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ABSTRACT

The gross composition of the human body is regarded as a mixture of water, lipids, and fat-free solids, the last consisting mainly of protein and inorganic mineral. The proportions of these constituents are important biological variables, both in normal and diseased states of the body, but their evaluation has been subject to considerable uncertainty. Total body water is the only constituent amenable to direct measurement in vivo, whereas the remaining constituents can only be estimated indirectly from the proportion of body water or from the whole-body density. The indirect methods for estimating fat and fat-free solids are subject therefore to both experimental error and biological variability. The consequent uncertainty in the estimates depends largely on the assumptions that are inherent in the methods that are used. Each of the procedures for estimating body composition that is based on body water, corporal density, or a combination of these measurements is examined for its basic premises, its formulation, and the limits of its validity. Formulas are derived for calculating total depot fat in humans and for calculating the standard deviation. In general, the uncertainty in fat estimated from density or water is about ± 4% of body weight, whereas combined body water and density yield an error of ± 2%.

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1. Introduction

In an era when quantitative in vivo measurement dominates many physiological investigations, it is hardly necessary to stress the need for reliable means for estimating the gross constituents of the human body. With in vivo techniques now available, however, only the fluid spaces of the body can be measured directly, whereas the body's burden of fat can be derived only approximately by indirect methods involving the fluid spaces and the density of the whole body. For the remaining principal constituents, protein and mineral, there is not even an indirect approach that can be said to be wholly satisfactory. While admittedly new and independent methods are needed perhaps more than refinements in existing techniques, still the fluid spaces and body density are likely to remain essential measurements in any analysis of gross constituents, even if practicable direct methods are in the future devised for other constituents.

The procedures for estimating body composition, and more particularly fat, from the size of the fluid space and corporal density are well established in principle. Quantitatively they have been open to a variety of interpretations ever since the development by Behnke[3] 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 procedures appear to be confirmed in laboratory animals by direct chemical analyses, but whether or not they can be applied without modification to humans, with expectation of equally reliable quantitative results, has been open to conjecture. It has not been possible to confirm them by direct chemical analysis, and there is compelling evidence that the human population at large tends toward greater variability in body composition than do laboratory animals. Thus, in some instances estimates of fat derived from fluid spaces and density have been treated with considerably more confidence than the underlying premises of the method would appear to justify. Then, too, estimates by one indirect method have been seemingly corroborated by another, whereas both (for example) total body water and density must necessarily give identical fat values--which need not, however, be the true value.

Keys and Brozek[9] have reviewed comprehensively the current status of our understanding of body composition and the problems inherent in its investigation. Nevertheless, the specific methods for estimating body composition that are derived from fluid spaces and density still bear closer scrutiny by analytical methods. Among the leading questions that still remain to be answered, the following in particular should yield to such an approach:

(a) Assuming that total body water, extracellular fluid space, and corporal density are the only quantities that can be measured, how are fat, protein and mineral estimated from any one or a combination of such measurements?

(b) What are the underlying assumptions in these methods and their range of validity?

(c) What uncertainty does biological variability as well as experimental error introduce into the final estimate?

(d) For practical purposes, what experimental accuracy is desirable in a given method?

These questions yield readily to analysis, and the results possess the advantage of generalized algebraic form that may then be evaluated by the investigator in accordance with the best available current data on the biological variables, such as the interrelationships between water, protein, and mineral.

In the following sections each of the potential methods for estimating body composition from the fluid spaces, corporal density, or their combination, is examined for its basic premises, its formulation, the inherent uncertainties, and general conclusions. The algebraic formulas are expressed in as general a form as the assumptions concerning a method permit, so that within the limitations of this framework they are valid for humans and animals alike. Differentiation into specific working formulas occurs only when numerical values are inserted. Although this has been done, it must be emphasized that such formulas are provisional until the numerical values rest on more definitive data. In any study of body composition one or another of the formulas is, of course, necessary, but its inherent uncertainties should be recognized.

2. General Principles

The sole constituents of the body are considered in the following analysis to be lipids, water, protein, and mineral. Water alone can for this purpose be regarded as two compartments; namely, the intra- and extracellular fluid spaces. For convenvenience in formulating the numerous algebraic expressions relating to gross body composition, the constituents are expressed as proportions of the total body weight, or of adipose tissue where this is indicated. Hence, fat is designated by f (kg fat/kg body weight, or of adipose tissue), with w, i, e, p, and m, similarly defined for total water, intra- and extracellular water, protein and mineral. A further division into carbohydrates and into "essential" and "nonessential" lipids is not warranted, partly because the former two constitute a relatively small portion of the body, but more particularly because none of the indirect methods for determining body composition is capable of differentiating such divisions.

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

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

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

Since the weight and volume of a constituent are related by weight = density x volume, a third basic expression may be derived that is more useful than Eq. (2) when densitometry is employed:

$$\frac{1}{d} = \frac{f}{d_f} + \frac{w}{d_w} + \frac{p}{d_p} + \frac{m}{d_m} , \tag{3}$$

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 g cm^{-3}.

Despite the elementary character of these expressions the definitions of f, w, p, and m must be explicit if an interpretation of the result 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 totals of these constituents, including these substances in the fluid spaces as well as in cellular matter.

In the numerical expressions that are given as examples of applications of the various methods, it is assumed that the densities of f, w, p, and m are relatively constant compared to other empirical factors that are inherent in every method, and the following values are used:

$$d_f = 0.900 \text{ g cm}^{-3} \text{ at } 37^\circ \text{ C} \quad \text{(Ref. 9)}$$

$$d_w = 0.993 \text{ g cm}^{-3} \text{ at } 37^\circ \text{ C}$$

$$d_p = 1.340 \text{ g cm}^{-3} \text{ at } 37^\circ \text{ C} \quad \text{(Ref. 8)}$$

$$d_m = 3.000 \text{ g cm}^{-3} \text{ at } 37^\circ \text{ C} \quad \text{(Ref. 5)}$$

The studies by Fidanza, Keys, and Anderson cited by Keys and Brozek[9] indicate a remarkable uniformity in the density of human fat irrespective of the site from which it is taken. If further investigation reveals that human fat density is essentially constant for all individuals, this result--though desirable--will be at variance with observations on the density and composition of animal fats, which appear to change somewhat with diet and environment. While otherwise the density of human fat seems well established, the reliability of the numerical values of d_p and d_m cannot be argued with great confidence. Proteins are known to differ in density and the value of 1.34 g cm^{-3} 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.

Nearly as fundamental as the three universal relations 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 illdefined, but nevertheless present in studies of body composition. When only one or even two properties, such as water and density, are measured, it is evidently 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,"[1, 4] and Brozek's "standard man."[6, 9] Each of these is intended to provide the basic and presumably 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 appended to the basic lean structure. Behnke's lean-body mass is essentially the same thing except for recognition that the lean body contains certain essential lipid substances such as phospholipids that are irreducible cellular constituents. This distinction, although valid, is one that present methods for determining body composition cannot make.

The view that the body may be regarded as a lean structure to which pure fat is added appears to obtain in small mammals, and is supported by some animal studies. The recent studies by Pitts[12] 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 Brozek[9] 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 has reported similar findings, though numerically somewhat different.[2,4] Moreover, Keys and Brozek found that the compsition of adipose tissue was not constant over the entire emaciation-obesity range, but was substantially different for extremely lean persons.[9] Under these conditions, the fat-free body could not serve as a suitable reference because its composition would depend on the fatness of the individual. Instead, Keys and Brozek adapted a "standard man" derived from the mean composition estimated for a selected group of young men.[9] It was believed thatadipose tissue in the range of, and greater than the 14% of body weight estimated for the standard man was nearly constant in composition, hence, the standard man would serve as a valid reference for most of the population, but would be inappropriate for very lean persons.

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. There is, indeed, reason to believe that a series of such references may be needed to bracket the emaciation-obesity range, and perhaps other variables, such as mineral, as well. This, however, does not solve the difficulty, because some new and independent means would be required to select the appropriate reference.

All these possibilities must nevertheless be introduced into any generalized formulation for calculating fat, protein, or mineral from fluid spaces and density. The analysis of each method, therefore, proceeds from a generalized reference body whose composition is $1 = f_0 + w_0 + p_0 + m_0$ and whose density is d_0. 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 constituents relative to unit weight of such tissue. The quantity A is therefore the adipose-tissue difference between subject and reference as employed by Keys and Brozek. From these definitions it is readily seen that the total proportions of fat, water, protein, and mineral in the normally hydrated person are

$$\begin{aligned} f &= (1 - A) f_0 + Af_1, \\ w &= (1 - A) w_0 + Aw_1, \\ p &= (1 - A) p_0 + Ap_1, \\ m &= (1 - A) m_0 + Am_1. \end{aligned} \tag{4}$$

With each of the methods formulated in these terms, one may choose a composition for adipose tissue and whatever reference seems appropriate.

In the following sections, the general formulation for each method will also be given in numerical form as derived from two very different references. The first is based on Brozek's "Standard Man,"[6] which is characterized by

$d_0 = 1.063$ g cm^{-3},

$f_0 = 0.14$ unit of body weight,

$w_0 = 0.61$ unit of body weight,

$p_0 = 0.19$ unit of body weight,

$m_0 = 0.06$ unit of body weight,

together with Keys and Brozek's estimate of the composition of adipose tissue:[9]

$d_1 = 0.948$ g cm^{-3}

$f_1 = 0.62$ adipose tissue weight,

$w_1 = 0.31$ adipose tissue weight,

$p_1 = 0.07$ adipose tissue weight,

$m_1 = 0.00$ adipose tissue weight.

The second example is evaluated on the basis of the fat-free body, assuming its proportions of 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:

$d_0 = 1.1$ g cm^{-3}

$f_0 = 0.0$ unit body weight,

$w_0 = 0.72$ unit body weight,

$p_0 = 0.21$ unit body weight,

$m_0 = 0.07$ unit body weight.

These two references--Brozek's "Standard Man" and the fat-free body--are used in the following sections for numerical evaluation of the methods, primarily because they illustrate opposite extremes in concepts of reference bodies. Despite this, it will be apparent in analyses of most methods that the choice of reference body may have less material effect on the estimated quantity of fat or 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 magnitude of the constituents.

3. Experimental Error and Biological Uncertainty

It would be a misleading simplification to assume that the accuracy with which body composition can be estimated hinges largely upon the accuracy with which corporal density or the fluid spaces can be measured. Even if experimental error were insignificant there would still remain in most methods for estimating body composition a residual uncertainty (Standard Deviation) that is about ± 4% of body weight. Each method includes, whether explicitly or implicitly, a fixed reference body, or the equivalent in a set of assumptions interrelating the 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 but equally likely empirical relationship. Since it can hardly be expected that all individuals will conform exactly to the same numerical constants in such relationships, particularly among humans, deviations from preset values, even though they may be accurate averages, result in an inaccuracy stemming from biological variability.

The empirical constants in fat-estimating formulas may at best represent an average for a given population, they may furthermore be correct in only a limited segment of the obesity-emaciation range. The variability in each biological factor 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 determinations of 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 an experimental technique that would in reality produce no significant improvement in the estimate of fat or other constituents, 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 the application of a specific method.

The algebraic formulas for calculating the variance (SD squared) in the fat estimates are expressed in terms of the biological variables and their variance, experimental and biological. Evidently, these experimental and biological uncertainties must be the same in every method for estimating body composition from density and fluid spaces, although their cumulative effect will in general be different.

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:	$\sigma_d = \pm 0.0025$	g cm^{-3},	(Refs. 9, 14)
	$\sigma_w = \pm 0.02$	body weight.	(Refs. 15, 16)
Biological:	$\sigma_\alpha = \pm 0.1$,		
	$\sigma_{w_0} = \pm 0.02$	reference body weight,	
	$\sigma_{d_0} = \pm 0.01$	g cm^{-3},	
	$\sigma_{d_1} = \pm 0.01$	g cm^{-3}.	

The experimental errors are based on the best techniques now applicable for routine practice.

The quantity α, the ratio of total mineral to protein, is discussed in Section 6. The standard deviations σ_{w_0} and σ_{d_0} include the uncertainty in the exact composition of the reference body and the much greater dispersion in body composition for the population. They are, in effect, measures of the deviation of individuals from a fixed reference and not so much the error in the reference. The standard deviation in d_0, the reference body density, is derived from σ_α and σ_{w_0} (see Appendix 2). The value of σ_{d_1} is estimated from the combined data of Keys and Brozek,[9] Behnke,[2,4] and Siri.[16]

4. Densitometric Method

A correlation between corporal density and fatness was suspected as early as 1901 by Stern,[18] but in the absence of an accurate technique for measuring body density, it was not possible to establish a well-defined relationship. After perfecting the underwater weighing method for determining density of the body by Archimedes' principle, and compensating for lung volume, Behnke[3] was able to demonstrate that a high correlation did exist between overweight and density. Using this method Rathbun and Pace[13] were in turn able to establish a quantitative relationship between density and the percentage of fat in guinea pigs by comparison with direct chemical analysis. The semiempirical 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 original constants for humans, 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 are known to contain a systematic error because they are based on an incorrect value of fat density. Keys and Brozek[9] and Behnke[2] later proposed somewhat different values based on more extensive though indirect human data and the correct fat density.

The estimation of fat from density alone is founded upon the broad generalizations described in Section 2. It requires the assumption that all adult humans are identical in composition except for individual differences in their proportions of adipose tissue. This is specified either by comparison with a fixed reference body whose composition is presumed to be known, or the equivalent, by assuming fixed relationships between water, protein, and mineral in both the lean body (not necessarily the fat-free body) and in adipose tissue. Thus the individual can be regarded as identically the reference body to which a proportion A of adipose tissue has been appended or removed.

In arriving at a general formulation of the densitometric method, however, it is not necessary to specify the numerical values beyond assuming that they are known and valid average values for the category of subjects to be examined. The formulas are in turn greatly simplified if expressed in terms of the densities of the reference body and of the generalized adipose tissue. The result is exactly equivalent to expressing the formulation in terms of the specific constituents of the reference and adipose tissue, as may be verified by making the substitutions.

An individual who differs from the reference by a proportion of adipose tissue A is characterized by a mean body density d, related to A by

$$\frac{1}{d} = \frac{A}{d_1} + \frac{1 - A}{d_0} , \qquad (5)$$

or, if terms are rearranged the estimating equation for adipose-tissue difference becomes

$$A = \frac{1}{d}\left(\frac{d_0\, d_1}{d_0 - d_1}\right) - \frac{d_1}{d_0 - d_1} . \tag{6}$$

The difference that is pure fat is then $\Delta f = Af_1$, whereas the total fat proportion of the individual is $f = Af_1 + (1 - A)f_0$, or more explicitly,

$$f = \frac{d_1\, d_0}{d}\left(\frac{f_1 - f_0}{d_0 - d_1}\right) - \frac{d_1 f_1 - d_0 f_0}{d_0 - d_1} \tag{7}$$

Equations (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 Brozek's standard man, and then on the basis of the fat-free reference body. For the first of these, $d_0 = 1.063 \text{ g cm}^{-3}$, $f_0 = 0.14$, and $f_1 = 0.62$.

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

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

These are essentially the equations proposed by Keys and Brozek,[9] although there is a negligible difference in the constants because fewer decimal places in d_0 and d_1 are used here.

If, on the other hand, the fat-free body is the correct reference, then $d_0 = 1.1 \text{ g cm}^{-3}$, $d_1 = 0.90 \text{ g cm}^{-3}$, $f_0 = 0.0$ and $f_1 - 1.0$. The fat-estimating equation then 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 g cm^{-3}, the original Rathbun-Pace formula yields 23.9%, Keys and Brozek's version, which is the same as Eq. (9) above, gives 18.9%, whereas Eq. (10) above gives 21.5%.

A true estimate of the uncertainty associated with the densitometirc method, as pointed out in Section 3, must include not only the experimental error but also the biological variability associated with the assumptions that are 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 in a population. The over-all uncertainty, expressed as the variance σ_f^2 in fat, then has the form

$$\sigma_f^2 = \left(\frac{d_1 d_0 (f_1 - f_0)}{d(d_0 - d_1)}\right)^2 \left[\frac{\sigma_d^2}{d^2} + \left(\frac{d - d_1}{d_0(d_0 - d_1)}\right)^2 \sigma_{d_0}^2 \right. \tag{11}$$

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

The variance in the determination of the difference in fat between subject and reference is also given by the equation above if f_0 is set equal to zero and the fourth term in the bracket is omitted.

Since estimates of σ_f and $\sigma_{\Delta f}$ require only approximate values of the quantities in the formulas to be generally valid, the values proposed by Keys and Brozek[9] given above may be used to evaluate the standard deviations in fat and fat differential for a subject of density 1.050 g cm^{-3}:

$$\sigma_f^2 = 14.56 \ \sigma_d^2 + 11.18 \ \sigma_{d_0}^2 + 0.23 \ \sigma_{d_1}^2 + 0.81 \ \sigma_{f_0}^2 + 0.01 \ \sigma_{f_1}^2, \tag{12}$$

$$\sigma_{\Delta f}^2 = 24.28 \ \sigma_d^2 + 18.66 \ \sigma_{d_0}^2 + 0.38 \ \sigma_{d_1}^2 + 0.01 \ \sigma_{f_1}^2 . \tag{13}$$

It remains only to substitute representative values for the separate variances. The standard deviation σ_d represents solely the experimental error in measuring the subject density, and for the present purpose is taken as ± 0.0025 g cm^{-3}. The remaining standard deviations reflect primarily biological variability; thus variations in the mineral-protein ratio and 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 Section 3, are

σ_{d_0} = ± 0.01 g cm^{-3}, σ_{d_1} = ± 0.01 g cm^{-3}, σ_{f_0} = ± 0.02 reference body weight and σ_{f_1} = ± 0.05 adipose tissue weight. The standard deviation in fat estimated by the densitometirc method becomes

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

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

Several useful 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 g cm^{-3}. 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, but 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 or of adipose tissue A, between subject and reference, is the same as or greater than that for total fat. While this result is not intuitively evident, it follows from the analysis above, which demonstrates that the same uncertainties affect both Δf and f. There is consequently no real advantage in estimating Δf or A rather than total fat in the expectation of achieving a more reliable quantity.

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 experimental error, which may be exceedingly small, 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.

5. 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,[11, 12] suggesting that--at least in a limited range of fatness--such animals consist of a basic lean structure to which pure fat is appended in varying amounts without greatly altering the proportions of water, protein, and mineral. If this conclusion is accepted, the proportion of fat is given on the average by the widely used formula

$$f = 1 - \frac{w}{w'}, \tag{14}$$

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 68% 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[7, 10, 19] as well as indirect[16] evidence to demonstrate 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.[2, 9] The greater variability in total mineral and 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 altered hydration or differences in the proportions of protein and mineral can be taken into account in estimating fat solely from total body water, but if water is associated with adipose tissue, this can be expressed in the formula relating fat to total body water if it is assumed that the water fraction of adipose tissue is constant. In principle, a somewhat more general equation than that above should be obtained.

For the same reasons that a reference body and a generalized form of adipose tissue are inherent in a general formulation of the densitometric method, they are equally necessary in deriving the body-water method for estimating fat or any other constituent. 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 in excess of, 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:

$$w = Aw_1 + (1 - A)\, w_0, \tag{15}$$

$$f = Af_1 + (1 - A)\, f_0. \tag{16}$$

Combining equations, we have the general relation between total fat and water,

$$f = \frac{w_0 - w}{w_0 - w_1} (f_1 - f_0) + f_0 \qquad (17)$$

The difference in adipose tissue between reference and subject is then $A = (w_0 - w)/(w_0 - w_1)$, while the difference in fat is $\Delta f = Af_1$. Equation (17) 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 Brozek's standard man as a reference (see Section 2) becomes

$$f = 1.016 - 1.600\ w. \qquad (18)$$

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

$$f = 1.000 - 1.390\ w. \qquad (19)$$

The validity of the total-body-water method for estimating fat rests upon the same assumptions as are inherent in the densitometric method; briefly, that gross body composition is constant for all humans with the same proportion of adipose tissue; that the composition of adipose tissue is constant and known; and that the reference body is a true average whose composition is known. 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. (17), in the form

$$\sigma_f^2 = \left(\frac{f_1 - f_0}{w_0 - w_1}\right)^2 \left[\sigma_w^2 + \left(\frac{w - w_1}{w_0 - w_1}\right)^2 \sigma_{w_0}^2 + \left(\frac{w - w_0}{w_0 - w_1}\right)^2 \sigma_{w_1}^2 + \left(\frac{w - w_0}{f_1 - f_0}\right)^2 \sigma_{f_1}^2 + \left(\frac{w - w_1}{f_1 - f_0}\right)^2 \sigma_{f_0}^2\right]. \qquad (20)$$

The corresponding variance in the differential fat estimate, Δf, may also be calculated by Eq. (20) after deleting the last term and f_0.

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 with Brozek's standard man (see Sect. 2) used as a reference. The numerical values of the standard deviations in w_0, w_1, f_0, and f_1 were discussed in Sections 3 and 5. The estimate of fat and the attendant standard deviation calculated with Eqs. (18) and (20) above are then

$$f = 23.6 \pm 4.8\% \text{ body weight},$$

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

Similarly, an estimate of fat in the same subject may be calculated from Eqs. (19) and (20) based on the fat-free body as a reference:

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

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.

The foregoing analysis of the total-body-water method leads to several conclusions of particular interest. It is seen at once that, in view of technical difficulties involved, increasing the accuracy of total-body-water measurement beyond ± 2% of body weight does not, in general, appear warranted. 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 ± 3.9%. Indeed, if there were no error whatever in total-body-water measurement the uncertainty σ_f in total fat would still be ± 3.6% of body weight because of irreducible variabilities in the other factors.

A particularly significant result is that the standard deviation of the differential fat estimate is, if anything, greater than for total fat estimate. 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 experimental error. Hence, neither the difference in adipose tissue nor in pure fat between subject and reference can be determined any more reliably than total fat.

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 reference body, and adipose-tissue composition, 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.

The absolute standard deviations in fat and fat difference calculated for the numerical example above will be approximately constant throughout the emaciation-obesity range. For a standard deviation in total-body-water measurement of ± 2% body weight, the uncertainty in fat estimation is not likely to be less than ± 4% body weight.

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 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, are incorrect.

6. Density—Total-Body-Water Method

Combined measurements of corporal density and of total body water yield the only 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 by the two measurements, but rather on a single formulation in which density and water occupy the roles of independent variables.[9,15] 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 wholly free of such uncertainties. Density and body water still do not provide all the information needed for the unambiguous determination of a system that contains four constituents. 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 Eqs.(1) and (2), which--it may be recalled--apply to a body of any description:

$$1 = f + w + p + m, \qquad (21)$$

$$\frac{1}{d} = \frac{f}{d_f} + \frac{w}{d_w} + \frac{p}{d_p} + \frac{m}{d_m} . \qquad (22)$$

One additional relationship is needed to complete the system, but it may be any assumption one chooses to introduce that relates two or more of the constituents by means of a constant.

Among the numerous possible relationships between constituents such as those formulated in preceding sections, two are best suited to the present method. The first assumes that the ratio of mineral to protein is constant (i.e., $m = \alpha p$), or the equivalent, that mineral forms a constant percentage of the mineral-protein fraction of the body. The second choice assumes that mineral forms a constant proportion of the fat-free body, i.e., $m = \beta\,(w+p+m)$. The latter choice has the disadvantage of involving total body water, and hence in states of abnormal hydration cannot be expected to be strictly valid. Furthermore, although it is equivalent to the first expression for the average normal person, if total body water is a variable fraction of the body even in normally hydrated persons, as recent studies suggest,[16] the second of these expressions introduces a needless uncertainty in the value of β. The relation $m = \alpha p$ is therefore subject to less variation and is used here in deriving the formula for fat. It may be noted, however, that for the average normally hydrated subject, both assumptions lead to the same result.

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

$$d_s = \frac{(1 + \alpha)\, d_m\, d_p}{d_m + \alpha d_p} . \qquad (23)$$

Combining these equations with Eqs. (21) and (22) above, we obtain the general formula for fat,

$$f = \frac{d_f}{d_s + d_f} \left[\frac{d_s}{d} - w\left(\frac{d_s - d_w}{d_w} \right) - 1 \right] . \qquad (24)$$

The value of α, upon which an estimate of d_s depends, rests on rather meager data for humans. Although it is relatively consistent in laboratory animals, with a value of about 0.25,[11, 17] the ratio appears to be substantially greater and more variable in humans. The direct analyses of five cadavers by Mitchel et al.,[10] Forbes et al.,[7] and Widdowson et al.,[19] whose results are summarized by Keys and Brozek,[9] yielded values ranging from 0.290 to 0.527. For the present purpose in illustrating a numerical form of the fat-estimating equation, a value of $\alpha = 0.35$ is adapted, 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 error in α does not in this method greatly affect the estimate of fat and of $p + m$.

The combined density of protein and mineral for $\alpha = 0.35$ is then $d_s = 1.565$ g cm^{-3}. When this and the numerical values for d_f and d_w are substituted into Eq. (24), the fat-estimating equation becomes

$$f = \frac{2.118}{d} - 0.780\, w - 1.354. \qquad (25)$$

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 experimental errors in measuring density and water. The magnitude of the uncertainty this produces can be estimated by applying the law of propagation to Eqs. (23) and (24) to determine the over-all standard deviation σ_f. The variance in d_s takes the form

$$\sigma_{d_s}^2 = \left[\frac{d_m\, d_p\, (d_m - d_p)}{(d_m + \alpha\, d_p)^2} \right]^2 \sigma_\alpha^2 = 0.308\ \sigma_\alpha^2, \qquad (26)$$

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

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

$$+ \frac{0.308\, d_f^2}{(d_s - d_f)^4} \left(1 - \frac{d_f}{d} - \frac{d_w - d_w}{d_w}\, w \right)^2 \sigma_\alpha^2 . \qquad (27)$$

If the numerical values for d_f, d_w, and d_s are inserted, the variance in fat reduces to

$$\sigma_f^2 = \frac{4.22}{d^2} \quad \sigma_d^2 + 0.608 \quad \sigma_w^2 + \left(1.126 - \frac{1.015}{d} - 0.106\, w\right)^2 \sigma_a^2. \quad (28)$$

The contribution of biological variability introduced through a 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 those referred to above from which to infer an estimate of σ_a, it is reasonable to assume that the standard deviation is not greater than ± 0.1, i.e., about 30% of the assumed mean value. For the purpose at hand only a rough estimate is needed to demonstrate the magnitudes involved, and the value would appear to be an adequate estimate of the dispersion in mineral-protein ratio.

The actual 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 and w = 0.55. Substituting σ_a = ± 0.1 and the experimental errors of σ_d = ± 0.0025 g cm^{-3} and σ_w = ± 0.02 into Eq. (28), we have the standard deviation

$$\sigma_f^2 = 0.251 \times 10^{-4} + 2.43 \times 10^{-4} + 1.40 \times 10^{-4},$$

or

$$\sigma_f = \pm\ 2.0\% \text{ body weight.}$$

From the preceding analysis several general conclusions may be drawn regarding the applicability and validity of the method.

First, the d-w method is strictly valid in any state of hydration. Moreover, since the isotopes of hydrogen can be used as solutes in measureing 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--extracellular water is in principle 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 Eqs. (27) and (28) that little is to be gained in measuring body density more accurately than ± 0.0025 g cm^{-3}. In fact an error as great as 0.004 g cm^{-3} does not greatly affect the over-all accuracy of the fat estimate. This conclusion would apply 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%. In many circumstances, however, the over-all gain of only 0.5% in reliability would not justify the considerable effort required to achieve an accuracy of 1% or less in the measurement of body water by the techniques now available.

Fifth, if the experimental errors were altogether negligible, the uncertainty in fat estimate would still remain about ± 1.2% body weight, unless σ_a were substantially less than ± 0.1. On the other hand, even if σ_a 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 uncertainty, given by $\sigma_s = (\sigma_w^2 + \sigma_f^2)^{1/2}$, is slightly greater.

7. Density – Extracellular-Fluid Method

Extracellular fluid space is the only distinct compartment other than total body water that has a direct bearing on gross body composition and is susceptible to in vivo dilution techniques. Intuitively it would seem advantageous to employ this added information when available by combining it with corporal density in a method similar to that of total body water and density for estimating fat. However, as the following analysis suggests, the greater exactness that might be expected is offset by the increased complexities of the assumptions that must be made and by the substantial uncertainties that extracellular fluid space introduces, both on theoretical and on practical grounds.[15]

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 the densitometric and the total-body-water methods, to introduce a reference body to which all individuals are assumed to conform except for a difference in the proportion of adipose tissue. A considerable array of possible relationships among the five constituents is available for a formulation of this method in addition to the basic equation above and the corresponding general equation for density:

$$\frac{1}{d} = \frac{f}{d_f} + \frac{i + e}{d_w} + \frac{p}{d_p} + \frac{m}{d_w} . \qquad (29)$$

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:

$$m = \alpha p \quad \text{or} \quad m = \beta(1 - f - h),$$
$$i = \mu(1 - f - h), \qquad (30)$$
$$g = \nu i,$$

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 relates 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.

As in the preceding sections, it is simpler but equivalent to formulate the method in terms of the generalized reference body and adipose tissue. 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

$$\frac{1}{d} = \frac{1 - A - h}{d_0} + \frac{A}{d_1} + \frac{h}{d_w}, \tag{31}$$

where the subscripts 0 and 1 signify reference body and adipose tissue respectively. When combined with the expression for total extracellular fluid, $e = (1 - A - h)e_0 + Ae_1 + h$, the difference in adipose tissue between reference and subject becomes

$$A = \frac{d_0 d_w K (1 - e_0)}{d} - e K(d_0 - d_w) - K(d_w - e_0 d_0), \tag{32}$$

where

$$K = d_1 / \left[d_w(d_0 - d_1) - e_0 d_0 (d_w - d_1) - e_1 d_1 (d_0 - d_w) \right].$$

The absolute proportion of fat is $f = (1 - A - h)f_0 + Af_1$, or--combined with Eq. (32) above--the estimating equation for total fat has the form

$$f = \frac{f_1 (1 - e_0) - f_0 (1 - e_1)}{1 - e_0} + f_0 \frac{1 - e}{1 - e_0}. \tag{33}$$

Examples of numerical forms of these equations may now be evaluated on the bases of the two reference bodies described in Section 2. With the values proposed by Keys and Brozek, the equations become

$$A = \frac{8.666}{d} - 0.684\, e - 8.044, \tag{34}$$

$$f = \frac{5.148}{d} - 0.573\, e - 4.612. \tag{35}$$

For a subject with $d = 1.050 \text{ g cm}^{-3}$ and $e = 0.14$, as an example, the proportions of adipose tissue and fat derived from these equations are $A = 11.3\%$ and $f = 21.0\%$ body weight.

If, however, the fat-free body is the more nearly correct reference, then $f_1 = 1$, $f_0 = e_1 = 0$, e_0 is about 0.18, and the general fat formula reduces to

$$f = A = \frac{4.475}{d} - 0.535\, e - 3.972. \tag{36}$$

When this is applied to the subject above, a value of f = 21.5% of 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 appears to be less than half the uncertainty associated with either formula. Thus, 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. Methods such as inulin infusion and radiosulfate appear to give reasonable values, but there has been no means for directly testing their validity nor for estimating systematic error. 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, which then introduces a systematic error into the fat estimate, whereas it may be a normal variation in the ratio of extra- to intracellular water.

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, such as in extensive edema or ascites, because of their slow diffusion rates into distinct fluid volumes compared to their rates of excretion.[15]

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.

8. Extracellular–Total-Body-Water Method

An analysis of methods for estimating body composition would not be complete without examination of the use of combined measurements of the extracellular fluid space and total body water. Let us overlook for the present the sometimes uncertain interpretation of extracellular fluid space in terms of actual water volume; a method utilizing determinations of both fluid spaces should then in principle be valid in abnormal as well as normal states of hydration.

The general assumptions described in the preceding 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, we have then the actual proportions of total water and extracellular water:

$$w = (1 - A - h)\, w_0 + A w_1 + h, \tag{37}$$

$$e = (1 - A - h)\, e_0 + A e_1 + h, \tag{38}$$

where the subscripts 0 and 1 designate quantities associated respectively with the reference body and adipose tissue. If these two equations are combined to eliminate h, then the difference in adipose tissue, A, between reference and subject may be expressed as

$$A = \frac{e\,(1 - w_0) - w\,(1 - e_0) + w_0 - e_0}{(w_1 - w_0)(1 - e_0) - (e_1 - e_0)(1 - w_0)}. \tag{39}$$

The difference in adipose tissue therefore is calculated in terms of the measured values and the presumed constant normal values of the fluid spaces in the reference and in generalized adipose tissue.

The total fat is evidently $f = (1 - A - h)\, f_0 + A f_1$, which, combined with Eq. (39), becomes

$$f = eK\left[f_1(1 - w_0) - f_0(1 - w_1)\right] - wK\left[f_1(1 - e_0) - f_0(1 - e_1)\right] + K\left[f_1(w_0 - e_0) - f_0(w_1 - e_1)\right], \tag{40}$$

where

$$K = 1/\left[e_1(1 - w_0) - e_0(1 - w_1) + w_0 - w_1\right].$$

These general formulas may now be evaluated on the bases of the two references. Inserting first the standard man of Brozek (see Section 2) and the values $e_0 = 0.16$, and $e_1 = 0.14$ proposed by Keys and Brozek,[9] we have the fat-estimating equations

$$A = 1.535\ e - 3.306\ w + 1.772, \tag{41}$$

$$f = 0.596\ e - 1.620\ w + 1.041. \tag{42}$$

If, on the other hand, the fat-free body is the correct reference, then $f_1 = 1$, $e_1 = w_1 = f_0 = 0$, and the fat-estimating equation becomes

$$f = A = 0.519\ e - 1.518\ w + 1. \tag{43}$$

It is of interest to note that on taking the difference between Eqs. (42) and (43) we find that the proportion of fat estimated on the basis of the two reference bodies always differs by less than 1.5% of body weight. For example, a person in whom total water constitutes 55% of body weight and extracellular water is 14%, consists of 22.3% fat by Eq. (42) and 21.8% by Eq. (43). This difference is far smaller than the inherent errors in either formula; consequently, the choice of reference, the assumed composition of adipose tissue, and other assumptions that may be introduced are relatively unimportant. Conversely, the method cannot be expected to give a very reliable estimate of body composition.

Essentially the same conclusions are reached concerning this method as those described for combined density-extracellular fluid space in the preceding section. 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. For technical reasons the method is of questionable value in the presence of excessive hydration, while, on the other hand, 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.

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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 variance (standard deviation squared),

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

where $(\partial F/Fa)$ 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 g cm^{-3} in the value of the reference-body density is more a measure of the dispersion of body composition in the population than the experimental error in the specific reference that may be selected. The magnitude of the uncertainty in d_0 is based here on the dispersion in normal total body water of σ_w = ± 2% body weight together with a dispersion of ± 0.1 in the mineral-protein ratio for persons with identical adipose tissue. The resultant uncertainty in d_0 is then derived as follows, assuming $m = \alpha p$:

The reference body density may be expressed as

$$\frac{1}{d_0} = \frac{f_0}{d_f} + \frac{w_0}{d_w} + \frac{(1 - f_0 - w_0)(d_m + \alpha d_m)}{(1 + \alpha) d_m d_p} .$$

In applying the law of propagation of errors it is assumed that fat is constant, i.e., the standard deviation in d_0 is to reflect the dispersion in body composition for persons with identical proportions of fat or adipose tissue. The variance is then

$$\sigma_{d_0}^2 = d_0^4 \left(\frac{1}{d_w} - \frac{d_m + \alpha d_p}{(1 + \alpha) d_m d_p}\right)^2 \sigma_{w_0}^2 + d_0^4 \left(\frac{(1 - f_0 - w_o)(d_p - d_m)}{(1 + \alpha)^2 d_m d_p}\right)^2 \sigma_\alpha^2$$

$$= 0.164 \; \sigma_{w_0}^2 + 0.0042 \; \sigma_\alpha^2 .$$

With σ_{w0} = ± 0.02 and σ_α = ± 0.01, the standard deviation in d_0 becomes σ_{d_0} = ± 0.01.

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