BODY COMPOSITION MEASUREMENTS FOR NUTRITION RESEARCH

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INTRODUCTION: THE BASIS OF MEASUREMENTS

'A proper evaluation of the individual living body and its functional characteristics is not possible from the simple classification system of the butcher... '. With these remarks Keys & Brozek (1953) dismissed the contributions of anatomists to body composition measurements and introduced the concepts that still sustain modern investigators in this field. These are that meaningful nutritional and physiological investigations in energy metabolism are those focussed on a distinction between the relatively inert components of the body and those that are metabolically more active. The former consist of extracellular fluid, bone mineral and depot fat and the rest, muscle and muscle-free lean tissue. Similarly, Siri (1956) suggested that a distinction between fat, minerals, protein and water provided a physiologically useful compartmentalization. These views were in fact simply more elaborate statements of an earlier idea (Behnke *et al.* 1942) that the body could be thought of as consisting of two components, fat and lean tissue. Either way these propositions indicate first, what it may be useful to measure and second, that, with the addition of weight measurements, the size of a single unmeasured compartment can be calculated if estimates are available for the other components of the system.

Unfortunately, the simplicity of these arguments conceals a considerable difficulty. That is that since the size of an individual compartment is rarely measured directly, rather some constituent or property of it, the precision and accuracy of the basic measurement cannot easily be translated into equivalent values for the variable of interest. Account has to be taken of the consistency of the relationship between the measured constituent or property and the compartment to which it belongs and, when the size of an undetermined compartment is measured by difference between body-weight and the weight of other compartments, relative compartmental size determines the magnitude of the proportional errors. Examples of these problems will emerge in subsequent paragraphs.

https://doi.org/10.1079/NRR19880010 Published online by Cambridge University Press

ESTABLISHED TECHNIQUES

MEASUREMENT OF BODY DENSITY

The earliest application of compartment methodologies was the use of the measurement of average body density to assess the mass of two compartments (fat and non-fat) whose density is known. In most cases the subject is weighed in air and then under water, with a simultaneous measurement of residual lung volume; this may be as much as 2 litres. A constant correction is also made for gastrointestinal gas volume, never directly measured but usually assumed to be about 100 ml in an adult (Buskirk, 1961). The reproducibility of the measurement expressed as the standard deviation of repeated measurements of subjects thought to be of unchanged body composition ranges from 0·0008 kg/l (Mendez & Lukaski, 1981) to 0·0043 kg/l (Goldman & Buskirk, 1961). Siri's (1956) equation assumes that fat and fat-free tissues have densities of 0·900 and 1·100 kg/l respectively and may be written as

$$\text{kg fat} = \frac{4{\cdot}950}{d} - 4{\cdot}50\ \text{Wt},$$

where d is body density (kg/l) and Wt is body-weight (kg). Thus Burkinshaw (1985) showed that Durnin & Taylor's (1960) estimate of precision ($\pm 0{\cdot}0023$ kg/l) is equivalent to 0·72 kg fat for a Reference Man (Snyder *et al.* 1975) in whom fat is 19·3% of 70 kg body-weight, if it is assumed that weight is measured with no error. It will become apparent that in comparison to other methods available this level of precision is impressively small and consequently there is a considerable literature of measurements on healthy adult subjects. However, the need for total immersion makes the procedure unsuitable for very young, elderly and infirm, and sick subjects. For some of these cases complete immersion can be avoided by using newer plethysmographic techniques for which precision similar to that found for density measurements is reported (Garrow *et al.* 1979; Gundlach & Visscher, 1986). An acoustic pethysmograph intended for use with infants is also under development, but as yet is of insufficient sensitivity (Sheng *et al.* 1987).

MEASUREMENT OF TOTAL BODY WATER

Another much-employed procedure is to use the measurement of total body water. This assumes that lean tissue is hydrated to the extent of 73·2% (Pace & Rathbun, 1945) and that fat has no water associated with it. The value of 73·2% is a figure probably justified only by its regular use in the literature. In fact it is a value derived from animal experiments and very little information exists for man to justify its confident use in human studies (Sheng & Huggins, 1979). Over the years a variety of tracers for water have been used to estimate total body water by dilution but the bulk of the literature refers to findings using deuterium, tritium, or ^{18}O, and a variety of analytical techniques, i.e. scintillation counting for tritium, infra-red absorption and mass spectrometry for deuterium and ^{18}O. Mass spectrometry is rapidly becoming the procedure of choice, because measurements of the order of $<1\%$ precision on dilution values can be achieved and the radiation risks associated with tritium measurements do not exist for stable isotopes. There are, however, some unresolved problems. To begin with, deuterium and ^{18}O when used simultaneously do not yield the same results. The origin of the difference is thought to be rapid exchange of hydrogen isotopes with non-aqueous H (Culebras *et al.* 1977) to produce estimates of total body water that are larger than those obtained with ^{18}O. Animal experiments suggest that the latter values are close to the correct ones (Nagy, 1980; Whyte *et al.* 1985). A difference of 3–4% might be regarded as typical, but there is insufficient comparative information to allow us to suppose that this difference is constant either within or between individuals.

A second methodological difficulty arises as a consequence of the fact that dilution procedures such as these are not static measurements. The isotope given has to equilibrate within the body-water pool but is continuously excreted from it, thus the apparent isotopic-dilution space continuously changes. One way to overcome this problem is to calculate body water from the intercept of an isotope-disappearance curve measured over several days. This method has been usefully adopted when energy-expenditure measurements are made in the doubly labelled water method, but is inconvenient if body composition is the only variable of interest: samples have to be collected over several days and a substantial amount of analytical work is generated. An alternative procedure derived from the work of Halliday & Miller (1977) and adopted by Schoeller *et al.* (1985) is to allow several hours for isotope equilibration and to subtract from the dose given the amount of isotope excreted in the equilibration time. The observed dilution of the dose remaining will then give total body water. In practice, only urine is easily collected for the measurement of isotope loss during the equilibration period and if other insensible losses are ignored total body water will be overestimated.

The third and most widely adopted approach is to rigorously define dose and equilibration conditions and to sample body fluids at a fixed time after the dose has been given, making allowances for any water intake during this period. This procedure relies on the existence of a 'plateau' for isotope enrichment in body fluid at some time after dose administration. The existence of such a plateau is, however, a physiological impossibility (in reality, isotope enrichment will change slowly as equilibration of new isotope in body pools matches rates of egress, to produce an apparent plateau) and the problem is to identify an appropriate time at which to take samples. On average, blood, saliva and breath water reach such an equilibrium quickly (2–3 h) but urine may take longer (5–7 h) (see, for example, Schoeller *et al.* 1980; Trowbridge *et al.* 1984; Wong *et al.* 1988). However, the standard deviations of values for isotope dilutions, expressed relative to the 6 h value in the results of Wong *et al.* (1988) indicate that plateaus are reached at quite different times in individual studies and this adds to the uncertainty of total body water estimations made from single-point dilution determinations. At best, precision on such measurements will only be of the order of 2% for total body water or 1·12 kg fat in Reference Man.

MEASUREMENT OF TOTAL BODY POTASSIUM

Like water, K is present in fat-free tissue but does not occur in fat, thus, its measurement in the body can lead directly to estimates of lean body mass and indirectly to fat mass. Radioactive ^{40}K occurs naturally as a constant proportion of all K and its presence in vivo can be detected using whole-body counters. The attenuation of the count by a subject's body size and shape needs to be taken into account, and precision additionally depends on specific aspects of instrument design; values of 3–4% for standard errors are usually quoted for adults (Burkinshaw, 1985). It can be shown that this is equivalent to 2·16% fat for Reference Man, but there are other factors leading to further uncertainty. Reliable information on the K content of fat-free tissues is sparse and variable even for healthy individuals, K is not distributed evenly throughout lean tissues (Widdowson & Dickerson, 1960); more is found in muscle than other tissues (Snyder *et al.* 1975) and it varies with age, both during growth and development (Dickerson & Widdowson, 1960), and after maturity (Womersley *et al.* 1976); the concentration may be higher in people with greater fat-free mass (Morgan & Burkinshaw, 1983). There are no obvious advantages over and above density or water measurements.

ANTHROPOMETRY

Of the techniques discussed so far only the measurement of total body water by isotope dilution is feasible for all conceivable subjects in all possible locations. There is always,

therefore, a demand for more widely applicable techniques, for example Brozek & Keys (1951) pioneered the use of regression equations to predict body density from selected anthropometric measurements. Two basic assumptions additional to those in two-compartmental methodology are involved. These are that subcutaneous fat is a constant proportion of total body fat and that the chosen sites of measurement are representative of the mean skinfold thickness across the surface of the whole body; no attempt has been made to directly confirm these statements.

Durnin & Womersley (1974) used a logarithmic transformation of the sum of the skinfold thickness at four sites: biceps, triceps, subscapular, and suprailiac, whereas Jackson & Pollock (1978) recommend the sum of seven skinfolds: chest, axilla, triceps, subscapular, abdomen, suprailiac and thigh, with either a logarithmic or quadratic transformation, together with age, waist and forearm circumference. The equations of Durnin & Womersley (1974) are most frequently used but it is likely that their popularity can be attributed to simplicity rather than to any objective scientific criteria.

The regression equations were developed in a study of 209 male and 272 female subjects. The pooled standard error on the estimation of body density was 0·0084 kg/l (range 0·0073–0·0092) for men and 0·0102 kg/l (range 0·0082–0·0125) for women. Not surprisingly, different equations are required for the two sexes, and different age-groups, where the equations have similar slopes though differing intercepts. Possible causes for the changes with increasing age are that an increasing proportion of body fat is deposited internally rather than subcutaneously, skinfold compressibility is greater in older people or a loss of bone mineral results in a decrease in the density of the fat-free component of the body.

Regardless of the merits of particular regression equations there remains the basic problem that a methodology based on a prediction equation can never give any greater accuracy in fat estimation than that associated directly with the measurement against which it is regressed. Burkinshaw (1985) has calculated the standard error on the estimation of fat from skinfold thicknesses to be 2·2 kg fat in a 70 kg man. If we compare this with the standard error of fat from density of 0·72 kg it is apparent that in striving for a simpler technique the precision of the method has been compromised. Confidence in the method cannot be judged on the fact that the reproducibility of the measurement in skilled hands may be excellent, with a quoted precision of about 0·2 kg (Hill *et al.* 1978).

NEWER TECHNIQUES

ELECTRICAL CONDUCTIVITY AND IMPEDANCE

Just as skinfold thicknesses may be used to predict body density, so in recent years techniques have been developed to predict body water to simplify the means of determining body composition by this route.

Both total body electrical conductivity (TOBEC) and impedance measurements have been used for this purpose since these variables are primarily related to the body's water or electrolyte content. The former procedure is available in only a few laboratories and the cost of the necessary equipment is high. Because of this there is as yet no substantial body of literature available to allow judgement on the likely value of the technique. However, as a method for predicting total body water it relies on the development of appropriate regression equations (see, for example, Fiorotto *et al.* 1987; Van Itallie *et al.* 1987), just as the measurement of impedance does, without offering the advantages of portability. In contrast, the measurement of whole-body impedance is now being adopted with enthusiasm by both scientists and equipment manufacturers because instruments can be made that are both relatively cheap and portable. An additional commercial attraction is that these

devices can be supplied with an associated microcomputer and software to produce a product intended to allow measurements to be made by operators with little skill or training.

Impedance measurements were first suggested by Thomasset (1962) but it was Hoffer *et al.* (1969) who realized its potential as the basis of a simple field technique to measure total body water.

Lukaski *et al.* (1986) compared the powers of bioelectrical impedance, using a previously developed linear regression equation (Lukaski *et al.* 1985), and skinfold thicknesses using the equations of Durnin & Womersley (1974), to predict density. In respect to accuracy, the standard error of the estimates in bioelectrical impedance analysis was shown to be 2·7% body fatness (1·82 kg fat in Reference Man) and for skinfold thicknesses the value was 3·9% (2·8 kg fat). For precision this group of workers report a mean coefficient of variation of 2% (range 0·9–3·4%) (Lukaski *et al.* 1985) on impedance measurements over 5 d with fourteen subjects. In contrast, in a similar study of seventy-five subjects, Segal *et al.* (1985) showed that impedance did not predict body fatness determined by density measurements better than by an anthropometric procedure, and TOBEC was better than either procedure (standard error of the estimate 6·10% body fatness compared with 5·81% for anthropometry and 3·73% for TOBEC). There was also a tendency to overestimate lean body mass with increasing adiposity. On theoretical grounds one may suppose that the poor sensitivity is due to imperfections in the model of the human body as a simple geometric conductor, with a uniform and symmetrical distribution of water and electrolytes, or the use of height as an approximation for conductor length. Certainly bioelectrical impedance analysis in its present form cannot be used to give an accurate measure of body fat in an individual subject, though it may be a viable technique for comparing group means in population surveys.

ALTERNATIVE CONCEPTS IN BODY COMPOSITION MEASUREMENTS

Thus far, the errors in fat measurement that have been quoted relate only to the consequences of technical imprecision, and the implicit assumption has been that the measured and unmeasured components of the body bear a constant relationship to one another, exemplified by Reference Man. This is hardly likely to be the case and Siri (1961) calculated that a realistic estimate of errors consequent on density measurements was of the order of 4% body-weight or 2·8 kg fat if the error on a density measurement was 0·0025 kg/l. Similarly, variability in the degree of hydration of lean body mass might be expected to produce a total error of 2·45 kg fat if total body water, measured with a precision of 2% body-weight, is the basis of measurement. When either density or total body water are measured indirectly the situation will obviously be worse. Thus, if skinfold measurements predict density to $\pm 0{\cdot}01$ kg/l the error of a fat estimate would be about 3·20 kg fat, but an allowance for the inadequacies of the model would increase this to 4·19 kg fat. There is the further difficulty that the clinician, physiologist or nutritionist is generally more interested in changes rather than absolute values. This means that changes in the amounts of fat and lean tissue will only be measured correctly if the proportions of the constituents of lean tissue remain the same. Otherwise, a bias will be introduced into the measurements. Burkinshaw (1985) supposed that a subject of reference body composition underwent surgery and lost body mass of the composition reported by Kinney *et al.* (1968) for such patients, i.e. 13% fat, 77% water and 10% protein. Burkinshaw (1985) then calculated the true densities in the initial and final states and applied these values in Siri's (1956) equation. Because the initial state represented a Reference Man, for whom the

https://doi.org/10.1079/NRR19880010 Published online by Cambridge University Press

equation of Siri (1956) was designed to apply, initial lean body mass and fat values are virtually correct. However, in the final state, because of a deviation of the density of lean mass from its expected value, the values were not correct and calculated fat losses were 1·82 kg rather than 0·6 kg (Table 1). If fat mass were to be calculated from total body water measurements it would be observed that the absolute values are never correct (because in this example lean tissue is not 73·2% water) and the predicted change is a gain of 0·17 kg fat.

It is clear that the imprecision of the methods is limited more by the inadequacies of the model rather than analytical errors incurred in making the basic measurements. It follows, therefore, that improvements in techniques will emerge only from a fuller consideration of the models and what ought to be measured rather than by pursuing new methods for making the traditional measurements of density or water.

Burkinshaw (1985) has emphasized the importance of determining more components of body mass to improve estimates of components not usually directly measured and the in vivo neutron activation analysis (IVNAA) he and his co-workers have developed probably represent the best currently available technique. When the body is irradiated with fast neutrons, gamma rays are emitted as products of interactions between neutrons and the nuclei of elements in the body. The energy of the emitted radiation specifies the activated nuclei. At present the elements that can be measured in this way are O, carbon, H, nitrogen, calcium, phosphorus, sodium and chlorine. Thus for the measurement of fat, C, N and Ca are determined. Body protein is calculated on the assumption that all N is in protein and that it is 16% of protein mass. Body C is assumed to be present only in fat, protein and bone and is 77% of fat, 52% of protein and has a constant relationship to bone Ca (740 g/kg Ca). From these relationships simple equations are derived for predicting fat with an estimated analytical precision of 0·66 kg for Reference Man. The value for non-fat tissue is 0·68 kg. This is a comparable level of precision to that found for density measurements, but because the basic constituents of the body are directly measured there is substantially more assurance of accuracy in subjects with abnormal tissue composition. Unfortunately, the equipment required for this methodology is expensive and the use of ionizing radiation prevents its universal applicability to human subjects.

Another possibility is to attempt the direct measurement of the component of interest. It is, for example, theoretically feasible to measure body fat from the uptake of gases which are soluble in fat such as cyclopropane (Halliday, 1971; Lesser *et al.* 1971) and ^{85}Kr (Hytten *et al.* 1966), but procedures like this have not been adopted because the rates of uptake of the gases are slow and probably somewhat variable. Magnetic-resonance imaging has also the potential to quantify total fat mass (Fuller *et al.* 1987), and although this technique is extremely expensive, instruments are becoming increasingly available in clinical and hospital environments; progress in this area can be expected in the next few years.

A final little-exploited idea was suggested some years ago by Siri (1961). Realizing that the basic problems with body-composition measurements are more a consequence of incompletely defined models rather than the techniques for measurement, he suggested that there might be considerable profit in combining independent measurements of density and total body water. If it is assumed that bone mass remains unchanged and using the values for the density of individual components of body mass given in Table 1 it can be shown that

$$\Delta F = 2{\cdot}741\ \Delta V - 0{\cdot}7148\ \Delta W - 2{\cdot}046\ \Delta \mathrm{Wt},$$

and

$$\Delta P = 3{\cdot}046\ \mathrm{Wt} - 0{\cdot}2852\ \Delta W - 2{\cdot}741\ \Delta V,$$

where ΔF, ΔP, ΔW, ΔWt are changes in total body fat, protein, water and body-weight (kg) respectively, and ΔV is the change in total body volume (l) calculated from weight and

https://doi.org/10.1079/NRR19880010 Published online by Cambridge University Press

Table 1. *True and calculated body composition of a hypothetical subject (Adapted from Burkinshaw, 1985)*

	Initial		Final		Calculated from density		Calculated from body water	
Component	True mass (kg)	Density (kg/l)	True mass (kg)	Density (kg/l)	Initial mass (kg)	Final mass (kg)	Initial mass (kg)	Final mass (kg)
Water	40·9	0·993	37·7	0·993	—	—	—	—
Protein	12·0	1·340	11·6	1·340	—	—	—	—
Minerals	3·6	3·000	3·6	3·000	—	—	—	—
Fat-free	56·5	1·1004	52·9	1·1062	56·60	54·22	55·87	51·50
Fat	13·5	0·900	12·9	0·900	13·40	11·58	14·13	14·30
Total	70·0	1·0551	65·8	1·0586	—	—	—	—

density measurements. Applying these values to the values given in Table 1 produces predictions of the fat and protein changes of 0·60 and 0·40 kg, which are correct. Surprisingly, this technique appears never to have been used in any experimental situation where body-composition change is of interest.

It may seem strange to conclude a review on body-composition measurement published in the late 1980s with reference to the possible usefulness of a technique nearly 30 years old. However, in any critical examination of the literature it becomes evident that most of the techniques that have sprung into existence since then are unlikely to have much impact on nutritional science. Lukaski (1987), for example, listed fifteen techniques (many of them new) for the measurement of fat-free mass and fat, and assessed them in terms of technical convenience and achievable precision. While the majority of procedures were judged to be easier to perform than densitometry, only IVNAA scored as well as density measurements in terms of precision, and the majority of procedures were assessed as not being as good as the measurement of total body water with ^{18}O. No doubt balances can be sought between achievable precision and convenience in use but, as we have seen, even the best methods are not particularly precise or accurate and there is thus little or no room to compromise precision and accuracy for the sake of convenience. For these reasons it is evident that IVNAA is by far the most-significant development, the more so because it combines new technology with the development of the compartmental concepts used to derive functionally significant measurements.

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Printed in Great Britain