> *Only the man who is familiar with the art and science of the past is competent to aid in its progress in the future.*
>
> – Theodore Billroth

Classic Editor:
Harold Sandstead, MD
Professor, Department of Preventive Medicine and Community Health, University of Texas Medical Branch, Galveston, Texas, USA

Body Composition from Fluid Spaces and Density: Analysis of Methods

William E. Siri

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...Prospective Overview

Henry C. Lukaski, PhD
From the U.S. Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota, USA

Because methods for the assessment of human body composition are indirect and based on assumptions regarding the chemical or physical characteristics of various components of the body, an important issue is the variability to expect for estimates derived with any of various methods available. Siri convincingly addressed this fundamental issue more than 37 years ago when he evaluated the densitometric and isotope dilution methods, initially in a laboratory report[1] and later in a contribution in a conference proceedings.[2] The contribution serves as one of the landmarks in the field of body-composition methodology.

In the early 1950s, two techniques, hydrodensitometry or underwater weighing and hydrogen isotope dilution, were used routinely to assess body fatness in adults.[3] Each of these methods relies on assumptions regarding a unique chemical or physical property of the fat-free body (e.g., constant hydration and protein-to-mineral ratio) and fat (e.g., density of fat is less than that of bone, muscle, and protein) determined from chemical analyses. Although other investigators, e.g., Behnke et al.[4] and Keys and Brozek,[5] acknowledged the limitations of these assumptions, Siri[2] challenged the validity of these basic premises and formulated estimates of error in the prediction of body fatness based on the variability of the chemical composition of the fat-free body and adipose tissue.

Siri was troubled by the reliance on a "reference body" for which the relative chemical composition of the fat-free body was assumed to be constant or at least constant within narrow limits. Furthermore, it was assumed that, when body weight changed, fat was either added to or removed from the reference body without disruption of the basic assumption of constant composition of the fat-free body. Direct chemical analyses of animals indicated that these assumptions were valid.[6,7] However, studies of the body composition of humans during weight change associated with dietary changes suggested that adipose tissue, or the tissue that is gained or lost, is not only fat per se but also consists of water and cellular materials.[5,8] These observations led Siri to assess the components of error associated with the densitometric method.

The error associated with any indirect method of body-composition assessment has two components: measurement error and biological uncertainty. Siri concluded that measurement error is minor relative to the uncertainty associated with the interindividual variability in the assumptions of the chemical constancy of the fat-free body. He used a propagation-of-error model and determined that the error in estimating percentage body fat determined with densitometry was ~4% because of contributions from variability (standard deviation) in the water content (2.7%) and protein-to-mineral ratio (2.1%) of the fat-free body and adipose tissue composition (1.9%) in the general population. Siri indicated that this degree of uncertainty could be decreased if total-body water was used along with densitometry to estimate body fatness.

Siri emphasized the importance of treating measurements of components of the fat-free body as independent variables in the assessment of body fatness. This

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BODY COMPOSITION FROM FLUID SPACES AND DENSITY: ANALYSIS OF METHODS

WILLIAM E. SIRI

Introduction

The procedures for estimating body composition, and more particularly fat, from the volume of the fluid spaces and corporeal density are well established in principle. Quantitatively they have been open to a variety of interpretations ever since the early use by Behnke *et al.* (1942) of the underwater weighing technique for determining density, and the first uses of solutes for measuring extracellular and total body water. For the most part, these methods when applied to laboratory animals give results in close agreement with direct chemical analyses. Whether or not they can be applied to humans with expectation of equally reliable quantitative results is still open to conjecture, because the human population at large tends toward greater variability in some aspects of body composition than do laboratory animals. Because of this variability, estimates of fat derived from fluid spaces and density have in some instances been treated with considerably more confidence than the underlying premises of these methods would appear to grant. Moreover, estimates by one indirect method have been used to corroborate estimates by another, whereas, for example, total body water and body density must necessarily give identical fat values because the constants in the fat-estimated formulas are derived from the same basic assumptions.

Keys and Brožek (1953) reviewed critically the methods and concepts that had evolved in the investigation of body composition up to 1953. More recently Morales and Williams (1958) undertook an analysis of the densitometric method but failed to take into account some of the basic premises of this and the total-body-water methods for estimating fat. The specific methods for estimating body composition from density and fluid spaces still warrant closer analysis for the purpose of answering the fundamental questions: (1) How are fat, and protein plus mineral best estimated from total body water, extracellular fluid space, and body density, or a combination of such measurements? (2) What are the underlying assumptions in these methods and their range of validity? (3) What uncertainty does biological variability as well as error of measurement introduce into the final estimate? (4) For practical purposes, what accuracy is desirable in each of these measurements?

In the following sections each of the methods for estimating body composition from the fluid spaces, density, or their combination is examined in reference to its basic premises, inherent uncertainties, and general conclusions. The primary formulation is expressed in as general a form as the underlying assumptions in a method permit. Differentiation into specific working formulas occurs only when numerical values are assigned to the constants. Although this has been done, it must be emphasized that such formulas are provisional until the numerical values rest on more definitive data. Even then, however, the inherent limitations in the accuracy of any of the formulas should be recognized.

General Principles

The sole constituents of the body considered in the following analysis are lipids, water, protein, and mineral. The addition of carbohydrates, and a separation of lipids into "essential" and "nonessential" is not warranted, because none of the indirect methods for determining body composition is capable of differentiating such divisions. Water alone can for this purpose be regarded as two compartments, namely, the intra- and extracellular fluid spaces. For convenience in formulating the algebraic expressions relating to body composition, the constituents are expressed as decimal proportions of body weight, or of adipose tissue where this is indicated. Hence, fat is designated by f (kg fat/kg body weight, or if specified, kg fat/kg adipose tissue), with w, i, e, p, and m, similarly defined for total water, intra- and extracellular water, protein, and mineral.

All methods for deriving body composition have in common the two fundamental relations that the sums of the proportions of the constituents by weight and by volume must equal unit weight and volume:

$$(1) \qquad f + w + p + m = 1 \text{ (unit wt.)}$$

$$(2) \qquad F + W + P + M = 1 \text{ (unit vol.)}$$

A third expression may be derived that is more useful than Eq. (2) when densitometry is employed:

$$(3) \qquad \frac{1}{d} = \frac{f}{d_f} + \frac{w}{d_w} + \frac{d}{d_p} + \frac{m}{d_m}$$

in which d is the combined density, the density of the whole body, and d_f, d_w, d_p, and d_m are the separate densities of the constituents expressed in gm/cc.

The definitions of f, w, p, and m must be explicit if an interpretation of fat estimating equations is to be unambiguous. In any method involving densitometry, f consists of all substances that have essentially the same density as storage fat (triglycerides), but it is assumed that f includes only such fats. The same criterion necessarily applies to w, p, and m. In particular, water is regarded as pure water and not as body fluids, which are solutions mainly of proteins and inorganic salts and therefore have higher densities. Protein and mineral as expressed by p and m are the total of these constituents, including these substances in the fluid spaces as well as in cellular matter.

In the numerical evaluation of constants in formulas for estimating fat, it is assumed that the densities of f, w, p, and m are relatively constant compared to other biological factors, and the following values are used here:

$$\begin{aligned} d_f &= 0.900 \text{ gm/cc at } 37^\circ\text{C} \\ d_w &= 0.993 \text{ " " " "} \\ d_p &= 1.340 \text{ " " " "} \\ d_m &= 3.000 \text{ " " " "} \end{aligned}$$

The studies of Fidanza, Keys, and Anderson cited by Keys and Brožek (1953) indicate remarkable uniformity in the density of human fat irrespective of body site.[1] Further investigations are needed to establish that human fat density is essentially constant for all individuals. This result, though desirable, would be at variance with observations on melting points of human fat and the density and composition of animal fats, which appear to change somewhat with diet and environment. The reliability of the numerical values of d_p and d_m cannot be argued with great confidence. Proteins vary in density, and the value of 1.340 gm/cc is an average for fully hydrated protein *in vitro*. Whether or not it is the correct average for human protein *in vivo* has not been demonstrated. The same reservation applies to $d_m = 3.0$ as well, and some authorities will prefer 2.9 gm/cc.

Nearly as fundamental as the three universal relations stated above is the need for a reference body upon which all the methods except that of combined total body water and density are based. For the most part the reference body has been tacitly assumed and often ill-defined, but nevertheless present in every study of body composition. When only one or even two properties, such as water and density, are measured, it is necessary to assume that a constant relationship exists among the remaining constituents. In doing so, a reference body is implicitly introduced to which all individuals are presumed to conform except for differences in the proportion of adipose tissue.

The best defined reference bodies have been the "fat-free body," Behnke's "lean body mass" (Behnke *et al.*, 1953), and the Minnesota "standard man" (Keys and Brožek, 1953). Each of these assumes constant relationships between constituents that most indirect methods for estimating body composition cannot in themselves measure.

In the first of these concepts it is assumed that all adult normal humans are identical in their ratios of water, protein, and mineral, and that they differ only in possessing varying proportions of pure fat that is appended to the basic fat-free structure. Behnke's lean body mass is essentially the same thing except for recognizing that the body contains certain essential lipid substances such as phospholipids that are irreducible cellular constituents.

The view that the body may be regarded as fat-free structure to which pure fat is added appears to obtain in small mammals and is supported by some animal studies. The recent studies of Pitts (1956) appear particularly to support this contention in guinea pigs, at least in animals for which fat is less than 25% of body weight.

On the other hand, the extensive studies of Keys and Brožek on changes in body composition in humans during weight changes due to altered diet suggest that adipose tissue—or at least the tissue gained or lost—is not pure fat, but consists of water and cellular material as well. Behnke (1954; Behnke *et al.*, 1953) has reported similar findings, though numerically somewhat different. Keys and Brožek (1953) felt that the fat-free body could not serve as a suitable reference because its composition would depend, in part, on the fatness of the individual. Instead, they adopted a "standard (reference) man" derived from the mean composition estimated for a selected group of normal young men.

There is not as yet sufficient experimental evidence to formulate precisely what constitutes a satisfactory reference body, nor for that matter to assume that all adult humans must necessarily conform to any one reference. Nevertheless, a reference body is essential to most of the methods discussed here and must be introduced into any generalized formulation for calculating fat from fluid spaces or density. The analysis of each method therefore proceeds from a generalized reference body whose composition is $1 = f_o + w_o + p_o + m_o$ and whose density is d_o. It is then assumed that other individuals differ only in possessing a greater or smaller proportion of adipose tissue, A, whose generalized composition is $1 = f_1 + w_1 + p_1 + m_1$ with density d_1; where f_1, w_1, p_1, and m_1 are the proportions of the constituents in such tissue. The quantity A is therefore the "adipose tissue" difference between a given subject and the reference man in the sense that it was employed by Keys and Brožek but in more general form. The total proportions of fat, water, protein, and mineral in the normally hydrated person are therefore:

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[1] Editors' Comment (J.B.): See Fidanza, F., Keys, A., and Anderson, J. T., Density of body fat in man and other mammals, *J. Appl. Physiol.*, 6, 252-256 (1953).

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(4) \qquad
\begin{aligned}
f &= (1-A)\, f_o + A f_1 \\
w &= (1-A)\, w_o + A w_1 \\
p &= (1-A)\, p_o + A p_1 \\
m &= (1-A)\, m_o + A m_1
\end{aligned}
$$

One may now choose whatever composition seems appropriate for adipose tissue and for the reference body.

In the following sections, the general formulation for each method will also be evaluated for two extremes in reference body composition. The first is based on the Minnesota "reference man" (1952), characterized by $d_o = 1.063$ gm/cc, $f_o = 0.14$, $w_o = 0.61$, $p_o = 0.19$, $m_o = 0.06$, together with Keys and Brožek's estimate of the composition of "adipose tissue"; $d_1 = 0.948$ gm/cc, $f_1 = 0.62$, $w_1 = 0.31$, $p_1 = 0.07$, and $m_1 = 0.00$.

The second example is evaluated on the basis of the fat-free body, assuming the ratios between water, protein, and mineral are constant for all adult humans, and by identifying "adipose tissue" with pure fat. Under these conditions $f_1 = 1.0$, $w_1 = p_1 = m_1 = 0$, and the remaining quantities have approximately the following values: $d_o = 1.1$ gm/cc, $f_o = 0.0$, $w_o = 0.72$, $p_o = 0.21$, $m_o = 0.07$.

These two standards of reference are used primarily because they illustrate opposite extremes in concepts of reference bodies. It will be apparent in analyses of most methods that the choice of reference body may have less material effect on the estimate of fat or of protein and mineral than do the underlying uncertainties in the method. In view of the insensitivity of most methods and the consequent uncertainty associated with them, the characteristic values indicated above appear to be justifiable, even where there may be disagreement on the precise values of the proportions of constituents.

Technical Errors and Biological Uncertainty

It would be a misleading simplification to assume that the accuracy with which body composition can be estimated is dependent solely upon the accuracy with which corporeal density or the fluid spaces can be measured. Even if experimental errors were non-existent, there would still remain in most methods for estimating body composition a substantial residual uncertainty (standard deviation), estimated at about ±4% of body weight. Each method contains, whether explicitly or implicitly, a fixed reference body (or its equivalent) which incorporates a set of assumptions inter-relating constituents that cannot be measured directly. Thus, for example, all methods assume that mineral constitutes a fixed fraction of the fat-free body, or that it has a fixed ratio to protein, or that it conforms to some alternative empirical relationship. Since it can hardly be expected that all individuals will conform exactly to the same numerical constants in such relationships, individual deviations from the "standard" constitute an irreducible biological variability.

The empirical constants in fat estimating formulas may at best represent an average for a selected population. Furthermore, they are correct in only a limited segment of the obesity-emaciation range. The variability in each constituent therefore contributes its share to the uncertainty in an estimate of fat, protein, or mineral. Biological variability sets the limit of confidence one may have in estimates of body composition by methods now available, and it also sets a useful limit of accuracy that is desirable in measuring density and fluid spaces. This latter consideration is particularly significant from a practical standpoint. On the one hand, it may save the expenditure of great effort put into improving the accuracy of a measuring technique that would in reality produce no significant improvement in the estimate of fat, and on the other hand, would avoid interpreting an already precise measurement of density or fluid space as a comparably accurate determination of fat and body composition generally.

The over-all uncertainty in an estimate of fat must consequently include both biological variability and experimental error. Since the various methods can be formulated explicitly in terms of the biological variables, an estimate of this uncertainty expressed as standard deviation can be found by applying the Law of Propagation of Errors to the general formulas (See Appendix 1). This will also yield an estimate of optimum experimental accuracy that seems justified in applying a specific method.

The formulas for calculating the variance in the fat estimates are expressed in terms of the biological variables and their variance, experimental and biological. Obviously, the biological uncertainties must be the same in every method for estimating body composition from density and fluid spaces, although their cumulative effect may vary with the method used.

The standard deviations listed below are intended primarily to illustrate, when substituted into the appropriate formulas, the approximate magnitude of the uncertainty associated with each method. Nevertheless, their values are believed to be justified by the available data on body composition. The quantities to which they refer are indicated by subscripts.

Experimental: $\sigma_d = \pm 0.0025$ gm/cc
$\sigma_w = \pm 0.02$ body weight
Biological: $\sigma_a = \pm 0.1$
$\sigma_{w_o} = \pm 0.02$ reference body weight
$\sigma_{d_o} = \pm 0.01$ gm/cc
$\sigma_{d_1} = \pm 0.01$ gm/cc

The quantity α, the ratio of total mineral to protein, is discussed below under "Density—Total Body Water Method." The standard deviations σ_{w_0} and σ_{a_0} include the uncertainty in the exact composition of the reference body but more particularly reflect dispersion in body composition for the population. They are, in effect, measures of the deviation of individuals from a fixed reference. The standard deviation in d_o, the reference body density, is derived from σ_a and σ_{w_0} (See Appendix 2). The value of σ_{a_1} is estimated from the combined data of Keys and Brožek (1953), Behnke (1954); Behnke, *et al.*, (1953), and Siri (1956).

Densitometric Method

A correlation between corporeal density and fatness was suspected as early as 1901 by Stern (1901), but lacking an accurate technique for measuring body density, he could not establish a well-defined relationship.[1] By improving the underwater weighing method for determining density of the body by Archimedes' principle, and compensating for lung volume, Behnke, *et al.* (1942) were able to demonstrate a high correlation between overweight and density. Using this method Rathbun and Pacé (1945) formulated a quantitative relationship between body density and depot fat in guinea pigs by comparison with direct chemical analysis. The semi-empirical expression derived by these investigators has the form $f = (a/d) - b$, in which d is body density and a and b are empirical constants. The constants derived for humans on the basis of the guinea pig studies, which were related to body specific gravity rather than density, were $a = 5.548$ and $b = 5.044$. These values are still widely used although they contain a systematic error because they are based on an incorrect value of fat density. Keys and Brožek (1953) and Behnke (1954) later proposed somewhat different values based on more extensive though indirect human data and the correct fat density.

The formula for estimating fat from density alone is derived from the general formulations in the Section on General Principles. It requires that all adult humans be identical in composition except for individual differences in their proportions of adipose tissue. Thus the individual is necessarily regarded as a reference body of standard composition to which adipose tissue of some prescribed composition has been appended or from which it has been removed.

The formulas in the Section on General Principles are greatly simplified for the densitometric method if expressed in terms of the density of the reference body d_o and that of the generalized adipose tissue, d_1. An individual who differs from the reference body by a proportion of adipose tissue A is characterized by a mean body density d, related to A by

$$\frac{1}{d} = \frac{A}{d_o} + \frac{1 - A}{d_1} \tag{5}$$

Rearranging terms, the estimating equation for adipose tissue difference becomes

$$A = \frac{1}{d}\left(\frac{d_o d_1}{d_o - d_1}\right) - \frac{d_1}{d_o - d_1} \tag{6}$$

The difference that is pure fat is then $\Delta f = A f_1$, whereas the total proportion of fat in the individual is $f = A f_1 + (1 - A) f_o$, or more explicitly,

$$f = \frac{d_o d_1}{d}\left(\frac{f_1 - f_o}{d_o - d_1}\right) - \frac{d_1 f_1 - d_o f_o}{d_o - d_1} \tag{7}$$

Eqs. (6) and (7) are entirely general but still retain the form $f = (a/d) - b$ that was proposed originally.

The examples of numerical working forms of these equations may now be evaluated first on the basis of the Minnesota standard man, and then on the basis of the fat-free reference body. For the first of these, $d_o = 1.063$ gm/cc, $f_o = 0.14$, and $f_1 = 0.62$; hence,

$$A = \frac{8.764}{d} - 8.245 \tag{8}$$

$$f = \frac{4.206}{d} - 3.817 \tag{9}$$

These are essentially the equations proposed by Keys and Brožek (1953) except for small differences in the constants because fewer decimal places are used in d_o and d_1.

If, on the other hand, the fat-free body is the correct reference, then $d_o = 1.1$ gm/cc, $d_1 = 0.90$ gm/cc, $f_o = 0.0$, and $f_1 = 1.0$, and the fat estimating equation becomes

$$f = A = \frac{4.950}{d} - 4.500 \tag{10}$$

It is of interest, before examining the uncertainty in the method, to compare the values for fat derived from these and similar numerical formulas that have been proposed. For a man of density 1.050 gm/cc, the original Rathbun-Pace formula yields 23.9%, Keys and Brožek's version, which is the same as Eq. (9) above, gives 18.9%, whereas Eq. (10) above gives 21.5%. For a density of 1.000, the total fat estimated by these two formulas differs by 6% body weight.

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[1] Editors' Comment (J.B.): In the history of the densitometric analysis of body composition one should not overlook the contribution of W. Kohlrausch (Methodik zur quantitativen Bestimmung der Körperstoffe *in vivo*, *Arbeitsphysiol.*, 2, 28-45 (1930); Zur Kenntnis des Trainingszustandes, *Arbeitsphysiol* 2, 46-60.

A true estimate of the uncertainty associated with the determination of fat by the densitometric method, as pointed out in the Section on Technical Errors and Biological Uncertainty, must include not only the error of measurement in d, but also the biological variability associated with the assumptions made in formulating the method. The standard deviation in the estimated value of fat may be derived from the general Eqs. (6) and (7) by applying the Law of Propagation of Errors, recognizing that there will be dispersion in d_0, d_1, f_0, and f_1 due mainly to the variability in total body water and in the mineral-protein ratio among individuals with the same weight and fat. The over-all uncertainty, expressed as the variance σ_f^2 in fat and variance $\sigma_{\Delta f}^2$ in difference in fat between subject and reference body are given in explicit form in Appendix 3. Numerical evaluation of σ_f and $\sigma_{\Delta f}$ requires only approximate value of d_0, d_1, f_0, f_1 to be generally valid. Using the values proposed by Keys and Brožek given above, and a subject of density 1.050 gm/cc, the variances become

$$\sigma_f^2 = 14.56\sigma_d^2 + 11.18\sigma_{d_0}^2 + 0.23\sigma_{d_1}^2 + 0.81\sigma_{f_0}^2 + 0.01\sigma_{f_1}^2 \tag{11}$$

$$\sigma_{\Delta f}^2 = 24.28\sigma_d^2 + 18.66\sigma_{d_0}^2 + 0.38\sigma_{d_1}^2 + 0.01\sigma_{f_1}^2 \tag{12}$$

The standard deviation σ_d represents solely the error in measuring the subject's density and for the present purpose is taken as ±0.0025 gm/cc. The remaining standard deviations reflect primarily biological variability; thus, variations in the mineral-protein ratio in total body water introduce a dispersion into d_0, even though the reference body may be a true average for the population and its composition known precisely. The estimated values, which are discussed in the Section on Technical Errors and Biological Uncertainty, are $\sigma_{d_0} = \pm 0.01$ gm/cc, $\sigma_{d_1} = \pm 0.01$ gm/cc, $\sigma_{f_0} = 2 \pm 0.02$ reference body weight, and $\sigma_{f_1} = \pm 0.05$ unit adipose tissue. The standard deviation in fat estimated by the densitometric method becomes

$$\sigma_f = \pm 4.0\% \text{ body weight}$$

$$\sigma_{\Delta f} = \pm 4.6\% \text{ body weight.}$$

Several conclusions may be drawn from the foregoing analysis of the densitometric method. First, it is evident that little is gained, especially in view of the increased technical difficulties, in attempting to measure body density more accurately than about ±0.005 gm/cc. If there were no error whatever in measuring density, the uncertainty in fat estimate would still remain ±3.8% body weight primarily because of normal variability in body constituents, and also because of the uncertainty in attempting to establish the compositions of adipose tissue and reference man that are true averages for the category of subjects measured.

Second, the uncertainty in the estimate of difference in fat, Δf, or in adipose tissue, A, between subject and reference is the same or greater than the uncertainty in the estimate of total fat. While this result is not intuitively evident, it follows from the fact that the same uncertainties affect both Δf and f.

Third, the reference body cannot be formulated from densitometric analysis alone without danger of introducing a large systematic error. This error does not stem from lack of precision in measuring density, but from the impossibility of establishing body composition solely by measuring one quantity such as density or total body water. As a corollary to this, it may be noted that even if the densities of both subject and reference were determined with great accuracy, the uncertainty in the estimate of fat would still be 3.8% body weight.

Fourth, significant differences from the average in any of the gross constituents other than fat introduce a comparable indeterminate error in fat estimate. The method is obviously invalid, for example, in the presence of abnormal hydration.

Fifth, the nature of tissue gained or lost during weight change cannot be deduced from densitometry alone if other tissues in addition to adipose tissue are involved. It is conceivable, for example, that the apparent density of tissue lost could be less than that of pure fat, i.e., 0.9 gm/cc, if there occurred a gain in muscle mass concurrently with a loss of adipose tissue.

Total Body Water Method

Investigations of the gross composition of small animals by direct analysis reveal for the most part a relatively constant fraction of water in the fat-free body and a high inverse correlation between ether-extractable fat and total water. This has been demonstrated most extensively in the guinea pig (Pacé and Rathbun, 1945; Pitts, 1956), suggesting that, at least in a limited range of fatness, such animals consist of a basic lean structure to which pure fat is appended without greatly altering the relative proportions between water, protein, and mineral. If this conclusion is accepted, the proportion of fat is given on the average by the widely used formula

$$f = 1 - \frac{w}{w'} \tag{13}$$

where w is the measured total body water and w' the proportion of water in the fat-free body, which has been variously estimated from 67 to 74%.

There are, on the other hand, no comparable experimental data to support a similar conclusion for the constancy of the human body. On the contrary, there is some direct (Forbes *et al.*, 1953; Mitchell *et al.*, 1945; Widdowson *et al.*, 1951) as well as indirect (Siri *et al.*, to be published) evidence to demonstate that such a pattern is not followed quantitatively. Adipose tissue is thought by some investigators to consist in part of water and protein so that these constituents should increase in absolute amount with obesity (Behnke, 1954; Keys and Brožek, 1953). A greater variability in the ratio of mineral to protein among humans, compared to small mammals, would also affect independently the constancy of the total body water fraction, as would also transient and pathological alterations in hydration. There is no way in which either altered hydration or deviations in the ratio of mineral to protein can be taken into account in estimating fat solely from total body water. However, if water is associated with adipose tissue, this can be expressed in the formula relating fat to total body water, assuming the water fraction of adipose tissue is constant. In principle, a somewhat more general equation than that above should be obtained.

As we have seen, a reference body and a generalized form of adipose tissue are inherent in a formulation of the densitometric method. They are equally necessary in deriving the body water formula for estimating fat. Not only are the same assumptions required, but the reference body must be identically the same in the densitometric and total body water methods if they are to be mutually consistent. A subject who then differs in composition from that of the reference body is presumed to differ only in possessing a proportion A of adipose tissue that is greater or smaller than that of the reference body. The total water and fat in the normally hydrated person are then the sums of these constituents associated with the difference A in adipose tissue, plus that associated with the proportion $1 - A$ of the body that corresponds to the reference body:

$$w = Aw_1 + (1 - A)\ w_o \tag{14}$$

$$f = Af_1 = (1 - Af)\ f_o \tag{15}$$

Combining equations, the general relation between total fat and water is

$$f = \frac{w_o - w}{w_o - w_1}\left(\ f_1 - f_o\right) + f_o \tag{16}$$

The difference in adipose tissue between reference and subject is then: $A = (w_o - w)/(w_o - w_1)$, while the difference in fat is $\Delta f = Af_1$. Eq. (16) is the most general relation between fat and water that is consistent with what is presently known of body composition.

The choice of reference man, insofar as it is an accurate average in a given obesity range, is otherwise arbitrary.

The numerical form of the fat estimating equation based upon the Minnesota standard man (Keys and Brožek, 1953) as a reference (See the Section on General Principles) becomes

$$f = 1.016 - 1.600\ w \tag{17}$$

If, however, the fat-free body is the appropriate reference, the equation is then

$$f = 1.000 - 1.390\ w \tag{18}$$

The validity of the total body water method for estimating fat rests upon the same assumptions that are inherent in the densitometric method. The uncertainty associated with fat estimated by this method will consequently reflect the error in measuring total body water together with the actual and irreducible variability in body composition for the population, and of course, any uncertainty in reference body composition.

The variance in the estimate of fat, taking these factors into account, may be derived from Eq. (16), and is given in explicit form in Appendix 3.

The numerical magnitude of the uncertainty in the estimated fat may be illustrated with a subject for whom water constitutes 55% of body weight, and using Minnesota standard man as a reference (See Section on General Principles). The numerical values of the standard deviations in w_o, w_1, f_o, and f_1 were discussed in the Section on Technical Errors and Biological Uncertainty and the Section on Total Body Water Method. The estimate of fat and the attendant standard deviation calculated with Eq. (18) above and the formula for σ_f in Appendix 3 are

$$f = 23.6 \pm 4.8\%\ \text{body weight}$$
$$\Delta f = 12.4 \pm 5.5\%\ \text{body weight}$$

Similarly, an estimate of fat in the same subject may be calculated from Eq. (18) based on the fat-free body as a reference:

$$\Delta f = A = f = 23.6 \pm 3.5\%\ \text{body weight}$$

Although in the example given here, in which $w = 0.55$, the calculated value of fat is the same by both formulas, in very lean and very obese persons the two formulas differ by about 3% of body weight. This, however, is still within the estimated uncertainty of the method.

It is seen at once, in view of technical difficulties involved, that reducing the error in total body water measurement below ± 2% of body weight is of doubtful value. More precise water measurement yields little improvement in the reliability of the fat estimate. If $\sigma_w = \pm 1\%$, the uncertainty in fat would be reduced only to $\pm 3.9\%$. Indeed, if there were no error whatever in total body water measurement the uncertainty σ_f in total fat would still be $\pm 3.6\%$ of body weight because of irreducible variabilities in the other factors.

A particularly significant result is the fact that the standard deviation associated with the differential fat estimate is, if anything, greater than that for the estimate of total fat. The reason for this is explicit in the formulas for σ_f and $\sigma_{\Delta f}$, both of which contain the same factors affected by biological variability and error of measurement.

No attempt was made to evaluate systematic errors inasmuch as they may vary widely with techniques used. Such errors include hydrogen exchange in measuring body water with hydrogen isotopes, errors in the estimate of the compositions of the reference body and adipose tissue, and possibly the use of a reference body of one composition for the whole of the emaciation-obesity range. Altered hydration will, of course, render the method invalid.

Finally, it may be noted that the densitometric and total body water methods are not independent means for estimating fat. Aside from errors in measurement, both methods must in the strictest sense yield identical values, for they are derived on precisely the same premises in whatever formulation one chooses to accept. If, on the average, the two methods, when used separately, lead to different values for fat, it can only mean that inadvertently two different reference bodies were implicitly involved and consequently the constants in the density or in the total body water equation, or in both, must be readjusted.

Density—Total Body Water Method

Combined measurements of density and total body water yield a method for estimating body composition that does not require a reference body nor an explicit description of the composition of adipose tissue. The method is based, not on separate estimates of fat by the two measurements, but rather on a single formulation in which density and water occupy the roles of independent variables (Keys and Brožek, 1953; Siri, 1956). Although it is the method that appears to be the least affected by biological variability, because it requires the fewest assumptions concerning interrelations between constituents, it is not, nevertheless, wholly free of such uncertainties. On the other hand, since only one assumption need be made, it is possible to choose an empirical relationship for which the associated biological variability has relatively little effect on the reliability of the fat estimating equation.

A formulation of the method is derived directly from the fundamental equations (1) and (2) which, it may be recalled, apply to a body of any description.

One additional relationship is needed to complete the system, but it may be any assumption one chooses that relates two of the constituents by means of a constant. However, among the numerous relationships between constituents that can, and have been postulated, only one is best suited to the present method. It assumes that the ratio of mineral to protein is constant, i.e., $m = \alpha p$, or its equivalent, that mineral forms a constant percentage of the mineral-protein fraction of the body. This ratio is not altered by abnormal hydration, and the effect that adiposity may have upon it is relatively small, but more important, the estimate of fat is not strongly affected by fluctuation or uncertainty in the mineral to protein ratio.

The formula for fat, as well as that for estimating the standard deviation, is greatly simplified by introducing the substitution $s = p + m = p\ (1 + \alpha)$ and the combined density, d_s, of protein and mineral given by

$$(19) \qquad d_s = \frac{(1 + \alpha)\ d_m d_p}{d_m + \alpha d_p}$$

Combining these equations with the fundamental equations in the Section on General Principles, the general formula for fat becomes

$$(20) \qquad f = \frac{d_f}{d_s - d_f} \left[\frac{d_s}{d} - w \left(\frac{d_s - d_w}{d_w} \right) - 1 \right]$$

The value of α, upon which an estimate of d_s depends, rests on admittedly meager data for humans. Although it is relatively consistent in laboratory animals, with a value of about 0.25 (Pacé and Rathbun, 1945; Spray and Widdowson, 1950), the ratio appears to be substantially greater and more variable in humans. The direct analyses of five cadavers by Mitchell *et al.* (1945), Forbes *et al.* (1953), and Widdowson *et al.* (1951), whose results are summarized by Keys and Brožek (1953), yielded values ranging from 0.292 to 0.404. For the present purpose in illustrating a numerical form of the fat-estimating equation, a value of $\alpha = 0.35$ is adopted, which corresponds to total mineral of about 7% of the fat-free body. The exact value of α, either for the individual or for the average, is not needed however, for as shown below a considerable variation in α does not greatly affect the estimate of fat and of $p + m$.

The combined density of protein and mineral for $\alpha = 0.35$ is then $d_s = 1.565$ gm/cc. Substituting this and the numerical values for d_f and d_w into Eq. (20), the fat estimating equation becomes:

$$(21) \qquad f = \frac{2.118}{d} - 0.780w - 1.354$$

The reliance that can be placed in an estimate of fat by this method is affected by the one empirical constant, α, in addition to the errors in measuring density and water. The magnitude of the uncertainty this produces can be estimated by applying the Law of Propagation of Eqs. (19) and (20) to determine the over-all standard deviation σ_f. The variance in d, and in f takes the forms given in Appendix 3. Inserting the numerical values for d_f, d_w, and d_a, the variance in the estimate of fat reduces to

$$\sigma_f^2 = \frac{4.22}{d^2}\sigma_d^2 + 0.608\sigma_w^2 + \left(1.120 - \frac{1.015}{d} - 0.106w\right)^2\sigma_\alpha^2 \tag{22}$$

The effect of biological variability introduced through α depends somewhat on the fatness of the individual; it is greatest for very lean individuals and becomes smaller with obesity. Although there are no direct data other than that referred to above, it is reasonable on the basis of this and indirect data to assume that the standard deviation in the ratio of mineral to protein for humans is not greater than ±0.1, i.e., about ±30% of the assumed mean value of α.

The uncertainty to be expected in a determination of fat by the density-total body water method may be illustrated for a subject with $d = 1.050$ gm/cc and $w = 0.55$. Substituting $\sigma_\alpha = \pm 0.1$ and the experimental errors of $\sigma_d = \pm 0.0025$ gm/cc and $\sigma_w = \pm 0.02$ into Eq. (22) yields a standard deviation in fat estimate of $\sigma_f = \pm 2.0\%$ body weight.

From the preceding analysis several conclusions may be drawn regarding the applicability and validity of the method. First, the *d-w* method is valid for all states of hydration. Moreover, since the isotopes of hydrogen can be used as solutes in measuring body water, the method is for practical reasons the only one that appears to be generally valid in estimating fat when extensive edema, pleural effusion, or ascitic fluid is present. In some circumstances the test solutes for extracellular water, which in principle is the only alternative measure of excess hydration, cannot be expected to give a correct fluid volume because of their rapid disappearance and slow diffusion. Second, the estimate of fat and of $p + m$ is relatively little affected by biological variability. Third, it is evident from Eq. (22) that little is to be gained in measuring body density more accurately than ±0.0025 gm/cc. In fact, an error as great as 0.004 gm/cc does not greatly affect the over-all accuracy of the fat estimate. This conclusion applies even if the error in water measurement were reduced to ±1% of body weight. Fourth, the error in measuring total body water, set here at 2%, introduces the largest single source of error. In the example given above, a reduction in the water error from ±2% to ±1% of body weight would reduce σ_f to ±1.5% of body weight. Fifth, if the experimental errors were altogether negligible, the uncertainty in fat estimate would still remain about ±1.2% body weight unless σ_α were substantially less than ±0.1. On the other hand, even if σ_α were as great as ±0.2, the resulting uncertainty in fat would be only ±1.7%. Sixth, an estimate of total protein plus mineral is just as valid as that for fat, although the relative error is slightly greater.

Density—Extracellular Fluid Method

Intuitively, it would seem advantageous to combine extracellular fluid space and corporeal density in a method similar to that of total body water and density for estimating fat. However, the reliability that might be anticipated is offset by the increased complexities of the assumptions that are inherent in such a method and by the substantial uncertainties that extracellular fluid space introduces both on theoretical and practical grounds (Siri, 1956).

With the introduction of extracellular fluid, the body must be regarded as a system of five components instead of four, i.e., $1 = f + i + e + p + m$, where i and e are the intra- and extracellular water proportions of the body respectively. The additional compartment necessarily increases the number of assumptions needed to relate f, i, e, p, and m. It is also necessary, as in other methods to introduce a reference body and a prescribed form of adipose tissue. A considerable array of possible relationships among the five constituents are available for a formulation of this method in addition to the basic equation above and the corresponding general equation for density:

$$\frac{1}{d} = \frac{f}{d_f} + \frac{i+e}{d_w} + \frac{p}{d_p} + \frac{m}{d_m} \tag{23}$$

To include the possibility of abnormal hydration, it is necessary to regard e as the sum of a component g associated with the normally hydrated person and a component h representing the excess as in edema, or deficit as in dehydration. Whatever approach is then taken, the following relations are inherent in a formulation of the method:

$$\begin{aligned} m &= \alpha p \text{ or } m = \beta\,(1 - f - h) \\ i &= \mu\,(1 - f - h) \\ g &= \nu i \end{aligned} \tag{24}$$

where α and β are empirical constants relating mineral to protein, μ is a constant relating intracellular water to the fat-free body, and ν is a constant relating extracellular to intracellular water. In particular it is necessary to the validity of the method to assume that intracellular water is in no way affected by abnormal hydration.

A person who differs from the reference by a proportion of adipose tissue A and possibly an abnormal proportion of extracellular water h must then have a density given by

$$\frac{1}{d} = \frac{1 - A - h}{d_o} + \frac{A}{d_1} + \frac{h}{d_w} \tag{25}$$

where the subscripts o and 1 signify reference body and adipose tissue respectively. When combined with the expression for total extracellular fluid, $e = (1 - A - h)e_o + Ae_1 + h$, and that for total fat, $f = (1 - A - h)f_o$, the estimating equation for total fat has the form

$$f = \frac{f_1(1 - e_o) - f_o(1 - e_1)}{1 - e_o} + f_o\frac{1 - e}{1 - e_o} \tag{26}$$

The constants in the equation may now be evaluated for the two reference bodies. With the values proposed by Keys and Brožek, the equation becomes

$$f = \frac{5.148}{d} - 0.573\,e - 4.612 \tag{27}$$

For a subject with $d = 1.050$ gm/cc and $e = 0.14$, as an example, $f = 21.0\%$ body weight.

If, however, the fat-free body were the more nearly correct reference, then $f_1 = 1$, $f_o = e_1 = 0$, e_o is about 0.18, and the general fat formula reduces to

$$f = A = \frac{4.475}{d} - 0.535\,e - 3.972 \tag{28}$$

When applied to the subject above, a value of $f = 21.5\%$ body weight is calculated.

In the middle range of fatness, i.e., 15 to 30%, the difference between the two estimating formulas is negligible, while in the extremes of leanness and obesity, the difference is never greater than 3% of body weight. Even under the extreme conditions, the difference in the fat estimates derived on the basis of two references is far less than the uncertainty associated with either formula. So far as the method is concerned, it seems immaterial whether one chooses to think of adipose tissue as pure fat or some combination of fat, water, and protein. For the same reason it makes relatively little difference whether the fat-free body or some other reference is used.

A serious limitation in the reliability of this method stems from the large uncertainty in measuring extracellular fluid and the ambiguity in precisely what it means. Related to this is the difficulty in ascertaining the normal variability in extracellular water. By the method in this and the following section any deviation in the volume of extracellular fluid from that of the reference plus adipose tissue can only be interpreted as altered hydration, whereas it may be a normal variation in the extra- to intracellular water ratio, and a systematic error in fat is then introduced.

The method in principle takes into account abnormal hydration, but on the other hand, it is not always likely to do so in practice. It is questionable whether any of the solutes that are employed in measuring extracellular fluid can be expected to yield valid results in the presence of a substantial volume of transudate (Siri, 1956).

Additional uncertainties are introduced, as in the other methods, by the normal variability in total body water and the mineral to protein ratio among individuals in a population. These factors alone lead to an uncertainty in the fat estimate of about ±4% body weight.

In view of the great number of assumptions that are necessary and the possibility of large systematic error, it seems unlikely that the combination of density and extracellular fluid will yield an estimate of fat as reliable as that derived from density alone.

Extracellular—Total Body Water Method

An analysis of methods for estimating body composition would not be complete without examining the use of combined measurements of the extracellular fluid space and total body water. The general assumptions described in the last section governing the reference body and adipose tissue are again necessary in essentially the same form for this method. Assuming as before that an excess or deficit in total fluids, expressed as a fraction h of the body weight, is associated only with extracellular fluid space, the actual proportions of total water and extracellular water are then

$$w = (1 - A - h)\,w_o + Aw_1 + h \tag{29}$$

$$e = (1 - A - h)\,e_o + Ae_1 + h \tag{30}$$

where the subscripts o and 1 designate quantities associated respectively with the reference body and adipose tissue. Combining these two equations to eliminate h, and then with $f = (1 - A - h)\,f_o$ to eliminate A, the fat estimating equation becomes

$$f = ek\left[f_1(1 - w_o) - f_o(1 - w_1)\right] - wk\left[f_1(1 - e_o) - f_o(1 - e_1)\right] + k\left[f_1(w_o - e_o) - f_o(w_1 - e_1)\right] \tag{31}$$

where

$$k = 1 \Big/ \left[e_1(1 - w_o) - e_o(1 - w_1) + w_o - w_1\right]$$

The general formula may now be evaluated on the bases of the two references. Inserting first the constants for the Minnesota standard man and the values $e_o = 0.16$, and $e_1 = 0.14$ proposed by Keys and Brožek (1953), the fat estimating equation is

$$f = 0.596\ e - 1.620\ w + 1.041 \tag{32}$$

With the fat-free body as the reference, $f_1 = 1$, $e_1 = w_1 = f_o = 0$, and the fat estimating equation becomes

$$f = A = 0.519\ e - 1.518\ w + 1 \tag{33}$$

Estimates of fat on the basis of the two reference bodies never differ by more than 1.5% of body weight. This difference is far smaller than the inherent uncertainty of this method, consequently, the choice of reference, adipose tissue composition, or other assumptions that may be introduced, are relatively unimportant. Conversely, the method cannot be expected to give a very reliable estimate of body composition.

The introduction of extracellular space merely compounds the difficulties by adding greater uncertainties than those associated with estimating body composition solely from total body water. However, the most important conclusion is this: in the presence of edema, the method is subject to serious systematic error, and for normally hydrated persons, an extracellular-total body water method does not in fact exist. The latter conclusion may be demonstrated by formulating the method for conditions of normal hydration, in which case either the extracellular fluid space or the total body water cancels out of the formulation. One or the other measurement is redundant.

Appendix 1

If a quantity f is related by a function F (a, b, c, ...) to the quantities a, b, c. . . . ., each of which is subject to an uncertainty expressed as standard deviation, σ, the Law of Propagation of Errors provides the appropriate rule for calculating the cumulative uncertainty in f. For simplicity the formula is expressed below in terms of variances (standard deviations squared, σ^2):

$$\sigma_f^2 = \left(\frac{\delta F}{\delta a}\right)^2 \sigma_a^2 + \left(\frac{\delta F}{\delta b}\right)^2 \sigma_b^2 + \left(\frac{\delta F}{\delta c}\right)^2 \sigma_c^2 + \cdot\ \cdot\ \cdot$$

where $\delta F/\delta a)$ is the partial derivative of the function with respect to quantity a, and σ_a is the standard deviation in a.

Appendix 2

As explained in the text, the standard deviation of ±0.01 gm/cc in the value of the reference body density is intended as a measure of the residual dispersion in body density of humans after adjustment to the same proportion of fat as the reference body that was selected. The magnitude of the uncertainty in d_o is based here on the dispersion in normal total body water of $\sigma_w = \pm\ 2\%$ body weight together with a dispersion of ± 0.1 in the mineral-protein ratio. The resultant uncertainty in d_o is then derived as follows, assuming $m = \alpha p$:

The reference body density may be expressed as

$$\frac{1}{d_o} = \frac{f_o}{d_f} + \frac{w_o}{d_w} + \frac{(1 - f_o - w_o)(d_m + \alpha d_p)}{(1 + \alpha)d_p d_m}$$

Applying the Law of Propagation of Errors, with the condition that f_o is constant, the variance is then

$$\sigma_{d_o}^2 = d_o^4\left(\frac{1}{d_w} - \frac{d_m + \alpha d_p}{(1 + \alpha)d_p d_m}\right)^2 \sigma_{w_o}^2 + d_o^4\left(\frac{(1 - f_o - w_o)(d_p - d_m)}{(1 + \alpha)^2 d_p d_m}\right)^2 \sigma_\alpha^2$$
$$= 0.164\ \sigma_{w_o}^2 + 0.0042\ \sigma_\alpha^2$$

With $\sigma_{w_o} = \pm\ 0.02$ and $\sigma_\alpha = \pm\ 0.1$, the standard deviation in d_o becomes $\sigma_{d_o} = \pm\ 0.01$ gm/cc.

Appendix 3

A. Variance in densitometric estimate of fat.

$$\sigma_f^2 = \left(\frac{d_1 d_0(f_1 - f_0)}{d(d_0 - d_1)}\right)^2 \left[\frac{\sigma_d^2}{d^2} + \left(\frac{d - d_1}{d_0(d_0 - d_1)}\right)^2 \sigma_{d_0}^2 \right.$$
$$\left. + \left(\frac{d - d_0}{d_1(d_0 - d_1)}\right)^2 \sigma_{d_1}^2 + \left(\frac{d - d_1}{d_1(f_1 - f_0)}\right)^2 \sigma_{f_0}^2 + \left(\frac{d - d_0}{d_0(f_1 - f_0)}\right)^2 \sigma_{f_1}^2\right]$$

The corresponding variance in the differential fat estimate, Δf, is also given by the equation above if f_o is set equal to zero and the fourth term in the bracket is omitted.

B. Variance in fat estimated from total body water.

$$\sigma_f^2 = \left(\frac{f_1 - f_0}{w_0 - w_1}\right)^2 \left[\sigma_w^2 + \left(\frac{w - w_1}{w_0 - w_1}\right)^2 \sigma_{w_0}^2 + \left(\frac{w - w_0}{w_0 - w_1}\right)^2 \sigma_{w_1}^2 \right.$$
$$\left. + \left(\frac{w - w_0}{f_1 - f_0}\right)^2 \sigma_{f_1}^2 + \left(\frac{w - w_1}{f_1 - f_0}\right)^2 \sigma_{f_0}^2\right]$$

The corresponding variance in the differential fat estimate, Δf, may also be calculated by deleting the last term and f_o.

C. Variance in fat from combined density and total body water.

The variance in density of mineral-plus-protein has the form

$$\sigma_{d_s}^2 = \left[\frac{d_m d_p(d_m - d_p)}{(d_m + \alpha d_p)^2}\right]^2 \sigma_\alpha^2 = 0.308\ \sigma_\alpha^2$$

while the variance in f, after substituting for $\sigma_{d_s}^2$, becomes

$$\sigma_f^2 = \left(\frac{d_s d_f}{(d_s - d_f)d^2}\right)^2 \sigma_d^2 + \left(\frac{d_f(d_s - d_w)}{d_w(d_s - d_f)}\right)^2 \sigma_w^2$$

$$+ \frac{0.308\ d_f^2}{(d_s - d_f)^4} \left(1 - \frac{d_f}{d} - \frac{d_w - d_f}{d_w} w\right)^2 \sigma_\alpha^2$$

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...Prospective Overview (continued)

recommendation is timely today in that investigators assess the body composition of population groups whose fat-free body composition is altered by developmental status and ethnicity. As clearly shown by Lohman,[9] failure to use measurements of total-body water and bone mineral content in conjunction with densitometric determinations results in an overestimation of body fatness in children. Studies in elderly people[10] and different ethnic groups[11] also indicate the need for use of measurements of bone mineral and water to yield meaningful estimates of body composition.

The general model used by Siri,[2] fat = $a(\text{density})^{-1} + b$, is consistent with the equations published by other investigators who sought to use density to index body fatness.[4-8] Although this model is generally acceptable, Siri acknowledged that its major limitation is sample specificity[2]; it does not account for variations in hydration, obesity, or bone mineral density seen among individuals in the population. Furthermore, the validity of the general model has never been evaluated with direct chemical methods. The recent findings of Muscaritoli et al.[12] indicate the bias in the prediction of body fatness with the general Siri model based only on densitometric measurements and suggest the need for a modification of the model.

For more than 50 years, densitometry has been used as a reference method for the assessment of human body composition. Siri calculated the uncertainty of estimating body fatness from whole-body densitometry based on the variability of the chemical composition of the fat-free body. The importance of these calculations was, and continues to be, the acknowledgment of the need to use determinations of total-body water and bone mineral, together with body density measurements, to reduce error in the estimation of an individual's body fatness because of variation in the chemical composition of the fat-free body and varying amounts of adipose tissue. The significance of Siri's contributions on body-composition assessment is embodied in the current use of multiple independent measurements of components of the fat-free body to increase the validity of the body composition of children, elderly people, and various ethnic groups.

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