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Multi-Component Molecular-Level Body Composition Reference Methods: Evolving Concepts and Future Directions

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Abstract

Excess adiposity is the main phenotypic feature that defines human obesity and that plays a pathophysiological role in most chronic diseases. Measuring the amount of fat mass present is thus a central aspect of studying obesity at the individual and population levels. Nevertheless, a consensus is lacking among investigators on a single accepted “reference” approach for quantifying fat mass *in vivo*. While the research community generally relies on the multicomponent body-volume class of “reference” models for quantifying fat mass, no definable guide discerns among different applied equations for partitioning the four (fat, water, protein, and mineral mass) or more quantified components, standardizes “adjustment” or measurement system approaches for model-required labeled water dilution volumes and bone mineral mass estimates, or firmly establishes the body temperature at which model physical properties are assumed. The resulting differing reference strategies for quantifying body composition *in vivo* leads to small but under some circumstances important differences in the amount of measured body fat. Recent technological advances highlight opportunities to expand model applications to new subject groups and measured components such as total body protein. The current report reviews the historical evolution of multicomponent body volume-based methods in the context of prevailing uncertainties and future potential.

Keywords

Nutritional Assessment; Underwater Weighing; Dual-Energy X-Ray Absorptiometry; Isotope Dilution; Obesity; Total Body Fat

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Introduction

A seminal publication in 2005¹ reported a “correction” factor to be applied to the dual-energy X-ray absorptiometry (DXA) body fat mass measurements being collected in the National Health and Nutrition Survey (NHANES)^{2, 3} and linked Korean NHANES (KNHANES)³. Almost thirty thousand subjects were evaluated in these two projects designed in-part to establish the adiposity of non-institutional Americans^{2, 3} and Koreans³. Prompted by a sense that the selected DXA system was providing fat mass estimates that were too low relative to criterion methods, seven United States centers pooled the needed information used to re-calibrate the vast DXA data set¹. The adjusted percent fat values, about 3-4% above that measured, have since been used to set normative adiposity ranges^{2,4, 5}.

The problem encountered in NHANES and KNHANES is often compounded when research methods such as DXA are used as the reference for calibrating other simpler body composition methods such as bioimpedance analysis and anthropometry. As each new method is introduced a steady stream of publications follows that evaluates their value by comparison to a designated reference standard.

The question that arises from these collective observations is if there is a “correct” answer to how much fat mass an individual or population has? In other words, is there a single “gold” standard criterion measure of body fat against which the NHANES and KNHANES DXA or any other indirect body composition method could be adjusted for?

The aim of our review is to address this question by critically examining the currently accepted “molecular” level body composition reference methods with the goal of identifying information gaps, ambiguities in application, and promising advances that potentially can improve body composition reference methods. One of five body composition levels, the molecular level includes chemical components such as fat, protein, and water⁶. Our review builds on the historical development of reference methods with a focus on illuminating the various assumptions and models required to quantify body composition in living humans, with particular attention to assessing body volume. Our focus is on model design and development as other references provide reviews of model propagated measurement error⁷⁻⁹.

In Vivo Chemical Analysis

The most accurate approach to measuring human body composition is by direct chemical analysis of cadavers. This strategy was taken by Harold H. Mitchell and his colleagues at the University of Illinois who published their highly cited classic cadaver study in 1945¹⁰ (Figure 1). Total body ether extract (lipid), water, nitrogen (protein), phosphorous, and calcium (bone mineral) were quantified from a 35-year old man who had died suddenly from a heart attack. Several subsequent cadaver analyses by Mitchell and colleagues¹¹ and others¹² provide us with a limited but important and enduring compendium of information on human chemical composition.

The Mitchell approach to body composition analysis is obviously untenable on a wide scale, and the result is that many “indirect” approaches have evolved to measure body lipid, water, protein and minerals in living humans from birth onward¹³.

***In Vivo* Neutron Activation Approach**

One of the first modern attempts to replicate Mitchell's “complete” chemical analysis *in vivo*, and thus establish a molecular level reference method, was reported by Cohn and Dombrowski in 1971¹⁴ (Figure 1). These pioneering investigators used *in vivo* neutron activation analysis and whole-body counting to measure total body N, Ca, P, Na, K, and Cl and the Brookhaven National Laboratory group went on to develop and report several molecular level reference methods¹⁵, including a six-component model based on these elements consisting of total body fat, water, protein, osseous minerals, soft tissue minerals, and glycogen¹⁶. Although important studies were later conducted at Brookhaven¹⁷ and other centers¹⁸⁻²⁰ using this approach, the cost, required expertise, and subject radiation exposure limited the generalizability of Cohn and Dombrowski's *in vivo* chemical analysis method. Today there are only several research centers in the world that have the capacity to measure four or more molecular level components using neutron activation analysis in healthy adults or patients with chronic disease.

Body Volume Approach

Two-Component Model—A more applicable reference approach had its roots in the development of Albert R. Behnke's two-component underwater weighing method first introduced in 1942²¹ (Figure 1). Behnke conceived of the human body as two components, fat and “lean body mass”, each with an assumed stable density of 0.900 g/cm³ and 1.095 g/cm³, respectively²². By measuring body mass under water and on land along with residual lung volume, Behnke was able to derive an estimate of body volume (V_B) and density (D_B) that with a two-component model could then be used to derive the mass of fat and lean. Debate centered over the next decade on model specifics, but the two-component body-volume model reported by Siri in 1961 was the eventual survivor^{8, 23} now applied by most investigators.

Siri assumed that human body mass (M_B) consists of two components, fat (M_F) and fat-free mass (M_{FFM}). The densities of fat (D_F), largely ether-extractable triglyceride, and fat-free mass (D_{FFM}) at body temperature (37° C) were assumed by Siri to be 0.900 g/cm³ and 1.100 g/cm³, respectively. These model coefficients are summarized in Table 1 along with Reference Man body composition estimates²⁴. Later workers revised mean body temperature to 36° C and adjusted D_F to 0.9007 g/cm³^{16, 25}. Siri's original model and temperature-corrected model combined with underwater weighing (Table 2) were often considered the “gold” standard for molecular level body composition research over the next three decades. Siri's model supplanted the other two-component models described between 1945 and 1959 that reported respective fat and fat-free mass density ranges of 0.889-0.918 g/cm³ and 1.095-1.109 g/cm³²².

Three-Component Model—The two-component approach assumes a stable relative fat-free mass composition and “constant” D_{FFM} . Fat-free mass includes four main components,

water, proteins, minerals, and glycogen and while these molecular level components maintain stable relations with each other under usual conditions, many factors are recognized to vary their proportions. These multiple factors (e.g., growth, pregnancy, race, duration of fasting, presence of disease, etc.) in turn influence D_{FFM} and impact on the accuracy of body composition estimates. Siri and others recognized this limitation of the two-component underwater weighing model and refinements in the model were introduced by Siri in 1956²³ and again in 1961⁸. Siri proposed adding total body water to the two-component molecular level model to create a three-component model consisting of fat, water, and non-fat solids (mineral, protein; $M_{MIN} + M_P$), now referred to as residual mass (M_R) (Table 1). To create his model (Table 2), Siri assumed based on the earlier cadaver studies that total body mineral mass is present in a stable ratio of 0.35 to total body protein mass. He then assigned a density to this combined residual mass component (1.565 g/cm³) reflecting the density of protein (1.34 g/cm³) and minerals (3.00 g/cm³) (Table 1). Siri then went on to derive his three-component model. Use of Siri's model including a water mass term was feasible during this period as Schloerb et al.²⁶ and even Siri²⁷ were among the first to use labeled water to metabolism *in vivo* with the radioactive isotope tritium and the stable isotope deuterium.

Four- to Six-Component Models—As with Behnke's two-component model²¹, Siri's three-component model⁸ by necessity pooled several components within the residual mass compartment including protein and minerals (osseous and soft tissue; $M_{MIN} = M_O + M_S$). Siri ignored labile body glycogen that ranges from ~0.3 kg to 0.5 kg in the fasting state⁶. Siri was, however, unable to further separate the residual mass component into its protein and mineral portions as the measurement approaches for these chemical groups had not yet been introduced. Both Siri and Behnke recognized the importance of separating out residual mass components in 1957 with analysis of osseous mineral contributions from standard X-ray films²⁸.

The limited ability of investigators to accurately quantify residual mass components changed in 1963 when Cameron and Sorenson introduced single photon absorptiometry (SPA)²⁹ for evaluation of osteoporosis and other metabolic bone diseases (Figure 1). The SPA approach provided a measure of bone mineral mass at two peripheral sites, the radius and ulna. In 1977 Selinger used SPA estimates obtained from an early commercial scanner along with a prediction equation to derive an estimate of total body bone mineral mass as part of his doctoral thesis at the University of Illinois³⁰. Selinger then estimated soft tissue mineral mass by assuming this small component accounts for 1.05% of total body mass. Now Siri's three-component model could be expanded to four components, fat, water, protein, and minerals by further separating out the main residual mass chemical components. Selinger was prompted to develop his four-component model as a means of quantifying adiposity in children who may not yet have reached “chemical maturity” and thus whose fat-free mass composition did not adhere to the stable water, protein, and mineral proportions required by two- and three-component models.

An important limitation of SPA as applied by Selinger was that bone quality evaluations outside of the distal portion of extremities was limited due to overlying soft tissue that attenuated photons in addition to that produced by osseous minerals. His radius and ulna

bone mineral evaluations had to be extrapolated to the whole body. Meanwhile, the growing clinical interest in bone quality beyond that of sites such as the radius led Mazess and his colleagues to develop and report in 1970³¹ the dual-photon absorptiometry (DPA) method (Figure 1). Whole body DPA scanners with a radioactive ¹⁵³Gd source were introduced in the early 1980s and were followed by more advanced and practical dual energy X-ray absorptiometry (DXA) systems in 1987. Both DPA and DXA now advanced SPA by providing measures of “total body” mineral mass along with estimates of soft tissue composition as fat and lean. By 1990, Heymsfield et al. reported development of a four component model that incorporated total body bone mineral mass measured by DPA with total body water measured by tritium dilution and the results of this model compared favorably to those obtained in healthy adults using Cohn and Dombrowski’s *in vivo* whole body counting neutron activation analysis reference method³².

Model and Method Uncertainties: These early multicomponent models are all founded on the concept that whole-body density reflects the densities of fat, water, protein, minerals, and to a less extent glycogen, essential lipids³³, and other chemical compounds present in small amounts. All body volume models (Table 2) are designed around the concept that the densities of these components are known and constant *in vivo* (Table 1). Model development starts from two equations, one for body mass and the other for body volume (component volume = component mass/density) and then working through simultaneous equations to solve for the component of interest, typically fat or protein mass⁶. Applying the model at present then requires four measurements, body weight, volume, water, and bone mineral content. Soft tissue mineral mass and other residual components as included in some five and six component models must now be estimated from one of the other four measured quantities. However, uncertainties exist when applying these models. In the following sections we examine aspects of “model” error by estimating the impact of model manipulations on the predicted %fat estimates of Reference Man²⁴.

Derivation of Soft Tissue Mineral Mass: Each model developer takes a slightly different derivation strategy (Table 2), hence the continued publication of new and potentially more advanced models. The first challenge is how to model the small unmeasured components, notably soft tissue minerals, glycogen, and other residual mass components. Selinger assumed in his 1977 model that soft tissue minerals are a constant fraction of body weight (1.05%)³⁰. Heymsfield et al. assumed in their model that soft tissue minerals represent a constant fraction (0.235) of bone mineral “ash”³². Neither of these models has a strong mechanistic basis as non-osseous minerals are distributed in the extracellular and intracellular water (ECW, ICW) compartments present in soft tissues and do not form “constant” ratios to either body weight or bone mineral mass. By 2002, Wang et al.⁶ reported soft tissue mass prediction equations and a five-component model based on ECW and ICW derived by a combination of labeled water and bromide dilution volumes. The authors then developed a simplified soft tissue mineral mass prediction equation and four-component model based on total body water that assumes stable fluid distribution in healthy adults (i.e., ECW/ICW of 0.97). Even relatively large differences in fluid distribution (e.g., ECW/ICW of 0.75) have only small effects on predicted soft tissue mineral mass (40 g increase from 1.29 kg for a 100 kg adult). Other strategies for deriving residual mass

components are included in some published models (Table 2) and no consensus yet exists on which model to apply when developing and referencing simpler body composition methods against a “gold” standard.

Derivation of Bone Mineral Mass: A second and not fully resolved challenge for model developers is to derive an estimate of total body mineral mass from the measured DXA bone mineral value. The early DPA system developed by Lunar Corporation (now GE Lunar, Madison, WI) reported a value for “total body bone ash (TBBA)” that was based on the system developer's bone calibration phantom. Ash is produced when bone mineral is heated to $>500^{\circ}\text{C}$ and labile elements are released. Lunar also calibrated their scanner with a bone phantom that included “physiologic” amounts of marrow fat in addition to bone ash. Selinger recognized the distinction between “bone ash” and bone mineral and he adjusted his estimates based on earlier studies reporting that during the heating process 1 g of bone mineral yields 0.9582 of ash^{30, 34}. Since then most model developers have adjusted DXA bone (osseous) mineral measurements to reflect the ashing processes (i.e., $M_{\text{O}} = M_{\text{TBBA}} \times 1.0436$).

However, the other major DXA developer, Hologic (Bedford, MA) has a different scanner calibration protocol. Hologic calibrated their system to tri-basic calcium phosphate, a readily available chemical standard that has an elemental composition and density very similar to calcium hydroxyapatite³⁵. This bone mineral standard was molded to resemble the lumbar spine and embedded into a tissue equivalent epoxy resin to make the original Hologic Spine Phantom.

To gain further insight into these DXA system bone mineral differences, we evaluated 75 healthy men and women with systems from both companies (GE Lunar iDXA; Hologic Discovery A) (Supplementary Material I). The two estimates of bone mineral mass were highly correlated (R^2 , 0.98; $p < 0.001$) with the iDXA values significantly larger by $13.3 \pm 3.6\%$ than that by the Discovery A ($2.76 \pm 0.56\text{ kg}$ vs. $2.44 \pm 0.47\text{ kg}$; $p < 0.01$). Significant bias was also present between the two methods as indicated by a Bland-Altman plot. The between-manufacturer mean difference in bone mineral estimates will lead to a 0.6% fat unit difference in percentage body fat based on Reference Man (Supplementary Material II) when applied in a typical four-component model. Moreover, calculating percentage fat with and without the ash weight adjustment leads to a separate 0.2% fat unit difference.

Derivation of Water Mass: Another area in which model developers sometimes differ is the conversion of labeled water dilution volumes to water mass. Total body water volume today is measured by labeled water dilution using either $^2\text{H}_2\text{O}$ (deuterated water) or H_2^{18}O (O-18 water). These two stable isotopes have largely replaced the radioactive isotope $^3\text{H}_2\text{O}$ (tritium) when applying multicomponent models, particularly in children. Water mass is calculated as the product of total body water volume and the density of water at mean body temperature.

^{18}O -labeled water exchanges with oxygen in non-aqueous molecules. This leads to a small theoretical total body water overestimate of less than 1.01%^{36, 37}. A similar result was

observed by Lifson et al.³⁸ who experimentally compared ^{18}O dilution volumes to total body water volume measured by desiccation in rats and found an overestimate ($X \pm \text{SD}$) of $1.02 \pm 0.01\%$. A review by Schoeller³⁶ of experimental desiccation studies reported a $1.6 \pm 0.6\%$ overestimate of total body water measured by H_2^{18}O dilution³⁶. Although no formal consensus exists, most investigators today use these observations and assume ^{18}O exchange is 1%.

Tritium and deuterium dilution volumes are also larger than total body water due to exchange with non-aqueous hydrogen, mainly in proteins and carbohydrates. The amount of exchange observed in experimental studies is variable as sample sizes tend to be small, measurement approaches vary, and several different calculation methods can be applied. In a comprehensive review, Schoeller found that the dilution volume of $^2\text{H}_2\text{O}$ and $^3\text{H}_2\text{O}$ is ($X \pm \text{SD}$) $3.7 \pm 1.7\%$ larger than total body water assessed by desiccation³⁶. Racette et al.³⁹ evaluated ^2H and ^{18}O dilution volume in 85 females and 14 males ranging in age between 4 and 78 years and the observed $^2\text{H}/^{18}\text{O}$ dilution space ratio was ($X \pm \text{SD}$) 1.034 ± 0.014 . These observations are similar to the maximal exchange of 5% derived by Culebras and Moore using theoretical calculations⁴⁰ for $^2\text{H}_2\text{O}$ and $^3\text{H}_2\text{O}$. While no consensus exists, as with ^{18}O , most investigators now assume $^2\text{H}_2\text{O}$ overestimates total body water by 4%.

For every 1% adjustment in total body water (Supplementary Material II) dilution volume Reference Man's body fat will change by about 0.4% fat units.

As noted above, Siri⁸ originally assumed a mean body temperature of 37°C when developing his two- and three-component models. Since then most workers, although not all, assume a mean body temperature of 36°C resulting in a higher water density. Body temperature is not a "constant", but varies from the deep core organs and tissues to surface structures such as skin and hair. Core temperature measured in the rectum, esophagus or tympanic membrane under basal and thermoneutral conditions is usually $\sim 37^\circ\text{C}$ while skin temperature is typically $\sim 34^\circ\text{C}$. Brožek et al. used these values²⁵ along with Burton's theoretical formula⁴¹ to arrive at a mean body temperature of 36°C and he adjusted Siri's 1961 two-component model accordingly. Nevertheless, not all multicomponent models are derived using fat and water densities at 36°C , as for example Forslund et al. derived their model using mean body temperature values at 37°C (Table 2)⁴². A consistent approach to deriving model component densities would be useful in light of these often confusing aspects of equation development.

Adjusting mean body temperature by 1° will change Reference Man percentage body fat by 0.15% fat units (Supplementary Material II).

Accounting for Essential Lipids: Solvent extraction of total body lipids provides two main components, so-called "essential" lipids (cholesterol, density 1.067 g/cm^3 ; phospholipids, density, 1.035 g/cm^3 ; etc.) and non-essential lipids or "fat" (i.e., triglycerides)³³. General agreement exists that the non-essential lipid density of fat (0.9007 g/cm^3 ; Table 1) reflects that of mainly triglyceride³³. Reference Man was reported to have 1.5 kg of essential fat in 1975²⁴ and 2% of "lean body mass" ($\sim 1\text{--}2\text{ kg}$) in 2002⁴³. Comizio et al.³³ extracted whole rat carcass lipid and found that 13% of the total lipid was essential and that the proportion of

essential lipid increased with weight loss brought about by reducing dietary energy intake or increasing energy expenditure with exercise. These concordant observations suggest that the amount of essential lipids in humans must approach that of components such as soft tissue minerals and glycogen. No recent multicomponent model as of yet includes a term for essential lipids and we do not know what, if any, errors in fat or protein estimation are introduced by this omission. Early workers developed a “residual mass” component including unmeasured substrates and assigned this component a density value⁴⁴. Future modeling efforts need to consider how to manage issues related to the essential lipid component.

Between-Model Differences: Published volume-based multicomponent models are all founded on the same general assumptions but differ in the various coefficients used and adjustments made to measured variables (Table 2). Estimates, such as those for body fat mass, are similar but not identical. This can be observed in a sample of 130 children and adults who had body volume, total body water, and bone mineral measured by air-displacement plethysmography (ADP; Bod Pod, Cosmed-USA, Concord, CA), deuterium dilution, and iDXA (GE Lunar, Madison, WI), respectively (Supplementary Material III). The mean ($\pm$ SE) percentage fat for each model is shown in Figure 2 and ranged from $31.0 \pm 1.0\%$ to $32.7 \pm 1.0\%$ (1.7% fat units). None of these models specifically include glycogen and the effects of this omission can be estimated by adding a glycogen term to Wang's five-component model⁶ to create a six-component model (Table 2). The addition of glycogen to Wang's model would lower Reference Man's percentage body fat by 0.6% fat units.

Between-model differences ($\pm$ SD) were not “constant” but showed bias in some cases. For example, mean values for Lohman⁷ and Withers⁴⁵ models for %fat were $31.1 \pm 1.0\%$ and $32.7 \pm 1.0\%$, a difference of $1.6 \pm 0.02\%$. A Bland-Altman analysis for bias was statistically significant with the difference in %fat $\sim 1.0\%$ at high body fat and increasing to $\sim 2.1\%$ at low body fat.

Estimation of Total Body Protein: Body proteins not only serve as a source of potential metabolic fuel, but perform non-energetic functions essential for survival. Energy balance models thus include estimates of protein balance^{46, 47}, although this component is not easily quantified *in vivo* and generally unavailable methods such as prompt- γ neutron activation analysis are considered the reference methods for human nutrition and metabolic research¹⁴. Surrogate measures, such as “fat-free mass” are thus typically used to replace protein mass in relevant human energy balance models and clinical studies^{48, 49}. However, multicomponent body volume models include a “protein” mass component or a closely related “residual mass” component. Most of the models summarized in Table 2 can thus be rearranged to solve for protein mass and some published examples are presented in the table.

The key in deriving a multicomponent model of total body protein is to adjust the model for non-protein components typically found in residual mass. The six-component model developed by Wang in this report (#11, Table 2) includes estimates for residual mass components soft tissue mineral and glycogen. As an example, we derived two estimates of total body protein (M_P) in healthy adults (Supplementary Material IV), one by the total body

N (M_N) reference approach prompt- γ neutron activation analysis³² and the other from Wang's six-component model. The multicomponent total body protein model prediction was (9.39 ± 2.30 kg) was minimally larger than the prompt- γ neutron activation analysis estimate (9.06 ± 2.14 kg; $r = 0.33 \pm 1.56$ kg). The two protein estimates were well correlated (R^2 , 0.57, $p < 0.01$; Figure 3) and there was no significant bias as evaluated by a Bland-Altman analysis.

Multicomponent body volume model estimates thus appear to be a reasonable alternative to neutron activation analysis for quantifying total body protein in subject groups, particularly when care is taken to evaluate participants who are fasting and relatively weight stable so as to minimize variability in body glycogen. Additionally, the metabolizable energy density of protein and glycogen are similar (~ 4 kcal/g) and therefore multicomponent models may be useful in estimating long-term energy balance in clinical trials.

Measurement Advances—The usual approach to developing a four-component model requires four measurements including body weight, body volume, total body bone mineral, and total body water. Of these, the most recent advances are in the area of body volume measurement.

Air Displacement Plethysmography: Behnke's underwater weighing system for body volume measurement has now largely been replaced by air displacement plethysmography (ADP) with its theoretical framework and human validation reported in 1995^{50, 51}. Since then there have been many validation studies with a detailed review by Fields et al. in 2002⁵² and a recent review with a focus on pediatric applications by Demerath and Fields⁵³. ADP systems are now available for use across the entire lifespan while underwater weighing usually cannot be carried out in young children and is prohibitive in infants, the frail elderly, and people with serious health conditions.

The accuracy of V_B estimates by ADP systems (Bod Pod; Cosmed-USA, Concord, CA) can be confirmed by use of calibrated inanimate phantoms varying in size⁵⁰. ADP provides similar non-biased D_B and thus V_B estimates to that of underwater weighing when evaluated in adults and children^{54, 55, 56}.

Early studies identified several technical and measurement factors that when appropriately considered improve the quality of V_B measurements including subject attire, hair conditions (e.g., presence of a beard), movement in pediatric subjects, room airflow and temperature control, and duration of rest and cooling period following a bout of exercise^{52, 57}.

An important initial concern when designing ADP systems was optimizing the relation between chamber and subject volume. The optimal chamber to subject volume ratio should be below 6 and this guide led to the introduction of a small-chamber system for infants (Pea Pod; Cosmed-USA, Concord, CA) that has been validated for use in subjects who weigh < 8 kg and are < 6 months of age⁵³. A pediatric option for the Bod Pod accommodates children between the ages of 2 and 6 years. Two issues are present for evaluating children in the gap between 6 months and two years of age, the main concern subject compliance and the other technical factors related to system chamber relative to subject volume.

Body volume models all assume that measured V_B values do not include air in the lungs and gastrointestinal tract. Lung volumes are therefore measured or predicted as part of the underwater weighing and ADP procedures. Body volume calculations include an estimate of gastrointestinal gas, typically ~ 0.1 L⁵⁸. Including that adjustment to measured V_B leads to a 0.7% fat unit difference in Reference Man's percentage fat (Supplementary Material II), but the actual individual range is large (0-0.537 L)⁵⁹. Gastrointestinal gas volumes are also larger several hours after a meal and ingestion of a carbonated beverage can lead to a 2-3% fat unit increase in estimated percentage body fat⁵². Gastrointestinal gas effects on body composition estimates may differ between underwater weighing and ADP, although presently the issue remains unresolved⁵². These observations again emphasize the importance of measurements being made in the fasting state.

Photogrammetry: Another advance in measuring body volume in adults and children is by photogrammetry, notably with the use of 3D laser imaging systems^{60, 61}. This technology is advancing rapidly and systems beyond laser-based devices are being introduced to the commercial market, notably for fitting clothing. A typical laser system provides a total body scan in less than 15 seconds and data processing yields body dimensions, including V_B , within several minutes. Wang et al.⁶² evaluated body volume in 63 adults and children with a laser scanner (C9036-02; Hamamatsu Photonics KK, Hamamatsu Japan) and underwater weighing and observed a high correlation between the two (R^2 , 0.99; SEE, 0.89 L) with a small but significant between system difference ($X \pm \text{SEM}$; laser, 81.9 ± 4.0 L; underwater weighing, 81.5 ± 4.0 L; Δ , -0.5 ± 0.1 L). Pepper et al.⁶³ constructed a rotary laser system for body composition assessment and found a high correlation between V_B measured by their instrument and V_B measured by underwater weighing (R^2 , 0.99; SEE 1.6 L) in a group of 70 healthy adults. The respective slope and intercept of this regression line were not significantly different from 1 and 0 and there was a non-significant difference between V_B measured by underwater weighing (67.6 ± 1.7 L) and that by the laser system (67.8 ± 1.7 L). We also recently found similar results comparing V_B for a 3D laser system (Human Solutions Vitus Smart XXL; Human Solutions North America, Cary, North Carolina) and ADP (Bod Pod, Cosmed-USA, Concord, CA) in a study of 96 children and adults (R^2 , 0.99; SEE, 1.4 L) with a slightly larger mean between system difference (77.9 ± 2.4 L vs. 76.8 ± 2.3 L; $p < 0.05$; Δ , 1.10 ± 0.37 L)(Supplementary Material V). As with ADP, a wide range of inanimate phantoms differing in size and shape can be used to confirm the accuracy of measured volumes⁶⁴

Use of photogrammetry system V_B estimates in four-component models would require either predicting or measuring lung volumes. In addition, factors such as subject positioning, breath holding, and garments worn during the scanning procedure are all important considerations yet to be fully established and standardized.

Dual-Energy X-Ray Absorptiometry: The most recent advance is to exploit DXA system attenuation properties to estimate V_B ^{65, 66}. DXA manufacturers currently use calibration phantoms made of biologically equivalent materials that have known composition to derive equations that predict fat and lean from measured pixel attenuation values⁶⁷. The approaches proposed by Wilson et al.^{65, 68, 69} reverses this process by creating calibration

equations to link phantom X-ray attenuation values to pixel volume and mass and total body volume. With their pixel volume method⁶⁵, the authors compared their DXA volume estimates with corresponding results from ADP in 11 adults and observed a high correlation between the two (R^2 , 0.99; $p < 0.001$) with non-significant bias present. The authors generated V_B prediction equations with their DXA method linking fat, lean, and bone mineral masses to corresponding ADP volumes with a 109 person calibration dataset and validated the equations with a 79 person dataset. Unlike ADP and underwater weighing, the DXA approach to measuring V_B does not require a residual lung volume measurement and would eliminate the modeling ambiguities related to gastrointestinal gas.

While the DXA approach for measuring body volume needs further development and validation, a four-component model based solely on DXA measurements (M_B , V_B and M_{Bone}) and water dilution volume (for M_W) appears feasible.

Potential Reductions in Propagated Measurement Error: The four-component model improves the accuracy of body fat estimates over that provided by the two- and three-component models by accounting for individual variability in total body water and bone mineral estimates. While our review focuses on the underlying foundation of four-component models and the impact of these formulations on method accuracy, equally important concerns surround propagated measurement error^{7-9, 45}. In a critical analysis of these errors, Friedl et al. found that the largest within-subject standard deviation for three repeated measurements over one week arose from the underwater weighing procedure (0.002 g/cm³; ~1% body fat)⁷⁸. The phasing out of underwater weighing and replacement by ADP and even DXA for body volume measurement may reduce these technical errors, an area worthy of future research.

Conclusions

Almost eight decades have passed since Mitchell's chemical analysis of a single human cadaver¹⁰. Since then indirect approaches to quantifying body composition at the molecular level have proliferated and intense interest in this topic has spread from the laboratory to the general public with introduction of low cost and even portable measurement devices. Regrettably, only rarely are the same fat mass results found by different measurement approaches (e.g., bioimpedance analysis and anthropometry) and discrepant results often present even when the same measurement approach (e.g. DXA) is taken by different manufacturers. The resulting confusion is compounded by a lack of agreement on a reference or "gold standard" method equivalent to that provided by direct chemical analysis as approached by Mitchell and others^{10, 12}. These issues are compounded by the growing availability of body composition measurements on a global scale and the concerns that arise when attempts are made to integrate this "big data" across measurement sites. Our review highlights some of the prevailing ambiguities on developing and applying body volume molecular-level reference models and methods with a clear need to formulate a consensus on some of these issues. This need is emphasized by the rapid technological advances that have yet to find their path into applied reference methods but promise to advance the field in the coming years.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ADP	air displacement plethysmography
D_B	body density
DXA	dual-energy X-ray absorptiometry
DPA	dual-photon absorptiometry
ECW	extracellular water
ICW	intracellular water
KNHANES	Korean NHANES
M_B	body mass
M_{BONE}	bone mass
M_F	fat mass
M_{FFM}	fat-free mass
M_G	glycogen mass
M_{LBM}	lean body mass
M_{MIN}	mineral mass
M_N	total body nitrogen mass
M_O	osseous mineral mass
M_S	soft tissue mineral mass
M_P	protein mass
M_R	residual mass

M_{TBBA}	total body bone ash mass
SPA	single photon absorptiometry
TBBA	total body bone ash
NHANES	National Health and Nutrition Survey
V_B	body volume

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Year (ref)	Model Development	Measurement Advances
1942 ^{21, 83}	Behnke 2-Component Model: $M_B = M_F + M_{LBM}$	Advanced Underwater Weighing Method Developed
1945 ¹⁰	HH Mitchel Chemical Cadaver Analysis	Provided Component Ratios Used in Subsequent Model Development
1950 ²⁶		Early use of Isotopes by Schloerb et al. to Measure Total Body Water
1956 ²³	Siri 2-Component Model: $M_B = M_F + M_{FFM}$	
1961 ⁸	Siri 3-Component Model: $M_B = M_F + M_W + M_R (P + Min)$	
1963 ²⁵	Brožek Reports Key Component Densities, Including for Minerals	Cameron & Sorenson Introduce SPA for Bone Measurement
1970 ³¹		Mazess et al. Introduce DPA for Total Bone Mass Measurement
1971 ¹⁴	Brookhaven Neutron Activation Model	
1977 ³⁰	Selinger 4-Component Model: $M_B = M_F + M_W + M_P + M_{Min (O \& S)}$	
1987 ⁸⁴		First DXA Systems Introduced for Bone Measurement
1990 ³²	4-Component Model with DPA & 3H_2O -measured M_O and M_W Validated Against Neutron Activation	
1995 ^{50, 51}		Introduction of ADP for Body Volume Measurement
2000 ^{4, 6}		Introduction of 3D Imaging Systems for Body Volume Measurement
2002 ⁶	Wang 5-Component Model: $M_B = M_F + M_W + M_P + M_O + M_S$	
2013 ^{68, 76}		Development of DXA Body Volume Measurement Method

Figure 1.

Milestones in the development and implementation of multicomponent body composition models. Abbreviations: ADP, air-displacement plethysmography; DPA, dual-photon absorptiometry; DXA, dual-energy X-ray absorptiometry; IVNA, in vivo neutron activation; M_B , body mass; M_F , fat mass; M_{FFM} , fat-free body mass; M_{LBM} , lean body mass; M_{MIN} , mineral mass; M_O , osseous mineral mass; M_P , protein mass; M_R , residual mass; M_S , soft tissue mineral mass; M_W , water mass; SPA, single photon absorptiometry.

Figure 2.

Percent fat ($\pm$ SE) predicted by the eleven multicomponent models presented in Table 2. Subjects were a cohort of 130 healthy children and adults. Additional sample details are presented in Supplementary Material III. Models: 1. Selinger³⁰ 2. Lohman⁷³ 3. Heymsfield et al.³² & Baumgartner et al.⁷⁴ 4. Lohman⁷, Lohman and Going⁷⁵, & Wilson et al.⁷⁶ 5. Friedl et al.⁷⁸ 6. Fuller et al.^{79, 80} 7. Withers et al.⁸¹ & Heymsfield¹⁶; 8. Siconolfi et al.⁸² 9. Forslund et al.⁴² 10. Wang et al.⁶; 11. Z. Wang, this report.

Figure 3.

Total body protein estimated from Wang's six-component model (Table 2, #11) versus corresponding estimates made by prompt- γ neutron activation analysis (PGNA). The correlation between the two volume estimates was significant at $p < 0.001$. Subjects were 50 healthy adults. Additional sample details are presented in Supplementary Material IV. The line of identity is shown in the figure.

Table 1

Molecular level component densities and mass in Reference Man[†].

Molecular Level Component(s)	Density (g/cm ³) (±SD)	Temp (° C)	Mass (kg) (%BW)	Comment
Fat ⁷⁰	0.9007 (0.00068)	36	13.3 (19.0)	D _F from subcutaneous and intra-abdominal human adipose tissue ether extracts. M _F includes 1.2 kg of essential fat.
Fat-free mass ⁸	1.100	NS*	56.7 (81.0)	D _{FFM} assumes a constant relative FFM composition as water, protein, minerals, and other residual components.
Glycogen ²⁵	1.52	37	0.47 (0.7)	M _G usually not considered in models or is pooled with the protein component in residual mass. Estimate is from the model of Wang et al. ⁶ and is not included in Reference Man body weight or density calculation.
Water ²⁵	0.99371 0.9934	36 37	42 (60.0)	M _W is calculated as the product of isotope dilution volume, an adjustment factor (usually 0.99 for O-18 and 0.96 for ² H ₂ O), and D _W .
Protein ⁸	1.34	36-37**	10.6 (15.1)	M _P for hydrated protein <i>in vitro</i> . Densities of individual proteins and protein families may differ from the global estimate of 1.34 g/cm ³ .
Minerals ²⁵				
Total	3.042		3.7 (5.3)	Assumes M _S /M _{TBBA} of 0.235.
Soft Tissue	3.317	40	1.0 (1.4)	Estimated from the weighted amount of each tissue mineral and electrolyte present in the non-osseous fluid space; apparent densities calculated at 40 °C.
Bone	2.982	36-36.7	2.7 (3.9)	Based on values obtained from animal long bones.
Residual				
Siri ⁸	1.565			Assumes total body mineral/protein = 0.35.
Allen ⁴⁴	1.399 (0.051)	37		Includes protein, non-osseous minerals, and glycogen.
Silva ⁷¹				Residual mass density varies with age, race, sex, and weight.
Total	1.058	36	70 (100)	Combined fat, water, protein, bone mineral, and soft tissue mineral mass. Mineral-ash adjusted D _B value. Total mass includes unspecified residual components.

[†]From^{6, 24, 72)}

* NS, not specified.

** Cited by Brožek²⁵ as 36° C and by Siri⁸ as 37° C.

Abbreviations: BW, body weight; DF, density of fat; DFFM, density of fat-free mass; FFM, fat-free mass; MG, glycogen mass (kg); MP, protein mass; MR, residual mass; MS, soft tissue mineral mass, MTBBA, total-body bone ash (kg); MW, total-body water mass (kg).

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Table 2

Selected body-volume based models for estimating molecular level components.

Model (ref)	Year	Equation
Two Component Models		
Siri ²³	1956	$M_F = 4.950 \times V_B - 4.500 \times M_B$
Siri (adjusted) ²⁵	1963	$M_F = 4.971 \times V_B - 4.519 \times M_B$
Three Component Models		
Siri ^{8, 23}	1961	$M_F = 2.057 \times V_B - 0.786 \times M_W - 1.286 \times M_B$ $M_{MIN} = \alpha M_P$, assume $\alpha = 0.35$ to derive residual mass $M_W = 0.993 \times V_W$
Lohman ⁷³	1986	$M_F = 6.386 \times V_B + 3.961 \times M_{MIN} - 6.09 \times M_B$ $M_W/M_P = 3.80$ with density of $(M_W + M_P) = 1.0486 \text{ g/cm}^3$
Silva ⁷¹	2004	$M_F = 2.122 \times V_B - 0.779 \times M_W - 1.356 \times M_B$ $M_O = M_{TBBA} = 1.0436$ $M_W = 0.99371 \times 0.96 \times {}^2\text{H}_2\text{O}$ dilution volume
Four to Six Component Models		
1. Selinger ³⁰	1977	$M_F = 2.747 \times V_B - 0.714 \times M_W + 1.129 \times M_O - 2.037 \times M_B$ $M_O = M_{TBBA} \times 1.0436$ and $M_S = M_B \times 0.0105$
2. Lohman ⁷³	1986	$M_F = 2.747 \times V_B - 0.714 \times M_W + 1.146 \times M_O - 2.0503 \times M_B$ Assumes stable ratio of M_S to M_O .
3. Heymsfield et al. ³² & Baumgartner et al. ⁷⁴	1990, 1991	$M_F = 2.747 \times V_B - 0.7175 \times M_W + 1.148 \times M_{MIN} - 2.05 \times M_B$ $M_O = 1.0436 \times M_{TBBA}$ and $M_S = 0.235 \times M_{TBBA}$, thus $M_{MIN} = 1.279 \times M_{TBBA}$ $M_W = 0.99371 \times 0.95 \times {}^3\text{H}_2\text{O}$ dilution volume

Model (ref)	Year	Equation
4. Lohman ⁷ , Lohman and Going ⁷⁵ , & Wilson et al. ⁷⁶	1992,1993 2012	$M_F = 2.747 \times V_B - 0.714 \times M_W + 1.146 \times M_O - 2.053 \times M_B$ $M_P = 3.050 \times M_B - 0.286 \times M_W - 2.146 \times M_O - 2.747 \times V_B$ $M_W = 0.99371 \times 0.96 \times \text{H}_2\text{O dilution volume.}$ <p>Assumes constant ratio of M_S to M_O; see ⁷⁷ for discussion of M_F model.</p>
5. Friedl et al. ⁷⁸	1992	$M_F = 2.559 \times V_B - 0.734 \times M_W + 0.983 \times M_O - 1.841 \times M_B$ $M_O = 1.0436 \times M_{TBBA}$ $M_W = 0.99371 \times 0.98 \times \text{H}_2\text{O dilution volume}$ <p>Protein mass replaced by residual mass with density of 1.39 g/cm³</p>
6. Fuller et al. ^{79, 80}	1992	$M_F = 2.747 \times V_B - 0.710 \times M_W + 1.460 \times M_{TBBA} = 2.05 \times M_B$ $M_P = 3.050 = 0.290 \times M_B - 2.734 \times M_{TBBA} - 2.747 \times V_B$ $M_O = 1.0436 \times M_{TBBA} \text{ and } M_S = 0.23048 \times M_{TBBA}, \text{ thus } M_{MIN} = 1.2741 \times M_{TBBA}$ $M_W = 0.99371 \times 0.96 \times \text{H}_2\text{O dilution volume}$
7. Withers et al. ⁸¹ & Heymsfield ¹⁶	1992 1996	$M_F = 2.513 \times V_B - 0.739 \times M_W + 0.947 \times M_O - 1.790 \times M_B$ $M_O = 1.0436 \times M_{TBBA}$ <p>Protein mass replaced by residual mass with density of 1.404 g/cm³</p>
8. Siconolfi et al. ⁸²	1995	$M_F = 2.7474 \times V_B - 0.7145 \times M_W + 1.1457 \times M_{MIN} - 2.0503 \times M_B$ $M_O = 1.14 \times M_{TBBA} \text{ and } M_{MIN} = 1.21 \times M_{TBBA}$ $M_W = 0.9937 \times 0.96 \times \text{H}_2\text{O dilution volume}$
9. Forslund et al. ⁴²	1996	$M_F = 2.559 \times V_B - 0.734 M_W + 0.983 \times M_O - 1.841 \times M_B$ <p>Assumed D_F of 0.9 g/cm³ and D_W of 0.99336 g/cm³ at 37° C</p> <p>Protein mass replaced by residual mass with density of 1.39 g/cm³</p>
10. Wang et al. ⁶	2002	$M_F = 2.748 \times V_B - 0.715 \times M_W + 1.129 \times M_O + 1.222 \times M_S - 2.051 \times M_B$ $M_F = 2.748 \times V_B - 0.699 \times M_W + 1.129 \times M_O - 2.051 \times M_B$ $M_O = 1.0436 \times M_{TBBA} \text{ and } M_S = 0.0129 \times M_W$

Model (ref)	Year	Equation
		$M_W = 0.99371 \times 0.96 \times \text{H}_2\text{O dilution volume}$
11. Z. Wang (this report)	2014	$M_F = 2.720 \times V_B - 0.715 \times M_W + 1.108 \times M_O - 2.020 \times M_B$ $M_S = 0.0129 \times M_W$ $M_G = 0.044 \times M_P$

Abbreviations: M_B , body mass (kg); V_B , body volume (L); M_F , fat mass (kg); M_{LBM} , lean body mass (kg); M_{MIN} , total mineral mass (kg); M_O , bone mineral mass (kg); M_P , protein mass; M_R , residual mass; M_S , soft tissue mineral mass, M_{TBBA} , total-body bone ash (kg); M_W , total-body water mass (kg). Modified from^{9, 73}.