New Approaches to Body Composition Research: A Reexamination of Two-Compartment Model Assumptions

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Overview

Major new technological advances are now expanding our ability to study human body composition in vivo. The wealth of new information derived from these methods is advancing our understanding of growth, aging, and many pathological states. Our focus in this review is to demonstrate how these powerful new methods can be effectively used to enlighten our understanding of widely used older technologies. Specifically, our discussion centers on the 2-compartment body composition methods used effectively over the last 4 decades. Proceeding in several areas of nutrition and metabolic research requires a critical examination of 2-compartment model validity. Unfortunately, until recently a project of this type was possible solely through cadaver analysis. Only a few such postmortem studies have been performed due to the extreme difficulty in executing and interpreting this type of research. Our review demonstrates that for the first time a detailed compartmental analysis can be performed in vivo that provides the information needed to critically examine 2-compartment model assumptions.

Body Weight and Compartments

Body weight is the primary body composition measurement. It represents the sum total of all somatic chemical constituents. Further partitioning of body weight into subcompartments (C) requires additional measurements (M) such that:

$$M = C - 1.$$

Hence a 2-compartment body composition model requires 1 measurement in addition to body weight. A 4-compartment model is based upon 3 measurements, and so on. Most investigators currently rely upon a 2-compartment model in which body weight is divided into fat and fat-free components. The main reason for this choice is that physiologically these 2 fractions of body weight are of primary relevance to workers in the areas of nutrition and metabolism. Another reason for choosing a 2-compartment model is convenience; the measurement needed in addition to body weight is usually simple, safe, and reliable. Much of our current knowledge of body composition and related energy expenditure is founded on 2-compartment studies.

2-Compartment Models

There are 3 main techniques of fractionating body weight into fat and fat-free components: hydrodensitometry [1], whole body counting for ^{40}K [2], and evaluation of total body water

(TBW) [3]. Hence measurement of body density (D_b), $40K$, and TBW allow calculation of the percentage weight as fat and fat-free body mass (FFM).

Other methods of deriving 2-compartments are also available that share in common calibration against the above techniques. For example, skinfolds [4], body impedance analysis [5], and total body electrical conductivity [6] each measure a correlate of fat and FFM. Prediction equations for fat and FFM are developed by calibration against 1 of the 3 reference methods. A central reliance is thus placed upon the primary measurement technique's accuracy.

A worthy digression at this point is to explore the scientific foundations of 2-compartment methods. One example is selected for convenience, hydrodensitometry, although similar reasoning applies to $40K$ and TBW.

Hydrodensitometry

At the beginning of this century methods were sought for quantifying corpulence. The concept emerged that body specific gravity was inversely related to fatness. Unfortunately, technical problems beset investigators during this period, and no important advances were made until the 1940s. Improving on densitometry technology, Behnke and his colleagues in 1942 were able to accurately measure D_b [7]. In this and follow-up publications [8] the concept was advanced that fat and lean tissues each had a relatively constant density (d_f and d_{ffm}). Estimates of these densities were made by Behnke which were based upon approximate chemical compositions in the case of lean and on previous animal experiments in the case of fat. Once D_b is measured by hydrodensitometry, then fat and FFM are readily calculated based upon the assumed d_f and d_{ffm} . Subsequent workers focused on improving the d_f and d_{ffm} estimates [8,9]. Our emphasis here is on human studies, as one could argue that animal results cannot be extrapolated with confidence to man. In the 1950s Keys, Brozek, Fidanza, and others at the University of Minnesota [9,10] examined the estimated d_f and d_{ffm} in detail. Fidanza, using surgical specimens [10], found an average d_f of 0.9007 g/cc at 36°C. Unlike the largely single component fat (i.e. triglyceride), FFM represents a chemically

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diverse grouping of fluids and tissues. The Minnesota investigators developed a 4-compartment model based upon the major chemical categories that included fat (F), Water (A), protein (P), and mineral (M). The densities of A and P at body temperature were known, and the density of F was experimentally determined by Fidanza [10]. Mineral was divided into 2 fractions, osseous and cellular (M_o , M_c). Based upon the best estimates of the time, densities for M_o and M_c were calculated from the individual mineral components of bone and cells [9]. These densities and their reciprocal, specific volume, are presented in table 1. At the time there were 5 cadavers on whom the chemical composition was known in detail (table 2). Four were males and 1 was a female. One male with clearly abnormal hydration and the female were eliminated from the analysis. Through several assumptions the fractions (f) of body weight represented by F, A, P, M_o , and M_c were calculated for the 3 males (table 2). Body density and d_{ffm} were then calculated based upon the equation presented in table 3. The d_{ffm} of 1.10 g/cc agreed exactly with Behnke's estimated 1.1 g/cc. The now universally used Siri equation [11] for calculation fat from D_b is based upon these results, that is, a d_f and d_{ffm} of 0.90 and 1.10 g/cc, respectively. Today hydrodensitometry is universally applied as either the primary or calibration body composition method. However, some appropriate concern focuses on the assumed d_{ffm} . Several studies suggest that blacks have a higher mineral mass than whites, a finding which implies a relatively increased

dfm

blacks [11-13]. Men in general have a larger mineral mass than women, leading to speculation that the assumed dfm of 1.10 g/cc is lower in women. Aging, especially in women, is associated with a loss in mineral and a potential decrease in dfm. Hydration may increase in the obese, resulting in a lowering of dfm. Ethnicity, gender, aging, and obesity are all thus associated with potential changes in lean composition that could alter the assumed dfm density of 1.10 g/cc. The magnitude of error in fat analysis caused by an inaccurate dfm is shown in figure 1. While our emphasis here is on hydrodensitometry, similar questions circumscribe other 2-compartment body composition methods. A major development over the last decade are multicompart-

Table 1. Densities and specific volumes of chemical constituents at 36 °C. Modified from [4]

Substance

Density, g/cc

Specific volume, g/cc

ment body composition models based upon neutron activation analysis [14], dual photon absorptiometry [15], and stable isotopes [16]. These in vivo methods can both resolve the concerns related to 2-compartment models noted above and provide new and useful physiological somatometric information per se. The remainder of this presentation will focus on our own efforts in developing these new methods.

Mineral

Until recently the most elusive body compartment was mineral. Now several options are available for determination of M₀ and M_c. First, whole body counting combined with delayed gamma neutron activation analysis allows accurate measurement of Na, K, Ca, P, and Cl in vivo [4]. As nearly all calcium is in bone, the M₀ fraction can be calculated from the known ratio of Ca to bone ash. The remaining M_c can be calculated by first subtracting the amount of Na, Cl, and K in bone ash (table 4). The remainder is assumed to represent cell mineral. The cell K is used to calculate intracellular P, which is then converted to phosphate (table 4). M_c is the sum of bone ash-adjusted Na, K, and Cl plus phosphate. Other minerals and trace elements are assumed to represent a negligible fraction of body weight.

Fig. 1. Effect of variation in the density (D) of lean body mass on percent body fat estimated from densitometry. From [21]

Table 3. Equation for calculating the density of a mixture of two or more substances

-1

Σ

$\sum_{i=1}^n \frac{M_i}{M_t} \times SV_{ii} = \frac{1}{D}$

'M = mass of substance i and total (t) mass; SV = specific volume at 36 °C (see table 1).

Table 2. Body composition from direct analysis of 3 male cadavers as reported by Brozek et al [9]

Age, years Sex Height Weight

cm kg

Total weight

Fat-free weight

water

fat

protein ash
water

protein ash

46	M	168.5	53.8	55.1	19.4	18.6	5.4	68.4	23.1	6.7
35	M	183	70.6	67.9	12.5	14.4	4.8	77.6	16.5	5.5
25	M	179	71.8	61.8	14.9	16.6	7.5	72.6	17.5	8.8

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Another new approach to estimating mineral is based upon dual photon absorptiometry (DPA) [13]. DPA systems of increasing sophistication are now becoming available at many medical centers. Unlike neutron activation analysis, the DPA approach is generally less expensive and delivers a smaller dose of ionizing radiation.

The St. Luke's-Roosevelt system is a Lunar DP-4, which operates on the two energy levels of gadolinium. Through use of appropriate software, the system partitions body weight into total body bone ash (TBBA) and soft tissue mass. The scan time is about 50 min and radiation exposure is 5 mrem. The accuracy of DPA bone ash estimates is reportedly $\pm 5-7\%$ based upon skeletal and intact cadaver studies [17]. The precision of repeated TBBA measurements on consecutive days is $\pm 1\%$. At our center we correlated DPA-derived TBBA with total body Ca measured by delayed gamma neutron activation analysis. Thirteen healthy subjects who were at or near their desirable weight underwent the TBCa measurement at Brookhaven National Laboratory and the TBBA study at St. Luke's-Roosevelt Hospital. The results are shown in figure 2. Note that TBBA and TBCa are highly correlated ($r=0.96$, $p < 0.001$) and the ratio TBCa/ TBBA of 0.34 is similar to the calcium content of bone ash. DPA can thus provide M_0 by correction of TBBA for labile components lost during ashing. We currently use the factor of 1.043 suggested by Brozek [9], although clearly more work is needed in order to refine this estimate.

Table 4. Cell mineral equations

Composition of adult femur (cortex, g/kg):

Ca 26.4 Na 4.6 K 2.0 Cl 1.8

Cell mineral equation (g)a

*From [22]

$Na = TBNa - TBCa (0.017)$ $K = TBK - TBCa (0.076)$ $Cl = TBCl - TBCa (0.0068)$ phosphate = $K \times 1.66$

Total Body Protein

The next compartment, total body protein, can now be quantified by prompt gamma neutron activation analysis [18]. Total body nitrogen is measured and then converted to protein ($TBN \times 6.25$). Our system at Brookhaven has a precision of $\pm 4\%$ and delivers a 50 mrem radiation dose per study.

Total Body Water

The third major compartment is TBW. Technically, the water space is precise (1.5 %) and simple to measure using one of several different isotopes. A more difficult problem is to correct for hydrogen exchange in the case of 3H_2O or D_2O . In our studies we assume that TBW (or A) is equal to $0.95 \times ^3H_2O$ [19]. An adjustment is then made in TBW for density at $36^\circ C$ (table 1) with the result aqueous mass (A).

4 Compartments

The combined sum of M, P, and A is equal to FFM. One approach to calculating fat is to assume that the difference between body weight and FFM presents F. This method was used by Cohn et al. [20] through measurement of TBCa (M), TBN(P), and TBW(A). One weakness of this difference method is that all unmeasured substances are lumped into the F compartment. For example, 400–500g of unmeasured glycogen would be classified erroneously as lipid. Our alternative is to measure fat per se using either DPA or total body carbon. Only DPA will be discussed here. Our DPA system generates a soft tissue R value, a numerical expression of attenuation ratio at 44 and 100 KeV. The Rvalue varies with composition; for example, a linear relation exists between R and the proportion of ethanol in water (fig. 3). Similarly, a highly significant correlation exists between R and the percentage fat in ground beef mixtures (fig. 3). As the DPA system already pro-

Fig. 2. Correlation between TBBM measured by DPA and TBCa measured by neutron activation analysis. $TBBM = 2.65 TBCa - 98.8$; ($r = 0.95$, $p < 0.001$); $SEE = 186.7g$. From [22].

Fig. 3. R_{st} versus % ethanol in ethanol-water phantoms or % fat in beef-lard mixtures. The following 2 correlations apply: % ethanol = $-978.1 R_{st} + 1410.7$, $r = 0.99$, $p \pm 0.001$; and % fat = $-499.0 R_{st} + 732.6$, $r = 0.99$, $p < 0.001$. From [22].

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Table 5. Comparison of lean composition and dffm observed in the present study for pooled subjects (n = 13) to previous cadaver studies (n=3)a

fM,

fM,,

0.054 0.008 0.062 1.096

a (See [2]); this report did not present breakdown of mineral into osseous and non-osseous components. The results shown represent our estimates based upon the suggestions presented in the original report. Similarly, df% was not presented for each subject, but only for the pooled n = 3. The values for each cadaver dffm presented in the table represents our estimates based upon the above-mentioned partition of total mineral into M_{os} and M_{r} .

fP

fMn

fA

Present study

X 0.7390.198

0.042 0.065

Range

lower 0.714 0.164 upper 0.771 0.222

Cadavers (gender/age)

M35 0.779 0.166 0.048

M25 0.729 0.195 0.056

M46 0.696 0.234 0.064

X 0.7380.194 0.056

0.008 0.049 1.082

0.010 0.078 1.105

0.007 0.055 1.084

0.020 0.076 1.129

0.006 0.070 1.110

0.012 0.068 1.100

measured Db (10.38 ± 0.020 g/cc) was $\pm 0.6\%$. These results are encouraging, and we believe improvements in the model are possible.

The next step involved comparison of calculated D_{ffm} to the 3 cadaver studies summarized in table 2. Our calculated D_{ffm} was 1.096 g/cc, with no obvious gender or age effect in this small sample. This result is in remarkably good agreement with the 3 male cadavers, in which the mean D_{ffm} was 1.10 g/cc. The distribution between A, P, M, and F was also similar between in vivo measurements and cadavers (table 5).

In an initial study we surveyed the body composition of over 200 white women between the ages of 20 and 90 years. A similar but abbreviated protocol to that described above was used. The women were divided into 2 groups, 1 with a low DPA bone density (< 0.9 g/cm², n=23) and the other with a bone density above 0.9g/cm² (n=187). The osteopenic women tended to be older than their non-osteopenic counterparts ($X \pm SD$, age = 52 ± 6 vs. 37 ± 4 years). The main finding is that osteopenic women had a significantly reduced d_{ffm} (1.093 g/cc) compared to women who had a normal bone density (1.102 g/cc, $P < 0.001$).

vides the soft tissue R, we first developed a fat prediction equation by scanning 7 frozen ground beef phantoms of chemically determined fat content. The subject's fat was then calculated from the DPA study R. We found a close correlation between DPA fat and fat measured by other conventional methods such as hydrodensitometry, TBK, TBW, and neutron activation analysis ($r=0.89-0.93$, all $p < 0.01-0.001$). We therefore use DPA fat in the following presentation, as no underlying assumptions are used in this approach that might be confounded by ethnicity, aging, gender, or obesity.

Summary

The field of human body composition research by necessity is based upon assumptions related to tissue chemical content. The 2-compartment model, which requires only 1 measurement in addition to body weight, is the cornerstone of current research in this field. Yet the assumptions upon which 2-compartment models are based were developed on a limited scale and their validity under specified conditions is questionable. Recent developments now allow quantification of previously unmeasured somatic constituents. The capacity to extend our models to 4 or more components is now at hand. Not only will this allow us to vastly expand our validation of two 2-compartment approaches, but our ability to explore new and important physiological questions is within reach.

Chemical Measurement of Body Density in vivo

The preceding review of recently developed body composition methods indicates that all four major chemical categories related to body weight are now measurable. We therefore conducted a study that had a twofold aim: to compare Db calculated from chemical composition to Db measured by hydrodensitometry and to compare calculated d_{ffm} to the previously mentioned cada-verderived d_{ffm}.

The 13 subjects present above, 8 females and 5 males, underwent hydrodensitometry, prompt and delayed gamma neutron activation analysis, and DPA. Hydrodensitometry provided measured Db. Prompt gamma neutron activation allowed measurement of total body nitrogen, which we then converted to P. Tritium dilution and hydrogen exchange correction resulted in a value for A. DPA was used to measure M₀ (adjusted TBBA) and fat. Finally M_c was calculated

as the sum of bone adjusted TBK, TBNa, TBC1, and calculated phosphate (table 4). We found the sum of A, P, M₀, M_c, and F was an average of 3 % less than measured body weight. Presumably the unmeasured components of lean account for the missing 3 %.

Db calculated from chemical components agreed within 1 % of measured Db. The correlation between calculated and measured Db was highly significant ($r=0.96$, $p < 0.001$). The absolute difference between calculated ($X \pm SD=1.034 \pm 0.018\text{g/cc}$) and

Discussion

////(Auckland) placed practical aspects in the foreground. Which of these methods are relevant for patients, to what extent can they provide us with decisive help in the daily care of the sick? The sophisticated research methods provide basic conclusions, but are not available or permitted everywhere. The question of accuracy and precision arises in the case of simple techniques. Heymsfield (New York) indicated first of all a further problem which is of significance in the evaluation of the validity of various procedures. In examining the body composition of patients, we must be critical in testing whether the assumptions from which we are proceeding and upon which the subject is based are still valid. Many methods, for example, proceed from the assumption that the lean body mass consists of 73% water; a very questionable idea in cases of illness. Heymsfield then listed a range of various techniques; the most accurate methods are cadaver analysis and, as a direct in vivo measurement, neutron activation analysis (IVNAA). Then comes dual photon absorptio-metry. On a lower level there are important techniques such as the establishing of total body water (TBW), total body potassium (TBK) and hydrodensitometry based on various assumptions related to BC. Even further down the list are simple practicable processes such as, for example, anthropometry, impedance analysis (BIA) and total body electrical conductivity (TOBEC). These do not measure a tissue space directly, but are all calibrated with one of the methods mentioned above.

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Burkinshaw (Leeds) was sceptical regarding the accuracy of cadaver analysis. It is a very troublesome process, the chemical analyses are difficult and the results cannot easily be generalized, as most of those examined had succumbed to severe basic illnesses, with the changes to be expected in each case.

Hill saw a limit in the significance of hydrodensitometry, on account of a lack of practicability, the basic assumption and the difficulty of taking into account the air in the lungs and intestines. Shizgal (Montreal) pointed out first of all a basic problem. With several methods, the faulty measurements are to be found in the range in which the changes which we wish to measure are also found. Next, he spoke of the choice of procedure. The type of measurement in BC depends on the point of inquiry and on the answer which is being sought. In many cases, underwater weighing is probably adequate, but under other convincing circumstances certainly not.

When measuring the BC, we must bear in mind the purpose for which we need that measurement.

Burkinshaw established that the two-compartment model, as simple and as important as the division into fat mass and fat-free mass may be, is no longer sufficient to fulfil demands, and this for two reasons: firstly, the question of accuracy, and secondly, the fact that we can nowadays measure protein, water and other such things which interest us when people are ill.

Discussion summarized by D. Böhm

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