Review Article

Methods for the assessment of human body composition: traditional and new[1-3]

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ABSTRACT Renewed interest in the assessment of human body composition has stimulated the need for a balanced understanding of available methodologies of estimating fat-free mass and percent body fat. This review summarizes the physical bases and assumptions, describes applications, and discusses the theoretical and practical limitations of currently available indirect methods. Although standard methods are discussed, recent modifications and adaptations are emphasized. *Am J Clin Nutr* 1987;46:537–56

KEY WORDS Fat, fat-free mass, body water, protein, mineral

Introduction

Research to establish indirect methods of determining human body composition began during the 1940s in the laboratory of AR Behnke (1). Subsequently a variety of methods has been introduced. However, attempts to describe the theory and practice of individual methods have been limited. Early reviews (2–7) of body composition are detailed descriptions of few existing techniques while more recent efforts (8, 9) have been restricted to brief summaries of current approaches. The purpose of this review is to summarize the background and to describe the precision or error of each of the established techniques and to highlight the strengths and the limitations of methods.

Most body composition methods are based upon the model in which the body consists of two chemically distinct compartments, fat and fat-free (2, 10). The chemical composition of the fat-free body is assumed to be relatively constant with a density of 1.1 g/cc at 37 °C (2, 10, 11), a water content of 72–74% (6, 12), and a potassium content of 60–70 mmol/kg in men and 50–60 mmol/kg in women (13). Fat, or stored triglyceride, which is anhydrous and potassium free, has a density of 0.900 g/cc at 37 °C (14, 15).

During the derivation of the two compartment model, Keys and Brozek (2) divided the mammalian body into four chemical groups; water, protein, ash or bone mineral, and fat. Anderson (16) later used measurements of body potassium and water to estimate these components. Only recently has technology been available that allows for the in vivo determination of these four compositional variables.

The two and four compartment models served as the basis upon which all body composition methods were developed. The methods described in this presentation are classified as either traditional or new. The traditional methods represent the more established approaches to body composition assessment while the new methods reflect contemporary hypotheses and technology.

Traditional methods

Total body water

The findings that water is not present in stored triglyceride and that water occupies a relatively fixed fraction (73.2%) of the fat-free mass (12) have stimulated the determination of total body water (TBW) as an index of human body composition. Investigators have used the isotopes of hydrogen, deuterium and tritium, to quantitate body water volumes by isotope dilution in healthy and diseased individuals (6).

Some general assumptions of the isotope-dilution technique are that the isotope has the same distribution volume as water, it is exchanged by the body in a manner similar to water, and it is nontoxic in the amounts used (17). Because of the ease of liquid scintillation counting,

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some investigators relied upon tritium dilution to measure TBW (18). However, the use of a radioactive tracer such as tritium is contraindicated in research involving children or women of child-bearing age or in applications utilizing repeated measurements in a short period of time. Expanded use of deuterium occurred when newer techniques for the assay of deuterium in aqueous solution became available. For example, use of gas chromatography (19, 20), mass spectrometry (21), and fixed-filter infrared absorption (22) has facilitated the analysis of deuterium in biological fluids.

The typical procedure for using tritium or deuterium includes either the ingestion or the intravenous injection of a specified quantity of the tracer, an equilibration period, and a sampling period. The calculation of TBW volume is based upon the simplified relationship: $C_1V_1 = C_2V_2$, where C_1V_1 is the amount of tracer given, C_2 is final concentration of tracer in a biological fluid, and V_2 is volume of TBW. It is important that a correction be made for urinary loss of tracer.

The quantity of tracer given depends upon the type of tracer administered, the analytical system used, and the objective of the research. In general the smallest dose that allows good analytical precision and accuracy and provides the lowest risk to the subject should be given. For healthy men and women a 10 g dose of deuterium oxide (99.7% purity) mixed with ~300 mL of distilled-deionized water is used routinely in our laboratory (22). This procedure has an analytical precision of 2.5% using fixed-filter infrared absorptiometry (22). When using gas chromatographic methods, some investigators have given an oral dose of deuterium oxide of 1 g/kg body weight (19, 20). An oral dose of 2 g D_2O has been used with mass spectrometry (21). Among researchers using tritium as a tracer, a dose of 50 μCi (26 mrem radiation dose) is common (23). When repeated body-water determinations are to be performed over short periods of time (eg, every 2 wk or less) or in women or children, the use of deuterium as a tracer is suggested.

The length of time required for tracer equilibration depends upon the characteristics of the study sample. In healthy men and women equilibration of deuterium in saliva, plasma, and urine occurs 2 h after ingestion of the tracer and remains at a constant concentration in these biological fluids for the next 3 h (19, 20, 22). Among individuals suffering from water accumulation, equilibration may require 4–6 h (6).

Although plasma or serum tracer concentrations have been used to calculate TBW, saliva concentrations may be useful under some conditions (19, 20, 22). In field situations where obtaining pre- and postadministration venous blood samples may be difficult because of local beliefs or lack of adequate facilities, saliva and urine collection may permit greater participation by the group under investigation.

Recently, the use of oxygen-18 as a tracer to measure TBW was proposed (24) because it avoids the exchange of the label with nonaqueous hydrogen in the body that can overestimate body water by 1–5% (25). Implementation of this technique is difficult outside of a specialized research laboratory because of the need for laborious procedures and specialized equipment including a mass spectrometer. Also, the cost of oxygen-18 is prohibitive ($300/trial) for routine use. Comparative cost for the deuterium dilution procedure using 10 g of D_2O ($13) and fixed-filter infrared spectrophotometry ($4000) is much less.

Total body potassium

Chemical analyses have indicated that potassium is essentially an intracellular cation that is not present in stored triglyceride. Also, potassium-40, which emits a characteristic gamma ray at 1.46 MeV, exists in the body at a known natural abundance (0.012%). These facts have allowed investigators to estimate fat-free mass in humans and animals by external counting of potassium-40.

Quantitation of total body potassium (TBK) requires specially constructed counters that consist of a large shielded room (to reduce background radiation from cosmic and terrestrial sources) containing a gamma ray detection system connected to a suitable recording device. The detectors are of two types: large thallium-activated sodium iodide crystals, one or more of which are positioned near the subject, and large, hollow cylinders or half cylinders, the walls of which contain liquid or plastic scintillation material and into which the subject is placed so as to be completely or partially surrounded by the detector. These are referred to as 4π or 2π geometrical counters. The advantages of the crystal system include very good energy resolution and a low background rate. The plastic or liquid systems have very poor resolution, high background-interference rate, and high counting efficiency.

Initial attempts to measure potassium-40 used a single crystal placed adjacent to the subject, who was seated in a chair and counted for 30 min (26, 27). Later, a system was developed that allowed a single crystal to pass over the subject who was supine on a cot (28). Other investigators have utilized 4π potassium-40 counting (29). Regardless of which type of counting system is used, the reported variability of counting potassium-40 in an anthropometric phantom was > 5% and the error of potassium-40 counting in humans was ~5% (30). These estimates of counting precision were attributed to differences in detectors and interindividual variation in body geometry.

The most significant recent improvement in the whole-body-counting method is the development of techniques to provide absolute quantitation of potassium-40. Cohn et al (31) used a uniformly distributed cesium-137 source (0.5 μCi) and a 54-detector whole-body counter and associated computer facility to measure potassium-40 absolutely. By counting the gamma rays emitted from the cesium source, which is positioned briefly under the subject who is supine on a cot, attenuation factors are determined. These factors are used to correct for differences in body size and geometry and in gamma ray self-absorption for each subject. This method permits an accu-

racy and a precision of in vivo potassium-40 counting within 3%.

Another recent finding that can improve counting accuracy is reduction of background bismuth-214 contamination. Lykken et al (32) counted endurance cyclists before and after prolonged outdoor bicycling in North Dakota and found an apparent increase of 10% in potassium-40 counts of the legs. It was determined that the increased counts were due to gamma rays emitted from bismuth-214, which has one gamma ray in the potassium-40 energy level. Radiobismuth is a decay product of atmospheric radon. Thus, subjects should shower, wash hair, and wear clean clothing to under go whole-body counts.

Once TBK is determined one can estimate lean body or fat-free mass with a factor dependent upon the potassium content of the fat-free mass. From chemical analysis of a few human cadavers, Forbes et al (26) suggested values of 2.66 and 2.50 g potassium/kg fat-free mass in men and women, respectively. Boling et al (33) derived a new set of constants based upon the high correlation between potassium and water. They proposed 3.41 and 3.16 g potassium/L body water in men and women, respectively. Assuming a constant level of hydration of the fat-free body (73.2%) (12), these constants became 2.5 and 2.31 g potassium/kg fat-free mass for men and women, respectively. Behnke (34) calculated factors for converting total body potassium to fat-free mass to be 2.46 and 2.28 g for men and women, respectively. Using hydrodensitometry and absolute potassium-40 counting, Lukaski et al (35, 36) reported values of 2.46 and 2.50 g/kg fat-free mass in men.

Therefore, with the use of an accurately calibrated, well-shielded whole-body counting system, standard procedures for correction of counts due to body geometry and gamma ray self-absorption, and good control of bismuth-214 background interference, one can obtain absolute measurements of TBK. However, the cost of such determinations, including instrumentation and technical support, may be prohibitive for some research applications.

Urinary creatinine excretion

The origin of endogenous creatinine can be traced to the synthesis of its precursor creatine in the liver and kidney. Although many tissues take up creatine, the preponderance (98%) of it is located in skeletal muscle, mostly in the form of creatine phosphate (37). Creatinine is formed by the nonenzymatic hydrolysis of free creatine liberated during the dephosphorylation of creatine phosphate (37).

Since Folin (38) first proposed that urinary creatinine grossly was associated with body composition and Hoberman et al (39) demonstrated the direct proportionality of body creatine to urinary creatinine output using nitrogen-15 isotopic dilution, it generally has been acknowledged that urinary creatinine excretion is related to fat-free mass and muscle mass (40–43). However, some factors have been identified that affect the validity of this method.

The greatest drawback to this method is the large intraindividual variability in daily urinary creatinine excretion. The mean intraindividual coefficient of variation of daily creatinine output ranges from 11 to 20% for individuals consuming unrestricted, free-living diets (44–47) and can be reduced to 11% among people consuming meat-free diets (48). This large variability has been attributed to the renal processing of creatinine; it is both filtered and secreted at the kidney (49).

In addition to renal handling, diet also can affect daily creatinine excretion. Significant reductions (10–20%) in excretion occur in healthy men consuming meat-free diets for several weeks (44, 50). Changes in creatinine output were related directly to dietary creatine intake. Crim et al (51) consecutively fed healthy young men 0.23 g creatine/d for 9 d, 10 g creatine/d for 10 d, and then a creatine-free diet for 71 d. Urinary creatinine excretion increased 10–30% with creatine feeding and decreased during the creatine-free diet. Nitrogen balance was positive during all dietary periods. Lykken et al (47) derived a mathematical model with feedback that describes the creatine pool size and thus urinary creatinine excretion as a function of time after changes in the amount of creatine and protein consumed. These findings suggest that the body creatine pool is not under strict metabolic control and that urinary creatinine excretion is to some degree independent of body composition.

Another technical factor that must be controlled is accurately timed urine collections. Forbes (42) indicated that an error as small as 15 min in a collection period represents an error of 1% in the determination of 24-h urinary creatine excretion. It is generally advised to make three consecutive 24-h urine collections to assure representative creatinine excretion for an individual.

The precision of automated procedures using the Jaffé reaction to determine creatinine concentration in urine is 1–2% (43). When fat-free mass is estimated by creatinine excretion, error is high compared with reference values determined by densitometry or potassium-40 counting. Among men with a fat-free mass of 40–100 kg, errors are 3–8 kg (43). By use of total-body-potassium data fat-free mass can be predicted from urinary creatinine output with an error of $\sim$3 kg in children and adults (42).

Some investigators have proposed a constant relationship between creatinine excretion and muscle mass. Talbot (40) estimated that 1 g of creatinine excreted over a 24-h period was derived from 17.9 kg muscle mass. Cheek et al (41, 52), however, suggested that each gram of creatinine excreted in the 24-h urine sample was derived from 20 kg of muscle tissue. This apparent difference reflects sampling and methodological variation between these studies.

Forbes (42) addressed the issue of a constant ratio of muscle mass or fat-free mass to daily urinary creatinine excretion. His equation predicting fat-free mass from endogenous creatinine output by children and adults as well as other published regression equations (42) possess positive intercepts. This has been interpreted that excreted creatinine does not represent a constant fraction of either

muscle or fat-free mass over the range of subjects studied. Forbes contended that when the intercept is positive, the fat-free mass:creatinine ratio will decline hyperbolically with increasing values for creatinine excretion and will approach the slope of the regression line as an asymptote. Therefore, it apparently is inappropriate to use a constant ratio of creatinine:unit body composition in a population unless factors such as age, gender, maturity, physical training, and metabolic state are controlled (43).

Densitometry

The assessment of human body composition by measurement of whole-body density is a common method used for healthy people. This method assumes that the body is composed of two distinct components (fat and fat-free) and that it is possible to determine each of these components from the measured whole-body density. The mathematical derivation of this relationship was summarized by Brozek and Keys (2, 10).

Inherent in the application of the densitometric approach is the recognition of some fundamental assumptions. The chemical composition of the fat-free body is assumed to be relatively constant so that the density of the fat-free mass differs substantially from that of fat (1.100 vs 0.900 g/cc). These early assumptions formulated by Behnke (11) were confirmed later by direct chemical analysis of laboratory mammals (14, 15). Other assumptions include a constant level of hydration and a constant proportion of bone mineral (eg, skeleton) to muscle in the fat-free body.

These assumptions were questioned by Siri (3), who emphasized the normal variation in water content as the largest single source of variability in the density of the fat-free body and who calculated fat content of the body. By use of indirect methods, research in humans revealed a variation of 1–3% in the water content of the fat-free mass (53). Siri (3) used this estimate to calculate an error of 2.7% body fatness in the general population attributable to variability in the hydration of the fat-free mass.

Siri (3) also questioned the influence of variability of bone density on prediction of body fatness using densitometric procedures. After reviewing the data presented by Keys and Brozek (2), Siri (3) concluded that the variability in protein:mineral ratio could lead to a variation in percent body fat of 2.1% (0.005 g/cc) in healthy humans. Bakken and Struikenkamp (54) estimated a similar error in density (0.003 g/cc) caused by variation in bone density in the population.

Based upon these estimates of variability in bone mineral density and hydration of the fat-free body, Lohman (55) calculated the theoretical error of 3–4% for predicting body fatness in a population using densitometry. This error is similar to that suggested by Siri (3) to be associated with the uncertainty in density and chemical composition of the fat-free body.

The most widely used technique of measuring whole-body density is the determination of body volume according to Archimedes' principle, which states that the volume of an object submerged in water equals the volume of water the object displaced. If one measures mass in air and mass in water, the difference, corrected for the density of the water corresponding to the water temperature at the time of the underwater weighing, is the apparent body volume. With this technique it is mandatory to determine the lung volume during submersion (residual lung volume) which makes a sizable (1–2 L) contribution in the estimate of total body volume. A second volume, gastrointestinal gas, is considerably smaller in magnitude ($\sim$100 mL), and is never measured (56). Because the intraindividual variability in gastrointestinal gas volume can be quite large (50–300 mL), this variable can compromise the precision of the densitometric method (57).

Durnin and Satwanti (58) quantitated the effects of level of exhalation, preceding meal size, and consumption of a carbonated drink on estimates of body fatness. For each test condition residual lung volume was measured simultaneously with the determination of underwater weight. Relative to the values obtained at maximal exhalation, variations in level of exhalation and inspiration and meal size caused only $\sim$1% absolute difference in the estimated fat content. Relatively large volumes of gas in the alimentary tract resulted in an absolute difference of $\sim$1.5% in the estimation of body fatness. These deviations observed in the estimation of body fat by densitometry are within the errors of the method. Whether such variations could be detected using residual lung volume measurements made apart from the underwater weighing is doubtful.

The underwater weighing system originally described by Goldman and Buskirk (59) and later modified by Akers and Buskirk (60) has gained widespread use. Briefly, this system uses strain gauges mounted under the water on the floor of a stainless steel tank. On these gauges is positioned a cot on which the subject kneels. Use of these strain gauges provides a rapid and artifact-free measure of the subject's mass in water. In addition a pneumatic valve system is used to facilitate the determination of residual volume using the nitrogen washout of the lungs (61) simultaneously with the underwater weighing. The advantages of this system include fast and reproducible determination of mass in water while the subject retains control during the submersion procedure which reduces apprehension and promotes cooperation. The precision of body density measurements with this system is 0.0015–0.0020 g/cc, or $<$ 1% body fat in the groups studied (56, 60, 62). Thus, the absolute error (eg, kg of fat) of this technique is dependent upon the fatness of the subject.

It is important to emphasize the need to perform residual lung volume determinations at the time of the underwater weighing. Although one study (63) showed no difference in mean body densities calculated from residual lung volumes determined during submersion, predicted from standard tables, or estimated from vital capacity measurement, all subjects were healthy, nonsmoking, physically active students. It is unlikely that a similar finding would be obtained with middle-aged males and females on whom hydrostatic forces would enhance exhalation during submersion.

A second method of assessing body volume uses the actual water displacement technique using a body volumeter (64). The technique is similar to that of hydrostatic weighing except the actual volume of water displaced by the subject is measured rather than the loss in weight in water. Water displacement is measured when the subject submerges under water and the increase in water level is measured using a previously calibrated, fine bore burette connected to the tank. Residual lung volume must also be determined to calculate whole-body density. Because of the difficulties in distinguishing the changes in volume in the tank necessary to obtain the accuracy associated with the underwater weighing method (65) and a lack of control by the subject during submersion, this technique has not gained wide acceptance.

Very useful body compositional data can be gained from the hydrodensitometric technique. However, its use in populations unaccustomed to swimming or apprehensive about submersion in water may result in longer periods of time required to desensitize the subjects to obtain valid and reproducible body composition estimates. Because this system (60) includes many components (strain gauges, amplifiers, chart recorder, spirometers, and nitrogen analyzer), its cost is $25 000–$30 000. This system can be adapted for field use.

A recent development is the use of a plethysmograph which eliminates the need for total immersion of the subject (66). This system is a closed vessel in which the subject stands in water up to neck level; the volume of the subject is determined by measuring the pressure changes produced by a pump of known stroke volume. This system requires an apparatus more complex than for hydrodensitometry but it does not require the instrumentation for residual volume determinations. Thus, total body volume can be made with minimal subject cooperation and good precision (SD < 0.3 kg body fat). The validity and reproducibility of this technique have been established in only one laboratory (66).

A common equation for the calculation of percent body fat (f) from body density (D_b) is that proposed by Brozek et al (10): $f = (4.570/D_b) - 4.142$. Another equation was developed by Siri (3): $f = (4.950/D_b) - 4.50$. Within densities of 1.10–1.03, the two equations give results within 1% body fat. For subjects with > 30% fat, the Siri equation yields higher values than the equation of Brozek (55).

Anthropometry

The use of anthropometric data has facilitated the estimation of human body composition outside of the laboratory. Values from the determination of skinfold thickness at various sites and measurements of bone dimensions and limb circumferences can be used in multiple regression equations to predict body density and to calculate body fatness and fat-free mass. Descriptions of standard anthropometric measurements are presented elsewhere (34, 67).

Bone measures. The anthropometric estimation of fat-free mass is based upon the principle that a relatively constant proportion of the fat-free tissue is associated with a given skeletal size (68). Behnke (69) proposed the hypothesis that measurement of bone diameters could be used for estimation of skeletal mass and thus fat-free mass. Using a variety of bone measurements made with a broad-blade anthropometer (head length and width; biacromial, bideltoid, bi-iliac, and bitrachanteric diameters; knee, ankle, elbow, wrist, and chest widths), Wilmore and Behnke (70) developed prediction equations for body density and fat-free mass in college men. However, when these equations were tested in older men, smaller than expected correlation coefficients (r = 0.73–0.82) were found between predicted and densitometrically determined body-composition values (71). Similar differences (r = 0.77–0.80) between anthropometrically predicted and densitometrically determined density and fat-free mass also were observed in women (72). These findings indicate that the proposed anthropometric models were valid only for the segment of the population from which the model was derived.

Skinfolds. More emphasis has been placed on the use of skinfold thickness measurements to estimate human body composition. This approach is based upon two assumptions: the thickness of the subcutaneous adipose tissue reflects a constant proportion of the total body fat and the sites selected for measurement represent the average thickness of the subcutaneous adipose. Neither of these assumptions have been proven to be true. Despite the contention that subcutaneous fat makes up about half of the total body fat, there are no data to support this statement. Furthermore, because there is little information on the distribution of fat in the body of the population at large, the validity of using skinfold equations to predict body composition is restricted to populations from whom these equations were derived.

The measurement of skinfold thickness is made by grasping the skin and adjacent subcutaneous tissue between the thumb and forefinger, shaking it gently to exclude underlying muscle, and pulling it away from the body just far enough to allow the jaws of the caliper to impinge on the skin. Because the jaws of the caliper (calibrated to exert a constant pressure of 10 g/mm^2) compress the tissues, the caliper reading diminishes for a few seconds and then the dial is read. Duplicate readings are made at each site to improve the accuracy and the reproducibility of the measurements. In subjects with moderately firm subcutaneous tissue, the measurement is easy to perform; individuals with flabby easily compressible tissue or with not easily deformable very firm tissue present a problem for obtaining valid measures of skinfold thickness. Because a double fold (two layers of skin and subcutaneous tissue) is measured, any factor that affects the reproducibility and validity of the skinfold thickness measurement increases the error of the predicted body-composition value.

Many equations are available for the prediction of body density and thus body fatness from skinfold thickness measurements (55). However, in terms of general validity in the adult Caucasian population, some equations have

been recommended. Durnin and Womersley (73) developed a regression equation to predict body density using the logarithmic transformation of the sum of four skinfolds (triceps, biceps, subscapula, and iliac crest), age, and gender. Jackson and Pollock (74) estimated body density in men using logarithmic or quadratic transformation of the sum of seven skinfolds (chest, axila triceps, subscapula, abdomen, thigh, and suprailiac), age, and wrist and forearm circumferences. For women, Jackson et al (75) derived an equation to predict body density from a quadratic of the sum of three skinfolds (triceps, thigh, and suprailiac), age, and gluteal circumference.

It is important to emphasize that the use of a mathematical transformation (either as logarithmic or quadratic form) of the sum of skinfold thicknesses is needed because body density is not linearly related to subcutaneous fat mass (73). Also, the inclusion of age and gender reduces the error of prediction of percent body fat in cross-validation trials (74, 75).

The precision of a measurement of skinfold thickness is dependent upon the skill of the anthropometrist and the site measured. In general a precision of within 5% can be attained easily by a properly trained and experienced individual (67). This error can increase slightly if skinfold thicknesses either get very large (> 15 mm) or small (< 5 mm) (76). The error in estimating densitometrically determined body composition from anthropometry has been established to be ~5% body fat (55); depending on the prediction equation and the sample of subjects, some reported errors have ranged from 3 to 9% body fat (55, 77).

Arm circumference. Assessment of body fatness by standard techniques provides an estimate of nutritional status of an individual or population. Because of the impracticality of using laboratory methods in field studies, upper arm circumference and triceps skinfold have been used to assess nutritional status (78). Arm circumference alone, although being relatively age-independent and a useful, though limited, measure (79), does not yield a precise diagnosis of malnutrition. The triceps skinfold, which is relatively easy to measure, is an index of subcutaneous adipose tissue. The combination of these anthropometric measures have been used as an indicator of protein-energy malnutrition (80).

The estimated muscle arm circumference (C_m) gives an indication of the body's muscle mass and hence its main protein reserve. It (C_m) can be derived from the arm circumference (C_a) and the triceps skinfold (S) using the equation $C_m = C_a - \pi S$.

Using the cross-sectional fat (F) and muscle (M) areas is more logical in the assessment of energy and protein nutritional status than using the skinfold and arm circumference. Individually, each of these measurements is a weak predictor of energy and protein stores particularly in children. However, the combination of these variables appears to be a more precise indicator of nutritional status (81). The fat area has the additional advantage that the standard in adequately nourished children changes only slightly between ages 1–7 y, thereby providing an age-independent assessment of energy reserves (79). Calculation of these areas uses the following equations: $F = SC_a/2 + \pi S^2/4$ and $M = (C_a - \pi S)^2/4\pi$.

In practice the measurements made are arm circumference and triceps skinfold. Arm circumference is the mid-arm circumference measured to the nearest millimeter on the right arm midway between the tip of the acromion and olecranon process with the arm relaxed. The triceps skinfold is measured to the nearest 0.1 mm using a calibrated caliper at the same level as the mid-arm circumference on the posterior aspect of the arm.

The basic assumptions for the calculation of the arm and fat area include: the mid-arm is circular; the triceps skinfold is twice the average fat rim diameter; the mid-arm muscle compartment is circular; and bone, which is included in the anthropometric arm muscle area, atrophies in proportion to muscle in protein-energy malnutrition. Heymsfield et al (82) examined the validity of these assumptions for adults and found that each of these approximations was in error to some degree. The result was a 15–25% overestimate by anthropometry in arm muscle area while mid-arm fat area agreed within 10% to values measured by computerized axial tomography in adults. These investigators subsequently derived gender-specific equations to account for errors in each of the four assumptions. These equations reduced the average error for a given subject to 7–8% for arm muscle area (83). Corrected arm muscle (are (cAMA) equations for men and women are $[(MAC - \pi S)^2/4\pi] - 10$ and $[(MAC - \pi S)^2/4\pi)] - 6.5$, respectively, where MAC is mid-arm circumference and S is triceps skinfold (83). Using estimates of muscle mass derived from urinary creatinine excretion (42), these investigators proposed the following relationship to predict total body muscle mass from cAMA: muscle mass (kg) = (height, cm) (0.0264 + 0.0029 (cAMA). The error of this prediction ranges from 5 to 9%.

New methods

Neutron activation analysis

The development of in vivo total-body neutron-activation analysis has provided the only technique currently available for the measurement of the multielemental composition of the human body. Absolute content of calcium, sodium, chloride, phosphorus, and nitrogen can be determined safely.

Total-body neutron-activation systems designed for in vivo studies deliver a moderated beam of fast neutrons to the subject. Capture of these neutrons by atoms of the target elements in the body creates unstable isotopes such as calcium-49 and nitrogen-15. The isotopes revert to a stable condition by the emission of one or more gamma rays of characteristic energy. Radiation from the subject is determined using a recording of the radiospectrum of the emissions. The data are obtained from a subject positioned carefully with respect to a detector array in a highly shielded facility. Standard gamma spectrographic analysis is applied. The energy level identifies the element and the level of activity indicates its abundance.

Neutron activation is an analytical technique based on nuclear reactions rather than chemical reactions. The essential variables include neutron flux density, isotopic abundance, cross-section of the target element, half-lives of the product isotopes, and emission energy of the induced activity.

Total body calcium. The first application of neutron activation analysis to the assessment of human body composition was the determination of total body calcium (TBCa). Because calcium is a relatively constant fraction (38–39%) of bone mineral (ash) weight, estimates of TBCa can be used to quantitate total-body bone mineral. However, calcium mass is not always proportional to skeletal mass because extraosseous deposits occur in health and disease.

The availability of neutron activation facilities to measure human TBCa is limited to the University of Birmingham, Birmingham, UK (84); Battelle Pacific Northwest Laboratories, Richland, WA (85); and Brookhaven National Laboratory, Upton, NY (86). The first two facilities utilize cyclotrons to produce neutrons of 3.5 and 8 MeV energy levels, respectively. The Brookhaven facility uses 4.2 MeV neutrons from a plutonium-238, beryllium source, which is better suited for research purposes because it gives a lower radiation dose (~280 mrem) to the subject than the cyclotron sources (~500 mrem). Using the Brookhaven method (86), the accuracy and precision of the activation procedure in an anthropometric phantom is 1%. In healthy adults measured over a 4–5 y period, the precision of the repeated TBCa determination was 2.5% (87), which is suitable for studies of longitudinal changes (88).

To quantitate clinically meaningful differences in TBCa with aging or metabolic bone disease, specific procedures have been developed to normalize body calcium levels. Most importantly, normalization for anthropometric variables, such as age, gender, height, and lean body mass (eg, potassium-40 counting), is necessary to reduce variability (from 15–18% to 5–8%) in heterogeneous samples (89). The ability of TBCa to discriminate abnormality may be due in part to this normalization procedure.

This experimental approach has been used to estimate mean rates of body calcium loss in a cross-sectional study of American adults aged 30–90 y. In women, mean rate of calcium loss up to age 50 y was 0.37%/y (3.8 g/y) and after age 50 y it was 1.1%/y (7.6 g/y). The average loss for men is 0.7%/y or 7 g/y after age 55 y (90).

Total body nitrogen. The ability to measure total body nitrogen (TBN) levels by neutron activation analysis has provided valuable determinations of human body composition in health and disease. The first nuclear methods (91, 92) for direct determination of TBN in humans used the $^{14}N(n, 2n)^{13}N$ reaction and suffered from a lack of specificity because of interference from positron emitters from other body elements. Recently the development of the prompt-gamma technique (93, 94), using the reaction $^{14}N(n, \gamma)^{15}N$, has led to the recognition of the clinical usefulness of body nitrogen measures in body-composition assessment.

This prompt-gamma technique uses a portable plutonium-238 beryllium source to provide fast neutrons that are moderated before contacting the subject who is irradiated bilaterally. This method quantitates TBN absolutely by using body hydrogen as an internal standard (93, 94). By means of prompt neutron capture, the nitrogen and hydrogen nuclei interact with the moderated or slow neutrons to produce transiently (10^{-15} s) induced nuclides, ^{15}N and ^{2}H, emitting gamma rays of characteristic energy levels (10.83 and 2.23 MeV, respectively) that are quantitated simultaneously during the neutron irradiation. This procedure requires a 20-min neutron exposure and counting period and has a calculated whole-body radiation dose of 26 mrem. The advantage of this technique over the conventional method of analysis is that errors in counting resulting from differences in irradiation and detection conditions and from differences in size and shape of subjects are reduced considerably. This reduction in error makes sequential nitrogen measurements considerably more reliable, particularly when subject weight has changed significantly. The accuracy and precision of nitrogen determination in a phantom is 3%; the precision of repeated determinations in healthy humans is 2–3% (94).

The availability of methodology for absolute determinations of TBN values in connection with accurate TBN data allows the estimation of muscle and nonmuscle mass and their respective protein contents using the mathematical models of Burkinshaw (95). Knowledge of the muscle and nonmuscle components, bone mineral mass from TBCa determinations, and body mass allows the calculation of body fat by difference. This four-compartment model of human body composition (96) was shown to be useful in evaluating large differences in these compartments between healthy and diseased subjects (97).

More information can be gained from total-body neutron-activation analysis than by any other available method. However, factors such as the high cost (in excess of $400 000), the need for skilled operators, lack of mobility, and use of ionizing radiation generally preclude the routine application of this method to assess human body composition.

Muscle Metabolites

Total plasma creatinine. As discussed previously, 24-h urinary creatinine excretion has been used to estimate muscle mass and fat-free mass. Recently, the use of total plasma creatinine was suggested as an index of total body skeletal muscle mass.

Schutte et al (98) extended the original work of Talbot (40) and derived good relationships ($r = 0.82$, $p < 0.001$) between total plasma creatinine (plasma volume × plasma creatinine concentration) and 24-h urinary creatinine output by 24 healthy men. Total skeletal muscle mass also was estimated from 24-h urinary creatinine according to Talbot (1 g urinary creatinine = 17.9 kg skeletal muscle) (40) and Cheek (1 g urinary creatinine = 20.0 kg skeletal muscle) (41, 52). Using these estimates of skeletal muscle

mass, the authors calculated that each milligram of total plasma creatinine would account for 0.88 or 0.98 kg skeletal muscle.

To validate these calculations determinations of plasma creatinine and direct dissections of skeletal muscle were made in four mature dogs. The results indicated that each milligram of total plasma creatinine is the equivalent of 0.88 kg skeletal muscle, which confirms the findings of Talbot (40). A mean error of 3.9% (range: 0.5–10.8%) was observed between the predicted and observed skeletal muscle mass values.

Endogeneous urinary 3-methylhistidine excretion. The amino acid 3-methylhistidine (3-MH) has been suggested as a safe, noninvasive in vivo marker of muscle protein breakdown (99). It is located principally in skeletal muscle in which it is produced by the posttranslational methylation of specific histidine residues in the actin of all muscle fibers and in the myosin of white muscle fibers. During catabolism of the myofibrillar proteins, the released 3-MH is neither reutilized for protein synthesis nor metabolized oxidatively but is excreted quantitatively in the urine. These characteristics suggested that 3-MH could be useful in predicting human body composition.

Densitometrically determined fat-free mass was shown to be well correlated ($r = 0.90$, $p < 0.001$) with 24-h endogenous 3-MH excretion in 16 healthy men aged 23–52 y consuming a meat-free diet (48). Urinary creatinine was a weaker ($p < 0.05$) predictor ($r = 0.67$, $p < 0.01$) of fat-free mass in these men.

A follow-up study was conducted to ascertain the specificity of 3-MH as a marker of skeletal muscle mass (100). Muscle and nonmuscle mass and their protein contents for 14 healthy men were determined from measurements of TBN and TBK according to Burkinshaw (95). These men consumed two isocaloric isonitrogeneous diets in the sequence of a 4-d meat diet followed by a 7-d meat-free diet. Urinary 3-MH excretion during the meat diet (513 ± 21 μmol/d; mean ± SE) was significantly higher than excretion on day 3 of the meat-free diet (230 ± 10 μmol/d) after which the mean daily output was relatively constant with a mean coefficient of variation of 4.5%. The endogenous output of 3-MH was related significantly to skeletal muscle mass ($r = 0.91$; SEE = 2.0 kg) and was not associated ($r = 0.33$) with the nonmuscle fraction of the fat-free mass. Although endogenous 3-MH excretion was a better predictor than urinary creatinine output of densitometrically determined fat-free mass and of skeletal muscle mass, the daily excretion of these urinary metabolites was correlated ($r = 0.87$, $p < 0.001$), indicating the validity of these metabolites as indices of muscle and fat-free mass.

When the body composition data from these two studies (48, 100) are combined, one can estimate the predictive value of these relationships. The prediction of fat-free mass from endogenous 3-MH excretion ($r = 0.89$, $p < 0.001$) has an error of ~4 kg over a range of 50–82 kg (mean = 70 kg) fat-free mass. Similarly, the prediction of fat-free mass from urinary creatinine excretion has an error of ~5 kg in the same volunteers. Interestingly, the error in predicting daily 3-MH output from urinary creatinine excretion is only 4 μmol/d.

Recently the dominant role of urinary 3-MH as a significant predictor of fat-free mass and of oxygen consumption instead of creatinine was confirmed in 12 healthy men (101). This study identified the greater ($p < 0.05$) capacity of 3-MH to predict body composition and metabolic function during exercise. These data support the earlier findings that endogenous 3-MH excretion is associated with the fat-free component of the body.

Some drawbacks to implementing these muscle metabolites as useful indices of human body composition include the need for consumption of a relatively controlled meat-free diet (47, 48) and complete and accurate urine collections. Also, the inherent variability (10–20%) in creatinine excretion is a concern (44–46).

Use of 3-MH as a marker of muscle protein or body composition received criticism because of the potential influence of nonskeletal muscle protein turnover on its excretory rate (102). In the rat a significant contribution to the urinary output of 3-MH has been attributed to the skin and gastrointestinal tract proteins (103). However, this problem has not been explored in detail for humans. The relative rates of muscle to nonmuscle protein synthesis and turnover are higher for the adult human than for the rat (37 vs 12%) (99, 104), and skeletal muscle may be a more important source of urinary 3-MH in the human whereas intestines and other sources are more important in the rat.

In general, use of 3-MH as a marker for human body composition may not be reasonable in conditions of severe sepsis or significant physical trauma in which accelerated rates of protein degradation occur, especially in muscle. This is in contrast with states in which significant muscle protein depletion has already occurred or where prior inadequate nutritional intake has contributed to a diminished state of health (105). In these latter cases a reduction of muscle protein synthesis is the basis for loss of muscle mass.

The analytical precision of measuring 3-MH in urine is high. Methods using ion-exchange chromatography with ninhydrin or ninhydrin-orthophthalaldehyde have yielded a precision of 4% (48, 106, 107), which is similar to that observed using high-pressure liquid chromatography with precolumn derivatization with fluorescamine (108). The highest precision has been obtained with a semiautomatic ion-exchange method using a color reaction involving ninhydrin-orthophthalaldehyde (109).

Absorptiometry

The availability of monoenergetic radiation from radionuclide sources led to the development of photon-beam absorptiometric methodology. The principle of this technique is simple; bone mineral content is assumed to be directly proportional to the amount of photon energy absorbed by the bone being studied.

Single-photon absorptiometry. Direct photon absorptiometry has become widely used and accepted for biomedical investigation of local or regional bone measurement. This technique involves a transmission scan using a highly collimated beam, usually from iodine-125 (27 keV) or americium-241 (60 keV), and a collimated sodium iodide scintillation detector (110). The beam is passed across a limb bone and changes of beam intensity are analyzed; the integral of these changes is proportional to the bone mineral content in the beam path. With this technique the bone must be enclosed in a constant thickness of soft tissue. Water baths, tissue-equivalent substances, and local compression have been used as control materials for this purpose. Determinations of local bone mineral content can be performed with either the axial or appendicular skeleton. However, most investigators have utilized the lower radius at a site approximately one-third the distance from the styloid process to the olecrenon.

Single-photon absorptiometry of the appendicular skeleton using iodine-125 was shown to have a high long-term precision (1–2%) in vivo when care is taken to reposition the limb (111, 112). In field studies the precision may reach 5% because of repositioning error. This error may be minimized by using the ratio of bone mineral content to radius width as the index. The accuracy of the measurement has been 2–4% (112, 113). A typical measurement on the distal third of the radial shaft is well correlated (r = 0.9–0.95) with the weight of the radius and of other long bones and with total skeletal mineral and calcium (113–116). This prediction of total skeletal mass has an error of 150–250 g (~10%), which is equivalent to an error of 60–100 g in TBCa determined by neutron activation (114–116). Thus, for individual assessment, an error of 5–10% in normal subjects or of 10–15% in osteopenic subjects is too large and a direct measurement of TBCa is desirable. It is not feasible to scan the total body with the single-photon technique. Also, the use of single-photon absorptiometry measurements of the appendicular skeleton may not be as sensitive a predictor of osteoporosis as are determinations, made by dual-photon absorptiometry of the spine, a prime target of demineralization.

Dual-photon absorptiometry. Total-body bone mineral content and lean body mass are measurable using dual-photon absorptiometry, which eliminates the need for constant soft-tissue thickness across a scan path and allows measurements of hitherto inaccessible body areas. This method uses a whole-body rectilinear scanner and a high activity (0.5–1.5 Ci) source of gadolinium-153 that emits energy at two discrete peaks (44 and 100 keV). The source is mounted beneath a table and is opposite to a scintillation detector above the table. The source and detector are passed across the body at a traverse speed of 1 cm/s with data collected at 0.5 cm (0.5 s) intervals (117).

Attenuation measurements at a given number of energy levels are required to analyze an even number of substances by absorptiometry. However, due to experimenal uncertainties and because attenuation coefficients are correlated, the number of substances that can be determined with precision is limited. Thus, attenuation measurements at two discrete photon energies are needed to quantitate a two-component system (bone mineral and soft tissue).

The composition of bone mineral is essentially invariable; however, soft tissue is composed of variable amounts of fat and lean tissue. Variations in fat-lean tissue composition produce differences in attenuation coefficients for soft tissue at both energy levels. Thus, one must obtain an estimate of soft tissue composition to obtain accurate measures of bone mineral and fat or lean tissue masses. This is achieved by obtaining experimental attenuation at the two energies as described by Peppler and Mazess (117).

Briefly, the ratio of attenuation at 44 keV to that at 100 keV gives the quantity, R, which directly indicates the fat content of soft tissue (118, 119). A weighted average of the R value (weighted for mass of tissue in each pixel) is calculated for the number of pixels containing soft tissue alone. The percentage body fat derived from the R value is used to calculate fat mass and lean body mass. Total-body bone mineral is calculated from the bone-containing pixels (117).

Estimated precision of total bone mineral content measurements by dual-photon absorptiometry for skeletons (1–2%) and for humans (2–3%) are high. Accuracy of this method for skeletons has been reported as a SEE of 36 g, or an error of 1% (117). Comparisons of total-body bone mineral measured in vivo using the dual-photon technique and TBCa by neutron activation analysis showed a higher correlation coefficient between the methods (r = 0.99) with an error of 113 g for the predicted total-body bone mineral (120).

Mazess et al (121) compared estimates of total body composition derived by dual-photon absorptiometry and densitometry. Similar estimates for percent fat and for lean body mass were obtained from 18 volunteers (14 females, 4 males) aged 23–58 y. Correlation coefficients between the compositional variables by the two methods were good (r = 0.90) and were influenced by the total-body bone mineral mass:lean body mass ratio, which was variable (CV = 17%) and probably influenced the validity of densitometric prediction of body composition.

The advantages of the dual-photon absorptiometry technique includes portability, low radiation dose (2–10 mrem), and total-body determination of bone mineral and lean body mass by relatively direct analysis. Instrumentation can be transported safely in a van and repeated measures can be made in subjects without undue radiation risk. Also, improved determinations of body compositional variables can be made because bone mineral, perhaps the most variable component of the lean body, can be measured. Cost of this instrumentation ($65 000) may be a limiting factor for its use. In addition some theoretical assumptions, such as the influence of Compton scattering, beam hardening, and attenuation factors, require addi-

tional consideration before this method can be recommended.

Electrical conductance

Bioelectrical impedance. The method for determining body impedance is based upon the nature of the conduction of an applied electrical current in an organism. In biological structures application of a constant, low-level alternating current produces an impedance to the spread of the current that is frequency dependent. The living organism contains intra- and extracellular fluids that behave as electrical conductors and cell membranes that act as imperfect reactive elements. At low frequencies (~1 kHz), the current mainly passes through the extracellular fluids while at higher frequencies (500–800 kHz) it penetrates the intra- and extracellular fluids (122, 123). Thus, body fluids and electrolytes are responsible for electrical conductance (eg, 1/resistance) and cell membranes are involved in capacitance.

The hypothesis that bioelectrical impedance measurements can be used to determine fat-free mass is based upon the principle that the impedance of a geometrical system is related to conductor length and configuration, its cross-sectional area, and signal frequency. With a constant signal frequency and a relatively constant conductor configuration, the impedance to the flow of current can be related to the flow of current: $Z = \rho L/A$, where Z is impedance in ohms, ρ is volume resistivity in ohm × cm, L is conductor length in cm, and A is conductor cross-sectional area in cm^2. Multiplying both sides of the equation by L/L gives: $Z = \rho L^2/AL$, where AL equals volume (V). Thus, $Z = \rho L^2/V$.

In living organisms electrical conduction is related to the water and electrolyte distribution in the biological conductor. Because fat-free mass, including the protein matrix of adipose tissue, contains virtually all the water and conducting electrolytes in the body, conductivity is far greater in the fat-free mass than in the fat mass of the body (124). The hypothetical relationship between impedance and electrical volume was proposed by Nyboer et al (125) who demonstrated that electrically determined biological volumes were related inversely to Z, resistance (R), and reactance (Xc) where $Z = (R^2 + Xc^2)^{0.5}$ Because the magnitude of reactance is small relative to resistance and resistance is a better predictor of impedance than is reactance (36), volume can be expressed as: $V = \rho L^2/R$, where L is standing height in cm and R is in ohms. Although there are difficulties in applying this general principle in a system with as complex geometry and bioelectrical characteristics as the human body, this relationship has been used to derive models for the prediction of human body composition (36, 126–128) by assuming that the body is a series of connected cylinders.

Determinations of resistance and reactance are made using a four terminal impedance plethysmograph (RJL Systems, model 101, Detroit, MI). (Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty by the US Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that also may be suitable.) The tetrapolar method is used to minimize contact impedance or skin-electrode interactions (122, 128). As a general procedure measurements are made ~2 h after eating and within 30 min of voiding. The subject, clothes but without shoes or socks, lies supine on a cot. Aluminum foil spot electrodes are positioned in the middle of the dorsal surfaces of the hands and feet proximal to the metacarpal-phalangeal and metatarsal-phalangeal joints, respectively, and also medially between the distal prominences of the radius and the ulna and between the medial and lateral malleoli at the ankle. A thin layer of electrolyte gel is applied to each electrode before application to the skin. An excitation current of 800 μA at 50 kHz is introduced into the volunteer at the distal electrodes of the hand and foot and the voltage drop is detected by the proximal electrodes. (According to Ohm's Law the electrical impedance [Z] to alternating current of a circuit is measured in terms of voltage [E] and current, [I] as Z = E/I. By using phase sensitive electronics, one can quantitate the geometrical components of Z; resistance [R] is the sum of in-phase vectors and reactance [Xc] is the sum of out-of-phase vectors.) This technique provides a deep homogenous electrical field in the variable conductor of the body. Determinations of resistance and reactance are made using electrodes placed on the ipsilateral and contralateral sides of the body. The lowest resistance value for an individual is used to calculate conductance (ht^2/R) and to predict fat-free mass. The precision of this method, determined in 14 men in whom impedance was measured on five consecutive days, was < 2% (36).

The tetrapolar impedance method has been used to predict body composition in healthy adults. Using densitometric data but lacking accurate residual volume measurements, Nyboer et al (129) developed preliminary statistical relationships between conductance and body composition variables in college students. Segal et al (130) attempted to verify these regression equations and found unsatisfactory predictions of fat-free mass in men and women aged 18–50 y. We have used standard methods to establish models to estimate total body water and potassium and fat-free mass in healthy men (36). The respective errors of prediction of these variables were 2.1 L, 10.7 g, and 2.6 kg. Recently we conducted a study with 47 men and 67 women to validate the relationship between conductance and fat-free mass (131). Comparison of densitometrically determined and impedance-predicted fat-free mass yielded an error of prediction of 2–2.5 kg and a calculated error of relative body fatness of 2.7%. Relative to densitometry the observed error of calculated body fatness was larger by anthropometry (3.9%) than by impedance (2.7%).

From an analysis of intraindividual coefficients of variation in repeated measurements of resistance over five consecutive days in 14 men, it was suggested that the observed variability of 2% probably reflects small changes in body water compartments (132). A sample of 33 males and females aged 19–61 y underwent determinations of

TBW by deuterium dilution and extracellular fluid volume by bromide dilution. As expected, conductance was the best single predictor of TBW and extracellular fluid space ($r = 0.97$ and 0.94, respectively). Body height, weight, and age also were significant predictors of TBW ($R^2 = 0.970$; SEE = 2.1 L). Weight and reactance were additional significant predictors for extracellular fluid volume ($R^2 = 0.930$, SEE = 0.9 L).

Kushner and Schoeller (133) demonstrated the validity of the impedance method to predict TBW in patients with either inflammatory bowel disease and receiving total parenteral nutrition or diabetes. In a sample of 20 nonobese and 20 obese adults, an equation was developed ($R^2 = 0.986$, SEE = 1.75 L) to predict TBW from conductance, body weight, and gender. In the prospective patient sample, there was no difference between TBW predicted by the impedance model (42.9 ± 7.4 L; mean ± SD) and determined by deuterium dilution (41.9 ± 7.3 L). This finding indicates the usefulness of the impedance method to assess TBW in individuals with altered metabolic function.

Total body electrical conductivity (TOBEC). As with the impedance method, this technique relies upon the differences in electrical conductivity and dielectric properties of the fat free and fat tissues to estimate body composition (124). The instrumentation used is an adaptation of the commercial device developed for determination of lean tissue in meat and live animals (134).

The instrument is a long, uniform solenoidal coil driven by a 5 MHz oscillating radiofrequency current (Dickey-john Medical Instrument, Auburn, IL). The oscillating field with induce an electrical current in any conductive material placed within the coil. The actual mesurement consists of the difference between the coil impedance when empty and that when the subject is inserted (135).

Domeruth et al (136) compared conductivity measures with direct chemical analyses and live potassium-40 data in 45 mature pigs (average weight 100 kg). The correlation coefficient between potassium-40 and conductivity measures was 0.75. The weights of carcass water, fat, and protein were correlated better with live conductivity measures ($r = 0.87$, 0.32, 0.83) than with live potassium-40 data ($r = 0.78$, 0.09, 0.69).

Bracco et al (137) used a TOBEC instrument designed to estimate the lean component of ground meat (DjMe100 Ground Meat Fat Tester, Dickey-john Medical Instrument) to determine relationships between conductivity values (TOBEC scores) and body composition estimated by densitometry and carcass analysis of 30 rats weighing 197–433 g. TOBEC values were correlated highly with lean body mass by densitometry ($r = 0.97$, SEE = 13.6 g), fat-free mass by chemical analysis ($r = 0.97$, SEE = 14.2 g), total water ($r = 0.98$, SEE = 10.7 g), and total protein ($r = 0.95$, SEE = 5.1 g).

Using phantoms of infants composed of electrolyte solutions and corn oil, Klish et al (138) validated a TOBEC system developed for use with human infants. The TOBEC system was sensitive to changes in total electrolyte content and fluid volume of the phantoms. A strong linear relationship ($r = 0.97$) was observed between fat-free volume and the natural logarithm (ln) of the TOBEC value. Similarly, strong relationships were observed between the ln TOBEC signals and fat-free content of ground meat ($r = 0.91$) and live rabbits ($r = 0.99$).

A method using this technique has been developed for human body composition assessment (139). The subject lies supine on a stretcher on rollers and is inserted into the instrument. Ten consecutive readings are obtained over a 3-min interval and the average is displayed. The amount of electromagnetic radiation received by the subject during a single measurement period is $^1/_{100}$–$^1/_{1000}$ less than any established regulatory limits for continuous exposure to radiofrequency waves (135).

In a group of 19 adults measured on four separate occasions, the reliability of the measurements was high ($r = 0.99$) and the precision of the measurements was < 1% (139). Raw conductivity scores were correlated with anthropometric estimates of lean body mass, total body water, and potassium ($r = 0.69$, 0.86, and 0.87, respectively). In another group of 32 adults, a good correlation ($r = 0.90$) was observed between conductivity scores and densitometrically determined lean body mass (140). Use of height × conductivity as the independent variable improved the correlation with fat-free mass ($r = 0.94$, SEE = 4.0 kg). However, multiple regression analyses indicated that gender and height × conductivity yielded the best prediction equation ($r = 0.95$, SEE = 3.8 kg). A study with another group of 75 adults found that a transformation of the conductivity score, height^2 × conductivity, was a better predictor of lean body mass (130). When compared with densitometry, this method had a lower predictive error of estimating body fatness (SEE = 3.7%) than did skinfold thickness measurements (5.8%).

Recently, Van Loan and Mayclin (141) described a second generation instrument (TOBEC II) and developed prediction equations for densitometrically determined fat-free mass, TBW, and TBK in a sample of 40 males and females aged 19–35 y. This new instrument also is a solenoidal coil but it is powered by a 2.5 MHz source that generates a magnetic field ~79 cm in diameter and 185 cm long. In contrast to TOBEC I, the subject is passed through the magnetic field of TOBEC II. Movement through the magnetic field yields a phase curve representing the interaction of the magnetic field with the geometric shape and the distribution and amount of conductive material (eg, fat-free tissue). The shape of the phase curve does not correspond visually to the contour of the subject or the distribution of fat-free mass because the curve represents the sum of two components (conductive and dielectric masses) passing through the magnetic field. Because the phase curve is a periodic function, Fourier analysis, with the ability to represent a complex waveform with simple coefficients, has been applied to body composition analysis.

Multiple regression analysis using zero-, and first-, and second-order Fourier coefficients as predictor variables of fat-free mass, TBW, TBK yielded R^2 of 0.983, 0.961, and 0.891 with SEE of 1.43 kg, 1.57 L, and 11 g, respectively.

These results indicate that TOBEC II has the potential for increased predictive accuracy over the original TOBEC instrument. However, cross-validation trials are necessary to prove this contention.

The advantages of the bioelectrical impedance method are portability, safety, convenience, cost ($2500), and acceptable levels of reliability and accuracy of body composition estimates in healthy adults. It appears to be well suited for population or epidemiological surveys. Its drawback is the lack of validation in patients undergoing weight or compositional change and in patients with abnormal water or electrolyte distributions. Similar disadvantages are associated with the total body conductivity technique. In addition, cost ($70 000) may be a limiting factor for TOBEC.

Computerized tomography

Computerized tomography (CT) is a modern radiographic method to determine regional body composition. This approach relates small differences in x-ray attenuation to differences in the physical density of tissues to construct a two-dimensional image of the underlying anatomy in the scan area.

The CT system consists of a collimated x-ray source and detectors aligned at opposite poles of a circular gantry. Lying on a movable platform, the subject is advanced through the central aperture of the gantry. The field of vision, or the designated area to be scanned, is a plane through the middle of this aperture and parallel to the gantry. As the x-ray beam is rotated around the subject, information about the intensity of the attenuated x-ray beams is recorded and stored. The scanner computer than applies complex algorithms to the stored series of profiles to reconstruct cross-sectional images.

Each CT scan image or reconstruction is a matrix of pixels or picture elements, each about 1 mm × 1 mm, arranged in rows and columns. Because the depth of the scan or slice thickness is known (it can vary from as little as 1 mm to typically 10–13 mm), this is referred to as a voxel or volume element. For each individual volume of tissue, the CT scanner measures the x-ray attenuation within that voxel independently of the remainder of that tissue. The reconstructed picture represents not the image at the surface of a cut but rather an average representing the full thickness of the slice.

The magnitude of the x-ray beam attenuation is reflected in the degree of pixel shading and is scaled as the CT number in Hounsfield Units. The gray scale shown on a CT scan uses the same linear attenuation coefficients used for conventional radiographic film. For example, lower densities appear black and higher densities are white with air and bone at the low and high ends of absorption, respectively. Thus, high image contrast is observed between bone, adipose, and fat-free tissues.

Generally, the CT scanner requires about 10 s to complete each slice. The number of slices depends upon the purpose of the CT scan. The typical radiation dose for a CT scan is a peak of 1.5–3.0 rads (142).

Different approaches have been used with the CT scanner to analyze body composition (143). The structure of interest can be traced directly on the viewing console with a cursor. The cross-sectional area of adipose, bone, muscle, or visceral organ then can be determined for each image using sophisticated software programs. Because slice thickness is known, one can calculate the relative surface area or volume occupied by each organ or tissue in the reconstructed picture. These methods have been used to assess changes in muscle and adipose tissue in malnutrition (144) and to describe cross-sectional differences in abdominal fat distribution during aging (145, 146).

When no sharp boundaries between structures are apparent but the tissues differ markedly in radiographic density, the pixels in successive slices are plotted as a histogram separating the pixels into fat-free and adipose tissues (147). Because the volume of each pixel is known, the volume of the adipose and fat-free tissue in each slice can be determined from the number of pixels forming each slide and added for all slices performed.

Another method utilizes tissue matrices in which the individual components are smaller than one pixel. This approach has been helpful in diagnosing organ tumors (147–149), fatty liver (150), and tissue iron content (151).

Tomographic pixels derived from an area of adipose tissue represent adipocytes (triglyceride and protein matrix) and not just neutral fat. Thus, to determine fat mass one must first assume that a fixed fraction of adipose tissue is triglyceride. An alternate approach is to determine the volume of adipose tissue by adding the appropriate number of adipose voxels and assuming a constant density for adipose tissue (145, 146). Neither of these approaches has been validated against standard body-composition methods.

Although the potential of CT scanners for body-composition analysis is great, practical constraints limit its general use. Because of the exposure to ionizing radiation, routine whole-body scans, multiple scans in the same individual, and scans of pregnant women or children are not encouraged. Also, the cost and general availability of modern CT scanners prohibit the routine use of this instrumentation for only body-composition assessment.

Subcutaneous adipose tissue thickness

In addition to skinfold thickness measurements, an estimate of body fatness using the subcutaneous adipose-tissue layer can be made by soft-tissue roentgenograms, the ultrasonic technique, and infrared interactance. These alternative approaches were developed because investigators recognized some limitations in skinfold thickness measurements, including skinfold compressibility with age (152) and the inability to measure skinfold thickness at some sites in obese people (153).

Soft tissue radiography is more accurate than are skinfold measurements of subcutaneous adipose-tissue thickness (154). However, this technique is cumbersome and only can be used with a limited number of relatively safe sites because of the undesirable radiation exposure. Also,

tissue magnification and subject positioning must be monitored carefully.

Ultrasound. This approach uses an instrument in which electrical energy is converted in a probe to high-frequency ultrasonic energy, which then is transmitted into the body in the form of short pulses. As these ultrasonic waves impinge perpendicularly upon the interfaces between tissues which differ in acoustical properties, part of the ultrasonic energy is reflected to the receiver in the probe and is transformed to electrical energy. On an oscilloscope screen, this echo is visualized as a vertical deflection of the horizontal time baseline.

Commercial ultrasound instruments provide images of tissue configuration (B-scan mode) or depth readings of changes of tissue density (A-scan mode). Assessments of adipose tissue thickness are made with A-scan mode devices.

Initial trials established the validity of the ultrasound method to estimate adipose tissue thickness in humans. Correlation coefficients of 0.80 and greater have been reported between ultrasonic measurements and skinfold thickness assessed by the caliper technique at the triceps and subscapula (155–158). Also, ultrasonic data were found to be correlated highly with electrical conductivity measurements of subcutaneous abdominal adipose tissue (156), with needle puncture measurements of abdominal fat (155), and with soft tissue radiographs over the iliac crest (157).

Using ultrasonic and caliper techniques, Haymes et al (159) measured subcutaneous adipose thickness in adults at the triceps, subscapula, abdomen, and suprailiac sites. Reproducibility of ultrasound measures (r = 0.87–0.98) were marginally lower relative to caliper values (r = 0.98–0.99). Correlations between measures obtained by the different methods generally were higher among women than men, probably because of greater adipose depots at each site. In a subsample of subjects from whom soft tissue radiographs were made, differences in adipose thickness between ultrasound and roentgenograms of 1.9 ± 2.1 mm at the triceps and 4.7 ± 4.9 mm at the waist were attributed to distinct discontinuities corresponding to the fascial margins observed on the roentgenograms.

Borkan et al (160) evaluated ultrasound and skinfold methods to predict body fatness determined by potassium-40 counting in a group of 39 men. Although measurements made with the two techniques at the same site typically produced different mean estimates of adipose tissue thickness, the values were highly correlated ($r > 0.80$) with each other, indicating similar relative ranking by each technique. Skinfold thicknesses were correlated more highly with fat weight than were ultrasound measurements (r = 0.51 vs 0.39). These data suggest that skinfolds are more effective than ultrasound in assessing body composition, particularly when the large difference in cost is considered.

Recently, Fanelli and Kuczmarski (161) suggested that ultrasound was as good a predictor of body fat as the skinfold caliper method. Adipose tissue thickness at seven body sites was measured with skinfold calipers and ultrasound in 124 men aged 18–30 y. Body fatness was determined by densitometry to be 3.5–32.7%. On average, slightly higher correlation coefficients were observed with body density and skinfolds than with ultrasound measurements. For the caliper method, the triceps site was the best single predictor of body density (r = 0.75) while for ultrasound the waist was the best predictor (r = 0.74). Multiple regression analysis showed that the best prediction equation of body density using skinfold values had an r = 0.779 and SEE = 0.0083 g/cc and using ultrasound measures had an r = 0.809 and SEE = 0.0078 g/cc. This suggests equal predictive capacity of the techniques.

Although these data suggest a reasonable validity of the ultrasound approach, certain limitations have restricted its general use. The appropriate signal frequency of the probe had not been well defined. The literature contains a range of 2.5–7.5 MHz with the best predictive accuracy associated with the highest frequency (161). Another difficulty is the need for uniform and constant pressure applied by the probe to the scan site. Changes in pressure by probe application can affect the distribution of adipose tissue and prejudice the ultrasonic determination of adipose thickness. Also, validation trials should be conducted with heterogenous samples containing large ranges in body fatness.

Infrared interactance. Infrared interactance, a new method proposed for the assessment of human body composition, is based upon the principles of light absorption and reflection using near-infrared spectroscopy. When electromagnetic radiation strikes a material, the energy is reflected, absorbed, or transmitted depending on the scattering and absorption properties of the sample. Energy transmitted into the sample is scattered and reflected back out of the sample contains information about the chemical composition of the sample. This approach was developed by Norris (162, 163) to predict the starch, protein, oil, and water content of grains and oilseeds.

For estimations of human body composition, a computerized spectrophotometer is used with a single, rapid scanning monochromator and fiber optic probe. The instrument is operated in the transmittance mode and scans are made over midrange wavelengths of 700–1100 nm. The probe emits electromagnetic radiation from the monochromator to a selected site on the body, collects interactive energy, which is the combination of reflected and scattered energy, and conducts it to the detector. The signal penetrates the underlying tissue to a depth of 1 cm and composition is assessed only at the examined site.

Interactance (I) data are calculated by the instrument as the ratio of the energy received from a scan site to the energy received from a calibration standard, a 1 cm thick Teflon block. Data are transformed to log (1/I) to be similar to absorption spectra plotted as log (1/T) and are shown to vary linearly with concentration of a specific absorber in a mixture of other materials in agricultural food stuffs (164).

Analyses of spectra used the ratio of two second derivatives of log (1/I) data measured at two different wavelengths. This mathematical approach reduces the effects

of temperature and particle size, resolves problems of overlapping absorption bands, and cancels out light-scattering effects (164). From empirical calculations, wavelengths of 916 and 1026 nm were selected for use in calculating the ratios.

Conway et al (165) used this approach with 53 males and females who underwent determinations of TBW by deuterium dilution and measurement of infrared interactance, skinfold thicknesses, and ultrasound at the triceps, biceps, subscapula, suprailiac, and thigh. To evaluate the validity of the infrared technique to predict body fat, data from 36 subjects were used to develop a model that was tested in the other 17 subjects.

The prediction equation showed a good relationship between the ratio of the second derivative interactances at 916 and 1026 nm and percent body fat estimated by deuterium space ($r = 0.91$, SEE = 3.2%). In general, the infrared interactance method overestimated body fatness. Among all subjects significant correlation coefficients were found between percent fat values derived by infrared interactance and deuterium dilution, skinfold thicknesses, and ultrasound (0.94, 0.90, 0.89, respectively).

This method must be considered to be in the developmental stage. Questions arise about the validity of extrapolating compositional data from a limited (1 cm in depth) subcutaneous depot to the whole body.

A major drawback to the ultrasonic and infrared interactance approaches is the dependence upon regional adipose distribution to predict total body fat. Although these approaches may be useful in homogenous samples, their ability to generalize to the heterogenous population is questionable.

Magnetic resonance imaging

A method with great potential for the safe, noninvasive, direct assessment of human body composition is magnetic resonance imaging (MRI). This approach is based on the fact that atomic nuclei, made up principally of neutrons and protons, can behave like magnets. When an external magnetic field is applied across a part of the body, each nucleus or magnetic moment attempts to align with the external magnetic field. If a radio frequency wave is directed into body tissues, some nuclei absorb energy from the radio wave and change their orientation in the magnetic field. When the radio wave is turned off, the activated nuclei emit the radio signal that they absorbed. This emitted signal is used to develop an image by a computer (166).

The most frequently studied nucleus in biology is hydrogen, ^1H, and in particular the hydrogen atoms of water molecules in cells and tissues. Hydrogen is the most abundant element in the body when it is considered as number of atoms (or nuclei for MRI purposes) rather than as a percentage of body weight. The majority of these hydrogen atoms are present as parts of water molecules. The hydrogen nucleus is the most amenable to MRI detection. Not only is the natural abundance of ^1H high (99.98%), but the sensitivity of MRI to this nucleus, which is simply a proton, is greater than any other atomic nucleus.

Whereas conventional x-ray radiographic and computed tomography images depend on electron density, MRI depends on the density of hydrogen nuclei and the physical state of the tissue as reflected in the magnetic relaxation times. Anatomical information has been obtained by comparing MRI images and corresponding frozen cross sections of normal animals (167). Tissue contrast is high between fat and muscle and can be enhanced by changing the magnetic relaxation time variable of the magnetic resonance instrument. Application of MRI to differentiate malignant from benign processes have indicated differences in relaxation times between normal and cancerous tissues in rats (168) and humans (169, 170). Although exact interpretation of these observations is unclear (170), the data appear to indicate a correlation with degree of hydration of tissue (171, 172).

These findings have stimulated other investigators to estimate regional and total body water using MRI. Hayes et al (173) used conventional MRI to quantitate the water distribution of saline-filled and normal rat lungs in isolated and in situ preparations. Studies in isolated lung fragments showed an accuracy of $\sim$1%, and images of phantoms had an error of $< 3\%$.

Recently Lewis et al (174) used proton MRI to determine total body water in baboons. The hydrogen associated with water was measured as the amplitude of the free-induction decay voltage. Body water calculated by multiplying peak amplitude by the experimentally determined constant for a water standard was similar to that determined gravimetrically in the same baboons.

In contrast to images produced by x-ray radiography and computed tomography, MRI does not use ionizing radiation. It has the capability to generate images in response to intrinsic tissue variables and to represent gross chemical characteristics, such as level of hydration and fat content. In addition to hydrogen, MRI can image phosphorous and future prospects include carbon, nitrogen, sodium, and chlorine (175). It has the potential to quantiate total fat mass and to discriminate differences in regional fat distribution (176).

The optimism of future applications of MRI for body composition assessment must be tempered with the practical limitations of restricted availability and high cost in addition to the technical problem of spatial resolution. Nevertheless, MRI is an exciting new method that may have profound influence in assessing body energy stores.

Body-composition change

Changes in body weight generally reflect a change in water, protein, fat, and minerals. Using data from the semistarvation experiments done at Minnesota, Keys et al (177) demonstrated that changes in human body composition are dependent upon the amount and duration of the energy deficit. Comparisons of estimates of energy balance confirmed body-composition analyses that

showed the total weight loss was not due solely to reduction in body fat.

Yang et al (178) examined the composition of weight loss in six obese men consuming low-calorie ketogenic diets over short periods of time using the following procedures: energy-nitrogen balance, body water-nitrogen balance, body water, body potassium, and anthropometry. Body fat loss, expressed as a percentage of total weight loss, was similar by the energy-nitrogen and body water-nitrogen balances; body potassium and anthropometry overestimated (10%) and body water underestimated (20%) body fat loss. The energy-nitrogen balance method, although laborious and time consuming, yielded less variable results.

Attempts to quantitate changes in body composition after exercise training and limited caloric restriction have relied upon assessment by densitometry and anthropometry. For example, changes in body fatness were observed for lean and obese middle-aged women undergoing 12 wk of physical conditioning (179). Densitometrically determined body fatness was reduced by 1.0 and 1.8% in the lean and obese women, respectively. Similarly, the sum of skinfold thicknesses and calculated body fatness was reduced in both groups. When the predicted body fatness values from densitometry were plotted against those from anthropometry, the anthropometric approach apparently underpredicted fatness by 3.5%. This finding suggests that subcutaneous adipose tissue does not reflect total body fat. These observations highlight the limitations of indirect techniques that rely on the assumption of a constant composition of the fat-free mass.

The composition of weight gain also has been assessed by standard body-composition methods. Alleyene et al (29) reported increases in the creatinine/height and potassium/height indices for children recovering from infantile malnutrition. Because creatinine and total body potassium reflect muscle and fat-free mass, these ratios are useful indicators of nutritional rehabilitation (180). Comparisons of properly collected urinary-creatinine and total-body-potassium data from survey groups and healthy individuals of the same height may provide potentially useful information on nutritional status.

In a study of cancer patients undergoing hyperalimentation, Cohn et al (181) compared changes in body composition assessed by anthropometry and neutron activation analysis. For patients who gained weight, triceps skinfold and midarm circumference increased slightly (7 and 5%, respectively). Protein (4%), TBW (36%), and percent body fat (62%) increased and nitrogen:potassium ratio decreased (13%), indicating an increase in cell mass. Thus, anthropometry gave a qualitative index and activation analysis provided a more detailed description of compositional change.

Summary

The ideal method for assessing human body composition should be relatively inexpensive at initial purchase and for maintenance of operation should require little inconvenience for the subject, be operated by unskilled technicians, and yield highly reproducible and accurate results (8). Unfortunately, no method is available that meets these stringent criteria. In practice, however, there is a compromise between cost, ease of operation, and reliability.

Table 1 summarizes the characteristics of the various methods described in this presentation. Generally, techniques that are least expensive tend to have poor precision in estimating body composition and the most expensive methods have better potential of providing precise estimates of fat-free mass and body fatness. The appropriate question, therefore, is which existing method best meets the objectives of the proposed research.

All indirect methods of determining human body composition can be classified by which model of body composition they utilize. Methods such as water, potassium, densitometry, anthropometry, muscle metabolites, electrical conductivity, and impedance utilize the assumptions of the two-compartment model (eg, fat and fat-free components). Neutron activation analysis for nitrogen and calcium, together with determinations of total body water and potassium, utilizes the models of Burkinshaw (95) to give information on the four-component model (water, protein, bone mineral, and fat). Dual-photon absorptiometry yields data on fat, bone mineral, and fat-free tissues.

TABLE 1
Limitations of methods of determining human body composition*

Method	Cost	Technical difficulty	Precision	
			Fat-free mass	% Fat
Water				
Deuterium	2	3	3	3
Oxygen-18	5	5	4	4
Tritium	3	3	3	3
Potassium	4	4	4	3
Creatinine	2	3	2	1
Densitometry				
Immersion	3	4	5	5
Plethysmography	4	3	5	5
Skinfold thickness	1	2	2	2
Arm circumference	1	3	2	2
Neutron activation	5	5	5	5
Photon absorptiometry	4	4	4	4
3-Methylhistidine	2	3	3	?†
Electrical				
Conductivity	5	1	4	4
Impedance	2	1	4	4
Computed tomography	5	5	?	?
Ultrasound	3	3	3	3
Infrared interactance	4	3	3	3
Magnetic resonance	5	5	?	?

* Ranking system: ascending scale, 1 = least and 5 = greatest.
† Unknown at this time.

A number of factors should be considered when selecting one or more indirect methods for assessing human body composition for a specific study. The study sample should be limited to either adults or children. All body-composition methods have been developed with the assumption of chemical maturity (182). At some point during growth, the chemical composition of the fat-free body approaches that of the adult. Moulton (182) estimated that chemical maturity is reached in humans by the age of 3–4 y. Attempts to use adult-based equations in healthy prepubescent and adolescent children have generally led to inaccurate body-composition estimates because these children have a higher water content and lower bone density than adults (183).

Selection of a method may depend upon which compositional variable requires quantitation. Epidemiological surveys to identify the prevalance of obesity in a healthy adult population can use densitometry, conductance techniques, or anthropometry if appropriate models are available. However, assessment of composition in populations suffering chronic nutritional deprivation requires the use of neutron-activation techniques to determine elemental composition because the basic assumptions of the two-compartment model are not valid (97). Whether chronic mild-to-moderate reductions in energy, protein, or mineral consumption can affect the composition of the fat-free body has not been studied using methods that determine absolute elemental content.

Another factor to consider is whether cross-sectional or longitudinal data are to be collected. Large population surveys have used measurements of height, weight, bone lengths, and skinfold thickness to describe trends and differences between groups of people living under different ecological conditions (184, 185). Unfortunately, estimation of body composition from these data is not appropriate unless prediction equations have been developed and cross-validated for the population under study.

In longitudinal studies where the influence of a disease or nutritional therapy in malnourished patients may lead to subtle changes in composition, precise measurements are needed. Qualitative information can be gained from measurements of weight, skinfold thicknesses, and mid-arm circumference (186). Use of serial determinations of creatinine or total body potassium provides an index of change in the fat-free body (29, 180) but no quantitative measure of change in muscle or nonmuscle protein (181).

Within the limitations described in this presentation, a method or combination of methods for determining body composition can be selected to meet research objectives. However, the selection of a method depends on an understanding of practical considerations (cost, ease of operation, technical skills required, and subject cooperation) and the limitations of each method.

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