Improved 4-compartment body-composition model for a clinically accessible measure of total body protein\textsuperscript{1–3}

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ABSTRACT

Background: Muscle wasting is a consequence of many primary conditions including sarcopenia, cachexia, osteoporosis, HIV/AIDS, and chronic kidney disease. Unfortunately, there is not a clinically accessible method to measure total body protein, which is the functional mass of muscle.

Objective: We sought to derive a simple method to measure total body protein by using dual-energy X-ray absorptiometry (DXA) and bioimpedance analysis (BIA).

Design: We retrospectively analyzed a clinical convenience sample of individuals with numerous metabolic conditions from the Monash Medical Centre, Melbourne, Australia, who had a concurrent protein measure by using neutron activation analysis-derived protein (NAA-TBPro), water measure by using BIA, and whole-body DXA scan. The study was split into calibration and validation data sets by using simple random sampling stratified by sex, BMI category, and age decade. We generated a protein estimate direct-calibration protein (DC-TBPro) derived from BIA water, bone mass, and body volume. We compared NAA-TBPro with DC-TBPro and 2 protein estimates from the literature, one that used the DC-TBPro equation with fixed coefficients [4-compartment Lohman method for analysis of total body protein (4CL-TBPro)] and another that used fat-free mass, age, and sex [Wang equation–derived protein (W-TBPro)].

Results: A total of 187 participants [119 women; mean (±SD) age: 37.0 ± 15.4 y; mean (±SD) BMI (in kg/m\(^2\)) 24.5 ± 7.7] were included. When plotted against NAA-TBPro, DC-TBPro had the highest correlation [coefficient of determination (\(R^2\)) = 0.87], lowest root mean squared error (RMSE: 0.87 kg), and fewest outliers compared with 4CL-TBPro (\(R^2\) = 0.75; RMSE = 1.22 kg) and W-TBPro (\(R^2\) = 0.80; RMSE = 1.10 kg).

Conclusions: A simple method to measure total body protein by using a DXA system and BIA unit was developed and compared with NAA as proof of principle. With additional validation, this method could provide a clinically useful way to monitor muscle-wasting conditions. Am J Clin Nutr doi: 10.3945/ajcn.112.048074.

INTRODUCTION

Muscle wasting affects numerous individuals with a variety of pathologies. Between 5% and 13% of elders aged 60–70 y old and 11–50% aged ≥80 y suffer from sarcopenia in the United States (1). Nearly 10% of HIV/AIDS patients suffer from significant weight loss and wasting (2). Chronic kidney disease, which is commonly complicated with protein-energy wasting, has increased in prevalence by 43% from 1991 to 2001 (3). Cachexia, which is a term that describes the complex disorder related to total weight loss, functional mass reduction, and inflammation, is prevalent in >30 million Americans (4).

Current muscle wasting, shown in all of these conditions, is a loss of functional mass that results in decreased strength and reduced bone mass. Currently, there is no technique that directly measures functional muscle mass. Because most protein is in muscle, it could be argued that total body protein is a surrogate for functional mass. Unfortunately, there is no clinically useful measure for the quantity of or change in distribution of total body protein (5–7).

Neutron activation analysis (NAA)\textsuperscript{4} is considered the criterion measure for in vivo total body protein (8, 9), despite its cost and overall clinically inaccessibility. NAA assumes that protein has a specific chemical stoichiometry and a nitrogen to protein ratio of 0.16 (10) and contains all body nitrogen; all of these assumptions have been validated in cadaver studies (11). Because of the neutron radiation dose (0.32 mSv), the US Nuclear Regulatory Commission highly regulates access to NAA devices. To our knowledge, there is only one clinical NAA system currently used in the United States and fewer than a dozen internationally available for research studies. Because there is no

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\textsuperscript{4}Abbreviations used: ADP, air-displacement plethysmography; ADP-volume, air-displacement plethysmography total body volume; BIA, bioimpedance analysis; BMC, bone mineral content; C1–C3, 3 subjects in the calibration data set; DC-TBPro, direct-calibration protein; DXA, dual-energy X-ray absorptiometry; DXA-volume, total body volume estimated from dual-energy X-ray absorptiometry–reported mass compartments; FFM, fat-free mass; FM, fat mass; NAA, neutron activation analysis; NAA-TBPro, neutron activation analysis–derived protein; RMSE, root mean squared error; V1–V4, 4 subjects in the validation data set; W-TBPro, Wang equation–derived protein; 4C, 4-compartment; 4CL-TBPro, 4-compartment Lohman method for analysis of total body protein.

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direct, clinically accessible measure of functional mass, lean soft tissue mass has been used as a surrogate for functional mass and protein. However, lean soft tissue is composed primarily of water (12); in essence, lean soft tissue mass is driven by the muscle hydration level and not functional mass.

Lohman’s 4-compartment (4C) body-composition model decouples water from lean soft tissue and is considered a gold standard in body composition; this model solves for fat mass (FM) by using a dual-energy X-ray absorptiometry (DXA) scan, total body water by using deuterium dilution, and body volume by using air-displacement plethysmography (ADP) or underwater weighing (13). From Lohman’s 4C model, we can estimate protein from the residual mass component; unfortunately, this approach potentially generates a lot of uncertainty in the protein measure because the error in the protein compartment is the sum of the errors in the other compartments. This model is not practical because it involves direct volume measurement by using ADP, which is not in a typical clinical suite, takes >4 h to acquire deuterium-dilution measures, and generally requires days for clinical water analysis.

The objective of this preliminary investigation was to derive a clinically useful total-body protein measure with few procedures, optimized time, minimal cost, and adequate access for patients. In this article, we present a simplification of Lohman’s 4C body-composition model by making 2 modifications. First, we solved directly for protein by body volume estimated by using DXA whole-body measures; second, we substituted bio-impedance analysis (BIA) for deuterium dilution to measure total body water. We compared our method with 2 other protein estimates (13, 14). With our method, we were able to measure and report total body protein in <10 minutes.

SUBJECTS AND METHODS

Our study design was a retrospective analysis of a clinical sample of convenience. Each participant received a body-composition evaluation that included the following techniques and measures: NAA, BIA, ADP, whole-body DXA, weight, and height. Three different protein estimates described [4-compartment Lohman method for analysis of total body protein (4CL-TBPro), Wang equation–derived protein (W-TBPro), and direct-calibration protein (DC-TBPro)] were generated for each individual and compared with neutron activation analysis-derived protein (NAA-TBPro).

Participants

Participants were recruited at the Body Composition Laboratory, Monash Medical Centre, Southern Health (Melbourne, Australia) after inpatient and outpatient referral from clinical units. Laboratory measurements for clinical purposes received general approval from the Southern Health Human Ethics Committee, including procedures that involved radiation exposure. Study participants included patients with chronic renal disease, intestinal failure, anorexia, malnutrition, obesity, liver disease, inherited metabolic disorders, and others. We were given body-composition measures for 189 individuals, but one person was excluded because of invalid DXA-scan results. Although most individuals (n = 171) had measurements from a single visit, some individuals (n = 18) had both baseline and follow-up measurements. Only baseline measurements were used in the overall study population, which was split into 2 data sets (calibration and validation). The calibration data set was used to generate coefficients for the protein measure DC-TBPro, and the validation data set was used to compare all protein measures. The total study population was split into these data sets by simple random selection stratified by sex, BMI category, and age decade (by decade, with individuals >70 y of age in a single group) with a sampling rate of 0.5 for each of the stratified categories. The calibration population consisted of 109 individuals (68 women), and the validation population consisted of 78 individuals (51 women).

NAA

Total body protein was calculated from the direct measurement of total body nitrogen by using prompt-γ in vivo NAA, in which 1 g N = 6.25 g protein (15, 16). In addition, the nitrogen index, which is a normalized comparison of actual total body protein to predicted total body protein, was calculated, whereby the absolutely normal nitrogen index was 1.00 (with an SD of 0.80) (17). In practice, subjects laid supine on a movable bed, which was positioned to center the trunk section in a collimated neutron beam that measured 40 × 20 cm at a bed level 35 cm above a 10 μg 252Cf neutron source. Induced γ-ray characteristics of body nitrogen were measured over a 1000-s exposure by two 10 × 10 × 15 cm thallium-doped sodium iodide detectors, one positioned on each side of the patient. Subject measurements were calibrated against a phantom and correction factor applied to account for variations in body width and thickness. The effective whole-body radiation dose was 0.32 mSv, and the quality factor (Q) was 20 to overestimate any radiation exposure (18). The intraassay and interassay accuracy and precision were established in phantoms as CVs of >97% and <4% and of >98% and <5%, respectively (17).

BIA

A single-frequency tetrapolar bioelectrical impedance analysis was performed by using an 800-μA (50-kHz) alternating current. After fasting for a minimum of 4 h and bladder emptying, patients adopted a supine position with arms spread apart from the body and legs separated. Signal input and output electrodes were placed on the dorsum of the right hand and foot. Recording electrodes were placed ipsilaterally 5 cm proximal to the output electrodes. Total body resistance was measured in ohms by using an Impedimed DF50 body-composition analyzer (Impedimed). Total body water was estimated by using the equation of Lukaski and Bolonchuk (19).

ADP

ADP measures were acquired by using the Bod Pod capsule (COSMED USA) to measure total body volume. Before each measurement, the instrument was calibrated by placing a hollow cylinder with a known mass and volume into the Bod Pod. Participants wore a spandex or silicone swim cap and tight-fitting, spandex-like shorts and sport bra (for women) to reduce the effects of isothermal air. The participant was sealed in the Bod Pod capsule. Body volume was measured by using the body’s air displacement with corrections for residual lung volume and
surface-area artifacts (20). Thoracic gas volume was estimated from a nomogram that involved age, sex, weight, and height provided by the manufacturer’s software. The surface-area artifact was automatically calculated with the Bod Pod software. These 2 adjustments (thoracic gas volume and surface-area artifact) were factored into the overall calculation of body volume.

**DXA imaging**

Each participant received a whole-body DXA scan by using the GE-Lunar Prodigy device (GE Medical Systems Lunar) and analyzed with software (Lunar enCORE version 12, GE Medical Systems Lunar). Participants were positioned as instructed by the manufacturer. Briefly, each participant was scanned in the same clothing used for the ADP measure and centered on the table. The scan took ~7 min with an X-ray dose of 0.1 μSv. All participants were scanned by using the medium-thickness scan mode. The analysis regions used were the standard regions where the head, torso, arms, and legs were all subdivided. The analysis report included the total-body bone mineral content (BMC) (g), bone area (cm²), areal bone mineral density (g/cm²), FM (g), soft tissue lean mass (g), and total mass (g). Daily quality-assurance and calibration phantom scans were acquired for each day of use. The precision of phantom scans during the study period was 0.8% CV, and no calibration drifts were observed.

**Protein estimates**

Protein was estimated by using the following 3 different methods: one method was derived from a variant of the 4C Lohman model (4CL-TBPro) (13), another method was estimated according to the method of Wang et al (14) by using fat-free mass (FFM), age, and sex (W-TBPro), and another method was directly calibrated to NAA by using whole-body DXA data and BIA water (DC-TBPro).

**4CL-TBPro**

The standard 4C model solves for fat by using total mass, total body volume (TBV) by using ADP, total body water (TBW) by deuterium dilution, and BMC by using DXA. This model assumes specific compartment densities for water, fat, protein, and BMC (13). FM is estimated using the following equation:

\[
FM \text{ (kg)} = 2.747 \times TBV - 0.714 \times TBW + 1.146 \times BMC - 2.050 \times \text{mass}
\]

With the use of this fat measure, protein mass is estimated as the residual mass when fat, water, and BMC components are removed from the total mass, which results in the following equation:

\[
4CL-\text{TBPro (kg)} = 3.050 \times \text{mass} - 0.286 \times \text{TBW} - 2.146 \times \text{BMC} - 2.747 \times \text{TBV}
\]

In this study, we generated a variant of this Lohman model by using BIA to measure total body water because deuterium dilution was not available.

**W-TBPro**

A prediction model was derived by Wang et al (14) that exclusively used DXA FFM, age, and sex. The estimate was derived in 144 adults with a mean age of 44 y and age range from 20 to 94 y, which resulted in the following equation:

\[
W-\text{TBPro} = 0.156 \times \text{FFM} - 0.028 \times \text{age} + 0.55 \times \text{sex} + 2.77
\]

FFM is the sum of the lean soft tissue and bone mass; the study reported a root mean squared error (RMSE) to NAA-TBPro of 0.86 kg and coefficient of determination \((R^2)\) of 0.83.

**DC-TBPro**

This protein-measurement method is similar to the 4C Lohman method except that it solves for protein directly instead of treating it like a residual mass component. This method is done in a 2-step process by estimating volume and calibrating to protein. We previously reported how volume can be derived from DXA scans (21). In this study, we simplified the approach by defining DXA-derived volume [total body volume estimated from dual-energy X-ray absorptiometry–reported mass compartments (DXA-volume)] by using compartmental masses of lean, fat, and BMC such as previously reported (22) so that

\[
\text{DXA-volume} = v_{\text{lean}} \times \text{lean} + v_{\text{fat}} \times \text{fat} + v_{\text{BMC}} \times \text{BMC} + v_{\text{residual}}
\]

Coefficients \(v_{\text{lean}}, v_{\text{fat}}, \text{and } v_{\text{BMC}}\) should represent the inverse volumetric densities of lean soft tissue, fat, and BMC and were shown by equating air-displacement plethysmography total body volume (ADP-volume) to Equation 4. The coefficient \(v_{\text{residual}}\) represents the residual volume that the 3 DXA mass compartments (lean, fat, and BMC) do not fully capture in the total body volume.

Protein is estimated by generating an equation in a similar format as Equation 2. However, we showed calibration coefficients that related the same measures used to generate 4CL-TBPro (volume, mass, BMC, and water) to NAA protein directly as follows

\[
\text{DC-TBPro} = c_{\text{volume}} \times \text{volume} + c_{\text{mass}} \times \text{mass} + c_{\text{BMC}} \times \text{BMC} + c_{\text{water}} \times \text{water} + c_{\text{residual}}
\]

The coefficients (represented by \(c\) in Equation 5) should relate to the volumetric densities of the individual mass compartments (water, fat, protein, and BMC).

**Statistics**

All statistics were performed with SAS software (version 9.2; SAS Institute). The study population was split into calibration and validation subsets by simple random selection stratified by sex, BMI category, and age decade (by decade with subject >70 y of age in a single group) by using the surveyselect procedure in SAS with a sampling rate of 0.5 for each of the stratified
categories. We used multiple linear regression techniques on the calibration data set to generate coefficients for Equations 4 and 5. These coefficients were used to generate DXA-derived body volumes and directly calibrated protein measures (DC-TBPro) in calibration and validation data sets. All protein estimates (4CL-TBPro, W-TBPro, and DC-TBPro) were compared with the reference method NAA protein (NAA-TBPro) by using linear regression, which generated $R^2$, RMSEs, regression slopes, regression intercepts, and standardized residuals per observation for each data set. The pairwise, Bonferroni-adjusted $t$ test was used to test mean differences between NAA-TBPro and the 3 protein estimates individually. Statistical significance from zero was defined at the $P < 0.05$ threshold.

RESULTS

Population characteristics for the calibration and validation data sets are listed in Table 1. Calibration and validation populations had no significant differences in characteristics except for height (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Characteristics of calibration and validation data sets</th>
<th>Calibration population</th>
<th>Validation population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (n)</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>10–19 y</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>20–29 y</td>
<td>30</td>
<td>26</td>
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<td>4</td>
</tr>
<tr>
<td>70–79 y</td>
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<td>0</td>
</tr>
<tr>
<td>80–89 y</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>BMI (n)</td>
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<td></td>
</tr>
<tr>
<td>Underweight (&lt;18.5 kg/m$^2$)</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>Normal (18.5 to &lt;25 kg/m$^2$)</td>
<td>46</td>
<td>39</td>
</tr>
<tr>
<td>Overweight (25 to &lt;30 kg/m$^2$)</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>Obese I (30 to &lt;35 kg/m$^2$)</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Obese II (35 to &lt;40 kg/m$^2$)</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Obese III (≥40 kg/m$^2$)</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Anthropometric measures</td>
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<tr>
<td>Weight (kg)</td>
<td>67.42 ± 24.48$^2$</td>
<td>67.56 ± 20.74</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.56 ± 11.91</td>
<td>166.93 ± 7.72</td>
</tr>
<tr>
<td>DXA</td>
<td></td>
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<tr>
<td>Fat mass (kg)</td>
<td>22.19 ± 16.71</td>
<td>21.97 ± 15.47</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>42.52 ± 11.27</td>
<td>42.92 ± 10.29</td>
</tr>
<tr>
<td>Bone mineral (kg)</td>
<td>2.45 ± 0.64</td>
<td>2.49 ± 0.54</td>
</tr>
<tr>
<td>BIA</td>
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<tr>
<td>Total body water (L)</td>
<td>34.68 ± 10.47</td>
<td>35.33 ± 8.81</td>
</tr>
<tr>
<td>ADP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body volume (L)</td>
<td>65.60 ± 25.46</td>
<td>64.64 ± 21.80</td>
</tr>
<tr>
<td>NAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total body protein (kg)</td>
<td>8.63 ± 2.34</td>
<td>8.86 ± 2.42</td>
</tr>
</tbody>
</table>

$^1$Height was the only characteristic with a significant ($P < 0.05$) difference between calibration and validation data sets. Statistical differences between groups was determined by using the $t$ test. ADP, air-displacement plethysmography; BIA, bioimpedance analysis; DXA, dual-energy X-ray absorptiometry; NAA, neutron activation analysis.

$^2$Mean ± SD (all such values).

For the DC-TBPro method, total body volume was first estimated from DXA and used to measure protein. With the use of the calibration data set, coefficients for Equation 4 were generated (all with $P < 0.01$) as follows: $\nu_{\text{lean}} = 0.933$, $\nu_{\text{fat}} = 1.150$, $\nu_{\text{BMC}} = -0.438$, and $\nu_{\text{residual}} = 1.504$. Coefficients of lean, fat, and BMC generated for Equation 4 should correspond to the inverse densities of each of the DXA components. From our calibration data set, the average densities for lean, fat, and BMC were 1.072, 0.870, and $-2.283$ kg/L, respectively. The residual coefficient corresponded to a volume of 1.504 L that was not explained by the lean, fat, and BMC components. After generating these calibration coefficients, the DXA-volume measure was calculated in the validation data set. As shown in Figure 2, there was a high correlation between ADP-volume and DXA-volume (generated from Equation 4) in calibration and validation data sets. In the validation data set, DXA-volume explained 99% of the ADP-volume variation with a small RMSE (0.51 L) but no significant intercept. There was no significant difference between mean values of ADP-volume and DXA-volume by using the $t$ test for calibration and validation data sets.

Coefficients for DC-TBPro (Equation 5) were generated by using the calibration data set as follows: $\nu_{\text{vol}} = -0.607$ ($P < 0.0001$), $\nu_{\text{max}} = 0.623$ ($P < 0.0001$), $\nu_{\text{water}} = 0.150$ ($P < 0.0001$), $\nu_{\text{BMC}} = 0.132$ ($P = 0.61$), and $\nu_{\text{residual}} = 1.076$ ($P = 0.0004$). These new calibration coefficients were used to measure DC-TBPro in both data sets.

Linear regression results of NAA-TBPro compared with each of the protein-measurement methods (4CL-TBPro, W-TBPro, and DC-TBPro) for calibration and validation data sets, respectively, are displayed in Table 2. On the group level, there were only significant differences (by using the Bonferroni-adjusted $t$ test for multiple comparisons) from NAA-TBPro with 4CL-TBPro in the calibration and validation data sets. The correlation between NAA-TBPro and the 3 protein-measurement methods for the calibration and validation data sets is shown in Figure 2. Compared with other protein estimates in the calibration data set, DC-TBPro had the highest $R^2$ (0.90), the lowest RMSE (0.76 kg), an identity slope, and no intercept. In the validation data set, DC-TBPro still had the highest $R^2$ (0.87), lowest RMSE (0.87 kg), a slope close to identity (1.04), and no significant intercept. W-TBPro and 4CL-TBPro came in second and third places, respectively, in terms of a high $R^2$, a low RMSE, a slope close to identity, and an intercept close to zero for calibration and validation data sets.

Explorations of outlier data are important to understand possible causes of protein-estimate inaccuracy. The 7 individuals [3 subjects in the calibration data set (C1–C3); 4 subjects in the validation data set (V1–V4)] that had at least one significantly different (defined as $\geq 3$ SDs by using Studentized residuals from linear regression analysis) protein measure (4CL-TBPro, W-TBPro, or DC-TBPro) from NAA-TBPro are shown in Table 3. Subject V4 had the lowest NAA-TBPro and nitrogen index value in the validation data set. Subjects V1 and V4 had BMC values (1.9 and 1.6 kg, respectively) that were $>1$ SD below the mean for the validation data set. Subjects C1, C3, and V2 had weight and ADP-volume measurements (147.1, 146.6, and 145.4 L, respectively) that were $>1$ SD below the mean for the validation data set. Subjects C3, V3, and V4 had very different ratios of total body water to lean mass (0.98, 1.12, and 0.66, respectively) that were almost 1 SD away from the data set mean values. Subjects
C1 and V2 had low ratios of water to weight (0.34 and 0.34, respectively) that were almost 1 SD below the data set means. Subjects C2, V1, and V3 had 4CL-TBPro values significantly different from NAA-TBPro values. Subjects C1, C3, V2, and V4 had W-TBPro values significantly different from NAA-TBPro values. Subjects C3 and V4 had DC-TBPro values significantly different from NAA-TBPro values.

DISCUSSION

We have developed a simple and clinically accessible protein measure (DC-TBPro) that improves on Lohman’s 4C body-composition model but does not require expensive, uncommon measurement techniques such as NAA or ADP. DC-TBPro can be measured in <10 minutes with 2 clinically common devices (a DXA machine and BIA unit), uses a single setup for a large range of ages and body types (23, 24), and does not require the patient to move between measurements. Ultimately, this protein measure could be a surrogate for functional mass in muscle-wasting conditions if fully validated.

In a clinical data set that included a wide variety of conditions and body types from anorexia and muscle wasting to short bowel syndrome and severe obesity, our protein estimate (DC-TBPro) had a higher accuracy, lower RMSE, and fewer outliers to the reference-method protein measure (NAA-TBPro) compared with 2 other protein estimates (4CL-TBPro and W-TBPro).

Because NAA is not clinically feasible, techniques for the estimation of protein have been developed besides W-TBPro and 4CL-TBPro. The measure of midarm muscle area by using calipers has been used as a surrogate for total body protein because muscle contains a large percentage of protein (25). Unfortunately, caliper measures of subcutaneous adipose have poor accuracy and poor interobserver agreement (26), muscle area is directly affected by body hydration (27), and the midarm muscle area is poorly correlated to the actual protein status (28).

Lohman’s 4C model can be used to estimate protein mass and assumes the body is composed of fat, water, bone mineral, and a residual component (13). This residual mass contains all

| TABLE 2 |
| Linear regression estimates between NAA-TBPro and 3 protein estimates for calibration and validation data sets |
|-----------|----------|----------|----------|
| Calibration data set | 4CL-TBPro | W-TBPro | DC-TBPro |
| $R^2$ | 0.69 | 0.82 | 0.90 |
| RMSE (kg) | 1.31 | 1.00 | 0.76 |
| Intercept (kg) | 1.29 | −0.48 | 0.00 |
| Slope | 0.72* | 1.02* | 1.00* |
| Mean ± SD (kg) | 10.24 ± 2.72 | 8.93 ± 2.08 | 8.63 ± 2.22 |
| Difference from NAA-TBPro (kg) | 1.62 (1.33, 1.91)* | 0.30 (0.11, 0.49) | 0.00 (−0.14, 0.14) |
| Validation data set | 4CL-TBPro | W-TBPro | DC-TBPro |
| $R^2$ | 0.75 | 0.80 | 0.87 |
| RMSE (kg) | 1.22 | 1.10 | 0.87 |
| Intercept (kg) | 1.18* | −1.35* | −0.32 |
| Slope | 0.75* | 1.13* | 1.04* |
| Mean ± SD (kg) | 10.27 ± 2.80 | 9.05 ± 1.91 | 8.81 ± 2.17 |
| Difference from NAA-TBPro (kg) | 1.42 (1.10, 1.74)* | 0.19 (−0.06, 0.45) | −0.05 (−0.24, 0.15) |

1 Statistical significance from zero was defined at the $P < 0.05$ threshold. *Statistically significant ($P < 0.05$). DC-TBPro, direct calibration protein; NAA-TBPro, neutron activation analysis-derived protein; RMSE, root mean squared error; W-TBPro, Wang equation–derived protein; 4CL-TBPro, 4-compartment Lohman method for analysis of total body protein.  
2 Mean; 95% CI in parentheses (all such values).
other mass including protein, glycogen, and residual soft tissue minerals. Lohman’s 4C model solves for FM directly and lumps protein into the residual compartment. Because glycogen and soft tissue mineral account for \( \frac{1}{100} \% \) of total body mass, we initially thought that the residual compartment from this model could be a reasonable and accurate estimate of total body protein. However, we found 4CL-TBPro to be a very noisy measurement. We believe DC-TBPro has the lowest RMSE value because it initially solves the equation coefficients for protein compared with fat (Table 2) and because of the reduced number of measurement techniques. These aspects result in a higher \( R^2 \) and lower RMSE to NAA-TBPro than 4CL-TBPro.

Lohman’s 4C body-composition model has not been clinically accessible because it requires a total-body volume measurement, total-body water measure, and DXA scan (13). Underwater weighing, which was once considered a gold standard measurement of body volume, has been replaced with ADP devices that use air displacement instead of water (20). Total body water is typically measured by stable isotope dilution, which involves the ingestion of heavy water, waiting \( 4 \) h to fully equilibrate, taking a water sample (via blood, urine, or saliva), and doing fractional analysis by using one of several spectroscopic techniques to determine the isotope dilution. BMC is measured by using DXA. Together, these procedures require the subject to fast and wait for 4 h to collect enough water samples for full analysis. This model requires time-consuming procedures and potential subject travel from one device to another. In our approach, clinicians only need one location, a DXA scanner, and BIA unit to determine total-body protein levels.

4CL-TBPro had a few outliers compared with NAA-TBPro (labeled in Figures 1 and 2 and described in Table 3). The outlier of subject C2 underestimated protein (14.5 kg) because of regression line fitting; 4CL-TBPro is closer in value to NAA-TBPro (15.8 kg) than W-TBPro (13.3 kg) and DC-TBPro (14.2 kg). The outlier of subject V1 overestimated 4CL-TBPro because Equation 2 had a negative BMC coefficient, and the participant had a very low BMC (1.9 kg). The outlier of subject V3 underestimated 4CL-TBPro because of a possible overestimation of total body water, which was indicated by a water-to-lean ratio of 1.12. As shown by Equation 2, an overestimated water measure results in an underestimated protein measure because of the negative coefficient of water.

Wang et al (14) developed a model for protein (W-TBPro) by using only DXA FFM, age, and sex in a cohort of strictly healthy individuals. Although W-TBPro is more accurate than 4CL-TBPro, W-TBPro relies on FFM, which groups water, protein, and BMC into a single measure. Unfortunately, this model failed to account for the coupling of some water and protein into the DXA-reported fat and lean measures (29). With the inclusion of a direct measurement of water into DC-TBPro, we were able to decouple water from each component more accurately than with W-TBPro. In addition, W-TBPro was developed from a cohort of healthy individuals, which raises concerns for its use in diseased states. In our study, we had a cohort of subjects whose characteristics were similar to that in the study of Wang et al (14) but were not healthy.

Because W-TBPro is based on the FFM level, its change was, unsurprisingly, highly correlated to the change in water and lean
Seven individuals had ≥1 of 3 protein estimates >3 SDs away from the best-fit line with NAA-TBPro\(^1\). The protein outliers are also identified and labeled in Figures 1 and 2. ADP-volume, air-displacement plethysmography total body volume; BIA water, bioimpedance analysis water; C1–C3, 3 subjects in the calibration data set; DC-TBPro, direct-calibration protein; DXA-volume, total body volume estimated from dual-energy X-ray absorptiometry–reported mass compartments; IEM, inborn error of metabolism; NAA-TBPro, neutron activation analysis-derived protein; PKU, phenylketonuria; Pulm TB, pulmonary tuberculosis; SBS, short bowel syndrome; UCD, urea cycle disorder; V1–V4, 4 subjects in the validation data set; W-TBPro, Wang equation–derived protein; 4CL-TBPro, 4-compartment Lohman method for analysis of total body protein.

Discussion

Although DC-TBPro was the most-accurate protein estimate to that of NAA-TBPro in this study, there were a couple of outliers. Although subject C3 was considered an outlier, her DC-TBPro measure was closer in value to the NAA-TBPro measure than the other 2 protein estimates; subject C3 was probably considered an outlier because of regression line fitting. Subject V4, who had significant muscle wasting from pulmonary tuberculosis indicated by a nitrogen index of 0.59, had an overestimated DC-TBPro value. Although subject C3 was considered an outlier, her DC-TBPro measure was closer in value to the NAA-TBPro measure than the other 2 protein estimates; subject C3 was probably considered an outlier because of regression line fitting. Second, BIA may be clinically accessible but is not considered the gold standard for total body water. The agreement of total body water by using deuterium dilution to bioimpedance is reasonable with an \(R^2\) of 0.96 and RMSE of 1.6 L (19). However, we were not able to confirm the calibration of our particular bioimpedance unit to deuterium dilution in this study because of the retrospective nature of our data. Inaccuracies of bioimpedance-derived total body water could affect the coefficients in Equation 5.

In conclusion, a simplified method to measure total body protein by using a clinical DXA system and BIA unit was developed and compared against NAA as proof of principle. This method eliminates the need for an additional measurement of body volume and shows good correlation to the reference method of NAA. Our method could provide a more clinically accessible technique to measure total body protein quickly and effectively.

The authors’ responsibilities were as follows—JPW and JAS: designed and conducted the research; BJS: collected data; JPW, BF, FWD, and JAS: analyzed data; JPW, BJS, BF, FWD, and JAS: wrote the manuscript; JPW: had primary responsibility for the final content of the manuscript; and all authors: read and approved the final manuscript. None of the authors had a conflict of interest.

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