn *et al.*<sup>10</sup> The error caused by measurement of model components ( $\sigma_{\text{fat}}$ ) can be estimated in the present study subjects by assuming a mean body composition as shown in Table 2 and measurement precision as stated in the Methods section. Accordingly,

$$\begin{aligned}
 (\sigma_{\text{fat}})^2 &= (0.1)^2 + (42.39 \times 0.015)^2 \\
 &+ (6.525 \times 1.81 \times 0.018)^2 \\
 &+ (2.709 \times 0.834 \times 0.016)^2 \\
 &+ (2.76 \times 0.142 \times 0.032)^2 \\
 &+ (0.077 \times 0.018)^2 \\
 &+ (1.43 \times 0.062 \times 0.012)^2
 \end{aligned}$$

Solving this equation, the propagated measurement error ( $\sigma_{\text{fat}}$ ) of the six-compartment model is 0.68 kg of body fat for the present study subjects. Additionally, this model has minimal model error and has no assumptions that are known to be influenced by age, gender, race and disease. Although model and measurement errors are likely to be small, the six-compartment model, as with all *in vivo* methods, ultimately requires evaluation in human cadavers



**Figure 1** Inter-method comparison of total body fat mass (BF) measurements. The mean and standard error of estimation (mean  $\pm$  2 s.e.m.) between total body fat measured by the six-compartment criterion model and other 16 methods are indicated.

and animals. Moreover, as the methods used in developing the six-compartment model (prompt and delayed- $\gamma$  *in vivo* neutron activation analysis) are not widely available and include radiation exposure, there is little role for the model in routine subject testing. The importance of the six-compartment model is that it can be used to evaluate other body composition methods.

### Inter-method comparison

A method designed to quantify BF should be equally accurate in health and disease, and across all age, gender and race groups. The 16 evaluated methods, however, differed widely in their estimates of total body fat in our cohort of healthy ethnically mixed subjects (Table 3 and Figure 1). Each of the methods outlined in the table has two sources of error: 1. reference method error (for statistically-derived methods) or model assumption error (for model-based methods); and 2. measurement error.

Ideally, a body composition method should have low TE and high CR. The 16 currently used methods varied in the results of the inter-method analysis (Table 4), and they can be empirically organized into three groups as follows:

Group 1 methods all have TE's < 0.8 kg, CR's larger than 99.5%, and s.d. of method difference (i.e., Bland-Altman analysis) < 1.1 kg. These methods, Baumgartner, Heymsfield, Selinger, Siri-3C and Cohn all share in common their derivation from multi-compartment body composition models and a requirement for TBW estimates. Although water measurement is still required, model assumptions do not involve TBW which is the largest molecular level

component. Accordingly, the model error of these methods should be small and total body fat measurements by these five methods are not known to be age, gender, race and health status dependent. These methods form a core with the six-compartment criterion model. Because of their small TE's and high CR's these methods are well suited for accurate measurement of body fat. The Cohn neutron activation model systematically over-estimated total body fat by about 1.0 kg or 5%, presumably because the model ignores two small components, soft tissue mineral and G.

There are other published models in this group that we did not evaluate, such as the three and four compartment models of Fuller *et al.*<sup>35</sup> These methods were very similar to those in their first group of multicompartiment models and their results as analyzed in this report would be indistinguishable from those presented in Table 3 and Table 4.

As neutron activation facilities are limited and the six compartment model cannot be applied in most research laboratories, the present findings suggest that the simpler and reduced radiation methods proposed by Baumgartner, Heymsfield, Selinger and Siri-3C may serve as alternative practical reference methods. However, it should be noted that the present study subjects were relatively young, and comparative analyses such as those set forth in the present study should be extended to other populations such as the elderly.

Group 2 includes methods with TE's between 1 and 2 kg, CR's between 97% and 99%, and s.d.'s of method difference (i.e., Bland-Altman analysis) < 2.5 kg (Pace method's TE is 0.90 kg). These include the DXA, Pace, Siri-2C, Brozek and Lohman methods. The DXA, Pace, Siri-2C and Brozek methods, share in

common an assumption of constant hydration at 0.73, making these methods more appropriate in normal subjects than in disease states in which hydration may be abnormal. Lohman's method also assumes a constant ratio of TBW to protein.<sup>21</sup> This group of methods are therefore suitable for use in subjects with normal hydration and may not be applicable across all age, gender, and disease groups. There might be an error if these methods are applied to subjects with abnormal hydration such as AIDS and obese patients.

Among the 16 fat methods, the DXA method uniquely provides both whole body and regional body composition measurements. Use of DXA for assessing body fat is supported by several studies.<sup>7,11,28</sup> Table 3 and Table 4 establish the accuracy of the DXA method in healthy subjects. However, the errors of the DXA method are still of concern if it were to be used as the criterion. This is because the DXA method is dependent on the assumption of uniform hydration.<sup>18</sup> Recently, Stall *et al*,<sup>36</sup> pointed out that DXA is suited for use in well-dialyzed peritoneal dialysis patients. The question still exists if DXA can accurately estimate total body fat for other patients in whom hydration is abnormal. Finally, in a recent study, DXA failed to accurately measure the composition of packets containing various materials placed over selected anatomic sites in human subjects.<sup>37</sup> Although these experiments have been criticized,<sup>38</sup> there remain important questions related to underlying DXA geometric reconstruction models.<sup>12</sup>

Group 3 includes methods with TE's > 2 kg, CR's < 95% and Bland-Altman analysis s.d.'s of > 2.5 kg. These include the Forbes-TBN, TBK, Segal-BIA, and two anthropometric methods. Both Segal-BIA and anthropometric methods are statistically derived and are referenced against traditional two-compartment methods. For example, the Steinkamp *et al*<sup>31</sup> method used both the Brozek and Forbes-TBK methods as the reference. The error of these traditional methods is thus propagated into the anthropometric method. Moreover, the BIA and anthropometric methods have potential TE such as incorrect gel electrode position and skinfold estimation. Therefore, it is not surprising that the two anthropometric methods have the largest TE (> 3 kg) and the lowest CR (< 90%) of the 16 methods. This group of methods is therefore not suitable for situations in which accuracy is required, although they may still be applied in field studies where low cost and ease of performance are important.

Of the 16 methods, the Kehayias *et al*<sup>20</sup> total body carbon (TBC) method is virtually orthogonal to other fat estimation methods. It is derived from the proportions of carbon in the carbon-containing components including fat, protein, G and mineral. This method is not effected by hydration state and results are not dependent on age, gender, race and disease status. Moreover, the Kehayias *et al*<sup>20</sup> method has only a small model error because the quantitative proportions of carbon in the carbon-containing components is

determined by rigorous chemical relationships. As Table 3 and Table 4 show, however, there is a considerable difference in BF as estimated by the Kehayias *et al*<sup>20</sup> method and the six-compartment criterion model. This discrepancy could be explained by a measurement error of TBC in the present investigation. Previous studies on phantoms reported a 3% (CV) precision of TBC estimation by inelastic neutron scattering.<sup>20</sup> However, our group recently reported a poor precision (CV = 11.0%) when TBC was estimated in humans.<sup>29</sup> We also calculated expected carbon content of the present study subjects using total body fat measured by the six-compartment model ( $21.4 \pm 7.4$  kg) and calculated TBC was significantly larger than that estimated by inelastic neutron scattering ( $19.5 \pm 6.7$  kg,  $P = 0.0005$ ), although the two estimates are highly correlated ( $r = 0.97$ ). This indicates that our inelastic neutron scattering facility may now underestimate TBC by 1.9 kg or 8.9%. If calibration of our inelastic scattering system for measuring TBC can be improved, the Kehayias *et al*<sup>20</sup> method would then be accurate relative to the six-compartment model for total body fat estimation.

#### Study limitations

Although the present study was comprehensive in evaluating fat estimation methods, our approach nevertheless was not exhaustive. We therefore included widely used descriptive anthropometric and bioimpedance formulas for estimating total body fat, but there were many other equations which, for practical reasons, were not included in the present study protocol. The possibility exists to evaluate these formulas in future validation studies.

One reason we limited our analysis to 16 methods, is that our subject pool was relatively small and included 17 men and six women. Hence, evaluating method validity in larger and more diverse subject pools would be a useful extension to the present investigation. A larger subject pool would allow examination of method validity in specific subgroups such as within male and female, ethnic, and age groups.

## Conclusion

The present study reports a comprehensive comparison of fat measurement methods using a six-compartment model based on *in vivo* neutron activation analysis as the criterion. Results clearly show between-method agreement differences with criterion fat estimates, although empirically three groups emerged. Fat estimates by the first group of methods were strongly associated (for example, by technical error, coefficient of reliability and Bland-Altman analysis) with criterion fat estimates, which suggests they are applicable as reference methods in future studies. Methods in the second group showed less

agreement with criterion fat estimates, opening questions as to their specific roles in clinical evaluations and in research studies. The third and least-associated group of methods showed relatively poor associations with criterion fat estimates and may be of value only for group estimates in field studies. Finally, while the present investigation was comprehensive in terms of evaluated methods, future similar studies are needed in more varied and larger cohorts.

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