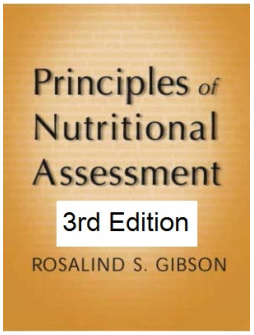

Gibson RS[1], Principles of Nutritional Assessment: Body Composition: laboratory Methods

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Abstract

Several indirect *in vivo* laboratory methods are available to assess body composition. Their selection depends on the study objective, the precision and accuracy required, the cost, convenience to the subject and their health, and equipment and technical expertise available. This chapter describes both non-scanning and scanning *in vivo* laboratory techniques. Non-scanning techniques include total body potassium, total body water (via isotope dilution or bioelectrical impedance analysis (BIA)), neutron activation analysis, densitometry (via under-water weighing or air-displacement plethysmography), and total body electrical conductivity. Four scanning techniques are included: computerized tomography, magnetic resonance imaging, dual energy X-ray absorptiometry (DXA), and ultrasound. Each technique generates body composition data in a different way, so the results are not interchangeable. For each technique, the characteristics, including both the advantages, limitations and assumptions applied to generate the body composition data, are outlined. In addition, their potential applications are summarized, with emphasis on the alterations in the relative proportions of the body components that may occur in certain life-stage groups or disease states. Such alterations invalidate the determination of fat and fat-free mass in a 2-component model of body composition when constants for the hydration and density of fat-free mass which ignore the inter-individual variability in these properties, are assumed.

Many factors have the potential to alter values for these assumed constants, especially growth and maturation in children, aging, pregnancy, and obesity. Consequently, researchers have developed specific constants for the hydration and density of fat-free mass specific for these circumstances. Their use will improve the estimates of fat-free mass when applying the simple 2-component model based on measurements of total body potassium, total body water, or whole-body density. From a comparison of nine *in vivo* body composition methods conducted by Field and coworkers (2015), measurement of densitometry via air displacement plethysmography was selected as the method with the highest degree of accuracy and reliability and with the least degree of technical error to track

and monitor whole-body composition across the lifespan, provided any alterations in the relative proportions of the body components, are taken into account.

In clinical patients with certain disease states, over- or underhydration and abnormalities in mineral mass may occur, resulting in substantial variability in both the hydration and density of fat-free mass. In these circumstances, application of multi-component models that include measurements of protein and/or bone minerals (via neutron activation or DXA) as well as total body water and density will minimize assumptions related to the structure, hydration, and density of fat-free mass. The 4-component model is considered the criterion method whereby whole-body composition can be most accurately assessed. A simplified version based on measurements from both DXA and BIA holds promise for monitoring conditions in certain disease states.

The four scanning techniques are widely used in clinical settings to quantify *in vivo* body composition, especially at the tissue-organ level, when investigations of bone density, skeletal muscle mass, and the deposition of visceral ectopic fat with their corresponding relationship with osteoporosis, sarcopenia, or cardiometabolic risk, respectively, are needed.

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14.0 An introduction to techniques used to measure body composition

Accurate methods for measuring body composition are required in investigations of obesity, malnutrition, weight loss following bariatric surgery, muscle wasting, sarcopenia, osteopenia, and osteoporosis. Body composition information is also used to establish the appropriate prognosis and treatment of hospital patients, and with longitudinal assessment, to monitor the effects of interventions on body composition (Lemos & Gallagher, 2017).

Selection of the method to measure body composition depends on the required precision and accuracy, the study objective, cost, convenience to the subject, their health, and equipment and technical expertise available (Lukaski, 1987). Methods based on multi-component models that include analysis of protein and minerals, minimize assumptions related to tissue density, hydration, and structure. This is important because in malnourished individuals, the elderly, and subjects with metabolic disturbances, the relative proportions of the body components is often altered, and losses of protein, fat, and bone mineral content may occur,

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often in association with the rapid accumulation of water. Changes such as these invalidate the determination of fat and the fat-free mass in the 2-component model.

Absolute validity cannot be assessed for any of the indirect *in vivo* body composition methods because the gold standard for body composition analyses is cadaver analysis. Instead, only relative validity can be assessed, defined as the comparison for each subject of the results from the "test" method with the results from another method, termed the "reference or criterion" method; the latter having a greater degree of demonstrated validity. A 4-component model is now considered sufficiently accurate to act as a reference or criterion method, but its use in many settings is limited because of the expensive and sophisticated technology required. Multiple statistical approaches can be used to establish the validity of the "test" method compared with a reference method. They include regression and correlation analyses, paired t tests, and more recently, Bland-Altman analysis; see Earthman (2015) for further details.

The characteristics of the various procedures used for measuring body composition are summarized in Box 14.1. This list includes both non-scanning and scanning techniques. Detailed sections (14.2-14.12) describe each of these indirect *in vivo* methods now available to assess body composition. Comments on these methods are also given, along with the assumptions used, and the advantages and disadvantages of each method. Scanning techniques such as computer tomography (Section 14.9), magnetic resonance imaging (Section 14.10) and whole body dual energy X-ray absorptiometry (DXA) (Section 14.11) are included together with a discussion of their clinical importance (Lee et al., 2019; Neeland et al., 2019). Each technique generates body composition data in different ways, so the methods are not interchangeable. Methods with the lowest cost are often the most imprecise.

Methods employing the 2-component model (i.e., body fat and fat-free mass) include total body potassium, total body water via isotope dilution or bioelectrical impedance, densitometry via hydrostatic weighing or air-displacement plethysmography, and total body electrical conductivity. Such methods are not suitable for clinical populations when the basic assumptions of the 2-component model are often invalid. Instead, in these populations, techniques using a 3, 4, or 5-component model should be applied. For example, DXA (Section 14.11) has the capacity to generate data that can be used with a 3-component model. See Lohman (1986) and Pietrobelli et al. (1996) for further details.

Three scanning techniques — computerized tomography (Section 14.9), magnetic resonance imaging (Section 14.10), and DXA (Section 14.11) —

can be used to quantify components (e.g., skeletal muscle, bone, visceral ectopic fat) at the tissue-organ level of body composition as well as to assess the relative proportions of the fat-free mass, body fat, and bone mineral content. Of these, only DXA has been recommended for the assessment of fat mass in patients with a variety of disease states; the use of DXA for the assessment of fat-free mass is not recommended for clinical populations because its validity for assessment of fat-free mass in any clinical population remains unknown (Sheean et al., 2020).

Box 14.1. Scanning and non-scanning laboratory techniques used to measure body composition.

14.1 Chemical analysis of cadavers
Cadaver analysis provides the gold standard data on body composition, but the use of such data is limited by ethical barriers. Results from a few older studies (1945-1968) are mostly based on adults who had died because of illness.

14.2 Total body potassium (TBK)
TBK is measured by counting radiation from naturally occurring ^{40}K in a whole body counter. Required equipment is only found in specialized facilities. Estimates of the fat-free mass can be derived from the TBK.

14.3 Total body water from isotope dilution (TBW)
A tracer dose of water, usually labeled with the stable isotope ^{2}H, is given orally or intravenously and then allowed to equilibrate. The concentration of the isotope in serum, urine, or saliva is measured and TBW calculated from dilution observed following equilibration. Obesity, pregnancy, and wasting disease increase TBW.

14.4 Multiple dilution methods
Typically, multiple dilution involves determining both total body water via isotope dilution and extracellular water (ECW), the latter using a tracer such as bromide that does not enter the intracellular space. The difference between these two measurements (i.e., TBW − ECW) reflects the intracellular water.

14.5 *In-vivo* activation analysis
Radioactive isotopes of N, P, Na, Cl, Ca are created by irradiating the subject. The resulting γ-radiation is measured using a whole body counter. Subjects are exposed to radioactivity. Sensitivity varies with the element. Required equipment is only found in specialized facilities.

14.6 Densitometry
Body density is derived from measurements of body mass and body volume. The latter is calculated from: (a) the apparent loss of weight when the body is totally submerged in water — difficult with young children, the elderly or sick patients — or, (b) air-displacement or water-displacement plethysmography.

14.7 Total body electrical conductivity (TOBEC)
Subject lies supine in a solenoid coil through which a 5MHz current is passed. The conductivity value of the subject is obtained by subtracting the background value when the coil is empty. Edema, ascites, dehydration, electrolyte balance and variations in bone mass all interfere with the conductivity reading.

14.8 Bioelectrical impedance (BEI)
The impedance to a weak electrical current passed between the right ankle and right wrist of a subject in supine position is measured. Edema, ascites, and dehydration invalidate single frequency measurements. Multifrequency measurements allow estimation of both total and extracellular compartments.

14.9 Computerized tomography (CT)
Method measures attenuation of X-rays as they pass through tissues, the degree of attenuation being related to differences in physical density of the tissues. An image is reconstructed from the matrix of picture elements. Exposure to ionizing radiation limits use of CT for pregnant women or children. Expensive equipment.

14.10 Magnetic resonance imaging (MRI)
Imaging involves placing a subject in a very strong magnetic field and observing the relative differences in behavior of ^{1}H protons in lean and adipose tissue. No exposure to ionizing radiation but equipment bulky and expensive.

14.11 Dual energy X-ray absorptiometry (DXA)
Utilizes the attenuation of a dual energy X-ray beam, often during whole-body scanning. New fan-beam technologies replacing earlier pencil-beam techniques lead to lower X-ray doses and improved spatial resolution. High precision method, but results are calibration dependent and differences between different equipment manufacturers can be significant.

14.12 Ultrasound
High-frequency sound waves from a combined ultrasound source and meter pass through adipose tissue to the adipose-muscle tissue interface. At the interface, some sound waves are reflected back as echoes, which are translated into depth readings via a transducer. CT, MRI, or DXA provides higher degree of structure resolution

14.1 Chemical analysis of cadavers

Studies of body composition by direct chemical analysis of human cadavers are limited. Most of the cadavers were analyzed between 1945 and 1968 and were adults of varying ages who had died because of illness; hence, the values obtained may not be representative of an average healthy adult.

14.1.1 Applications of cadaver use

Sex (y)	Water (g/kg)	Protein (g/kg)	Density (kg/m^3)	Potassium (mmol/kg)
Male (25)	728	195	1120	71.5
Male (35)	775	165	1083	–
Female (42)	733	192	1103	73.0
Male (46)	674	234	1131	66.5
Male (48)	730	206	1099	–
Male (60)	704	238	1104	66.6
Mean	724	205	1106	69.4
SD	34	28	17	3.3

Table 14.1: The contribution of water and protein to the fat-free weights of six adults. From Garrow (1983).

Table 14.1 presents data on the contribution of water and protein to the fat-free mass of six adult cadavers. The fat-free tissues of the cadavers were of a relatively constant composition, containing about 72% water and about 20% protein; the potassium content was also relatively constant (about 69mmol/kg). In contrast, the amount of fat was very variable (data not shown), ranging from 4.3% to 27.9% of body weight in the six cadavers.

14.2 Total body potassium (TBK)

A constant fraction (0.012%) of potassium exists in the body as the radioactive isotope ^{40}K (half-life = 1.3×10^9y). This isotope emits a high-energy γ-ray of 1.46MeV, allowing the amount of potassium in the body to be estimated by counting with a whole-body γ-spectrometer with sodium iodide detectors (Forbes et al., 1961). The isotope occurs in low concentrations so that the background counts from external radiation (cosmic rays and local sources of ionizing radiation) must be minimized. Hence, the whole-body counter must be shielded from the background radiation with lead, steel, or concrete shielding. Counting times of at least 15min are normally required for adults, with proportionately longer times for children and infants. Such times may be a problem for ill patients. Note that the required equipment is expensive, requires sophisticated technical support: availability is limited.

Calibration of the whole-body counter must be done carefully because the ^{40}K count detected by the whole-body counter is a function of the total body potassium concentration, the geometric configuration of the subject, and internal absorption of the 1.46MeV by the subject. As a result, the counter must be calibrated to allow for differences in the body build of the subjects. Hansen and Allen (1996) achieved this by using a phantom containing a known amount of potassium (as KCl solution) and gender-specific correction factors for weight and height. These authors reported CVs of 1.5% for precision and 4.5% for accuracy for measurements on adults. However, the accuracy of the method has not been confirmed by the analysis of human cadavers.

14.2.1 Application of ^{40}K measurements

~~As~~ Potassium occurs almost exclusively as an intracellular cation, primarily in the muscle and viscera. Negligible amounts occur in extracellular fluid, bone, and other noncellular sites. Measurement of total body potassium can therefore used as a marker for the body cell mass, and as an index of the fat-free mass in healthy subjects, on the assumption that the fat-free mass has a constant proportion of potassium. Body cell mass represents the total mass of cells in the body that consume oxygen and produce work (i.e., the metabolically active, energy-exchanging mass of the body); it is the nonfat cellular portion of tissues, of which the primary components are skeletal muscle, organ tissue mass, blood, and the brain (Wang et al., 2004). The estimates of fat-free mass generated from total body

potassium measurements are accurate and precise at all life stages and in conditions with uncertain hydration status (Naqvi et al., 2018).

Originally ^{40}K measurements were converted from the total body potassium content into the fat-free mass using a value of 69.4mmol K per kg fat-free mass or 2.71g/kg fat-free mass. These values were derived from cadaver analysis. However, a single value for all subjects is now known to be inappropriate and no longer accepted. The potassium concentration of fat-free tissue is a function of age and sex, with both men and women losing on average 5% of their original total body potassium per decade (Figure 14.1). Women have a lower potassium concentration in the fat-free mass than men. Note the reduction in total body potassium with age and the large variation within each age group. The latter emphasizes that total body potassium alone is a poor predictor of fat-free mass unless age- and sex-dependent equations are used (Ribeiro & Kehayias, 2014).

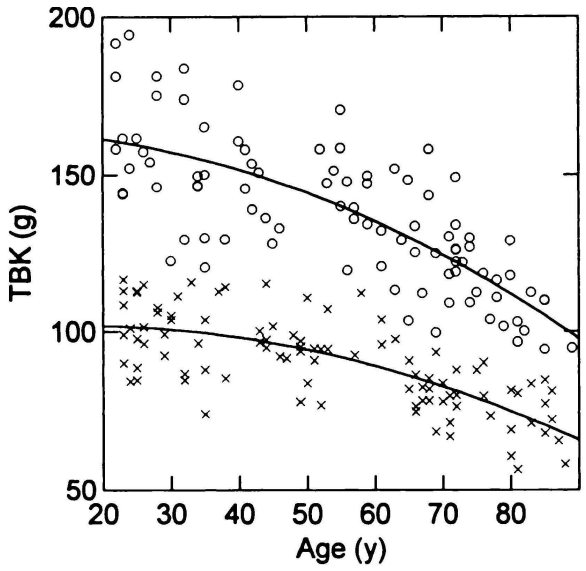

Figure 14.1. Total body potassium (TBK) measurements for females (x) and males (o) as a function of age. From a cross-sectional study with 188 healthy adults. The curves are the best quadratic fit. Modified from Kehayias et al. (1997)

The existence of racial / ethnic differences in total body potassium has also been reported (Ellis et al., 2000). In a recent study, Shypailo and Wong (2020) presented cross-sectional data for total body potassium (in g) measured by whole body counting by sex, race / ethnicity, for six age groups. Black children had higher amounts of total body potassium for certain age groups compared to white and Hispanic children.

The potassium concentration of the fat-free mass also declines with increasing obesity and during pregnancy due to the increased hydration of the fat-free mass. In obese subjects, some of the decrease may also be explained by the lower proportion of muscle in the fat-free mass and by a measurement error resulting from absorption of the γ-rays by adipose tissue (Womersley et al., 1976). During pregnancy, the potassium concentration of the fat-free mass is lower than for non-pregnant women (2.1g/kg vs. 2.3g/kg fat-free mass), but increases in early postpartum (Hopkinson et al., 1997).

Total body potassium may also be altered in clinical patients with a wasting disease such as cancer, when muscle mass is reduced (Cohn et al., 1981). Clearly, constants for the potassium concentration of the fat-free mass must consider age, sex, ethnicity, obesity, and pregnancy, or estimates of fat-free mass derived from ^{40}K measurements will be in error, sometimes by as much as 20%.

The simple 2-component model:

$$\text{Total body fat (kg)} = \text{body weight (kg)} - \text{fat-free mass (kg)}$$

allows the calculation of total body fat (kg) from the fat-free mass (kg). However, any errors and uncertainties in the calculation of the fat-free mass from ^{40}K measurements will be propogated into the derived calculation of total body fat. (Silva et al., 2013). These uncertainties can limit the application of the 2-component model in clinical populations with certain disease states, particularly a wasting disease such as cancer, when total body potassium measurements are low as a result of loss of muscle

mass. Consequently, in these circumstances, use of the 2-component model to derive indirectly total body fat is inappropriate. Instead, more sophisticated body composition models, involving 3- or 4-components, that reduce the need for underlying assumptions, should be used.

14.3 Total body water from isotope dilution

Either the stable isotope deuterium (^{2}H), the radio-active isotope tritium (^{3}H), or the stable isotope of oxygen (^{18}O) can be used to measure total body water. Of the three, deuterium is now most frequently used. Standardized conditions are necessary for the measurement because fluid and food intake and exercise can all affect total body water concentrations. As a result, samples should be taken in the morning, after an overnight fast and the bladder has been emptied, and with a restriction of fluid intake.

A tracer dose of sterile water labeled with an accurately known amount of the isotope (often deuterium oxide, D_2O) is administered either orally or intravenously to the subject and allowed to equilibrate. Two samples of serum, or urine, or saliva are collected; one prior to the administration of the tracer and a second after the isotope has equilibrated with the subject's water pool (usually 3-5h post-dose). The baseline sample measures the naturally present isotope in the total body water. The enrichment observed in the post-dose sample allows calculation of the total body water. The measurement of enrichment appears to be less reliable in urine than in serum probably because of the longer equilibration period of the bladder contents relative to blood.

Ideally, no food or water is permitted during equilibration, which may take 3-5h, depending on the isotope, the physiological sample, and the health condition of the patient. If fluid has been taken during the equilibrium period, a correction can be made, where necessary, to derive actual body water (Gutiérrez-Marín et al., 2019). Longer equilibration periods are necessary for urine compared to blood serum samples, for obese patients (Schoeller et al., 1980), or for those with edema, ascites, and shock (McMurrey et al., 1958). Table 14.2 demonstrates that the method is relatively robust and, independent of the different physiological fluids used. The calculation of total body water is based on the extent to which the isotopic dose is diluted by the total body fluid as shown below:

Samples (A) & (B)	Isotope	n	*Ratio	SD
(A) Saliva at 4h (B) Serum at 4h	^{18}O	33	1.006	0.019
(A) Urine at 6h (B) Serum at 6h	^{18}O	11	1.012	0.027
(A) Urine at 12h (B) Serum at12 h	^{18}O	14	1.006	0.010
(A) Saliva at 3h (B) Saliva at 4h	^{18}O	20	0.997	0.005
(A) Saliva at 3h (B) Saliva at 4h	^{2}H	43	0.996	0.007

Table 14.2: A demonstration of the relatively small variations in total body water (TBW), when calculated from isotopic enrichments of different physiological fluids and at different times postdose.
* Ratio: TBW_A/TBW_B.
From Schoeller et al. (1985).

$$\text{Total body water} = (V \times C) \,/\, (C_2 - C_1)$$

where V = volume of dose, C = concentration of administered isotope, C_1 = baseline concentration of isotope in serum/urine/saliva, and C_2 = concentration of isotope in serum/ urine/saliva sample after equilibration.

A correction may be necessary for urinary loss of the tracer. Details of the isotope dilution methods and the limitations encountered when measuring total body water in the field have been reviewed by Schoeller (1991). and Ellis (2001), respectively.

The isotopic tracer chosen and the method used for analysis are interrelated. Tritium (^{3}H) is easy to measure

with a scintillation counter but involves radiation to the subject, making the technique unsuitable for children and women of childbearing age (Schoeller et al., 1980), and when repeated measurements over a short time period are necessary. The nonradioactive isotope ^{18}O must be measured by mass spectrometry. The stable isotope deuterium is now the tracer of choice because it is much cheaper than ^{18}O and can be measured by infrared absorption, gas chromatography, an isotope ratio mass spectrometer, and more recently by Fourier transform infrared spectrophotometry (FTIR). Use of FTIR for deuterium (^{2}H) analysis is less expensive and quicker, although slightly less precise than the use of an isotope ratio mass spectrometer. Saliva is preferred to urine as the chosen body fluid when using FTIR for the measurement because urine may cloud the optical lens of the FTIR (Owino et al., 2017). For a comprehensive description of the use of FTIR for the analysis of deuterium in saliva, see:

https://nucleus.iaea.org/HHW/Nutrition/BodyComposition/RefsBodyComp/index. html#publ

Recently, an alternative FTIR spectroscopic method (ATR FTIR) has been developed which requires a smaller volume of plasma (10µl), and has shorter analyses times, making it suitable for use in pediatrics; for more details, see Ward (2021).

14.3.1 Application of body water measurements by isotope dilution

The measurement of total body water in both healthy and diseased persons is the most important application of isotope dilution techniques. All body water is present in the fat-free mass. Hence, total body water measurements can be used to estimate the fat-free mass. This estimation requires an assumed value for fat-free mass hydration, as shown below:

$$\text{Fat-free mass (kg)} = (\text{total body water (kg)}) \ / \ \text{hFFM}$$

where hFFM = hydration of the fat-free mass.

Based on the 2-component model, once the fat-free mass has been determined, total body fat (TBF) and percentage of body fat can be calculated as shown below:

$$\text{Total body fat (kg)} = \text{body weight (kg)} - \text{fat-free mass (kg)}$$

$$\%\ \text{body fat} = (\text{Total body fat (kg)} \times 100\%) \ / \ \text{body weight (kg)}$$

Age	Males Hydration (%)	Females Hydration (%)
5y	76.5	76.7
7y	76.1	75.5
9y	75.7	75.1
11y	75.3	75.0
13y	75.0	74.6
15y	74.4	74.1
17y	73.7	73.7
19y	73.4	73.6

Table 14.3: Median values for hydration by sex. Data from Wells et al. (2010) who also present data for intervening years,

In healthy adults, hFFM is assumed to be 0.732. However, use of a single value for the hydration of fat-free mass may lead to errors in derived values for the fat-free mass. For example, hFFM is known to vary during hormonal cycles in women and in certain disease states owing to under-hydration. Alternatively, over-hydration due to edema, may arise among children with severe acute malnutrition (Gutiérrez-Marín et al., 2019; Most et al., 2018). The hydration of fat-free mass is also known to change during growth and maturation. At birth hFFM is about 80%, after which it gradually decreases until reaching the adult value (Schoeller, 1989). The reference values for hFFM by age and sex in children aged 5-20y compiled by Wells et al. (2010) are shown in Table 14.3, and are similar to earlier values

reported for males but about 2% lower for females in mid-childhood (Lohman, 1989).

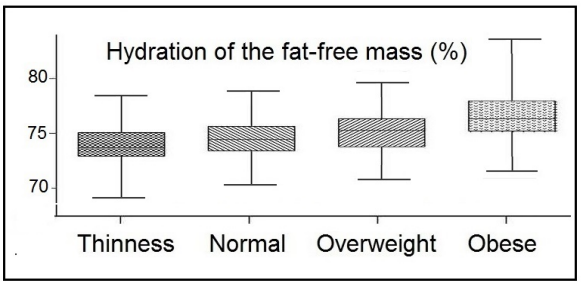

Figure 14.2 Values for hydration and density of fat-free mass based on the four-compartmental model and stratified by nutritional status grouped by BMI SD score for UK subjects aged 4-22y. Redrawn and abbreviated from Gutierrez-Marin et al. (2019).

Hydration values for the fat-free mass are also affected by obesity, the increase said to be due to an expansion of extracellular water, although other mechanisms may be involved (Leone et al., 2000); see Chumlea et al. (2007) for more details. Note the marked increase in hFFM (as %) in heavier BMI groups shown in Figure 14.2 based on a large study of subjects from 4-22y (Gutiérrez-Marín et al., 2019). This same trend has been reported previously in smaller studies of obese children (Haroun et al., 2005; (Haroun et al., 2005; Wells & Fewtrell, 2006) and adults (Waki et al., 1991), and highlights that reference data for hydration values for higher BMI groups are also needed to avoid bias.

Pregnancy is also associated with an increase in hFFM, as noted in Section 14.2. Published values for hFFM at specific time points in pregnancy are shown in Figure 14.3.

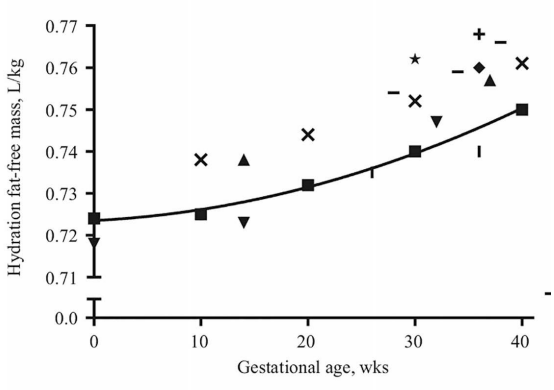

Figure 14.3 Published values for FFM hydration and density throughout pregnancy in published studies. The exponential regression line is based only on the data by van Raaij et al. (1988). Redrawn from Most et al. (2018) who also provide information on the sources of the individual data points.

The equations of van Raaij et al. (1988), based on ten-week intervals throughout gestation, are those most used in 2-component models to estimate maternal fat mass during pregnancy. A regression equation based on the van Raaij et al. (1988) data has been developed allowing estimation of hFMM for any given time throughout gestation; this regression line is also shown in Figure 14.3.

Author	Hydration constant	FFM (kg)	FM (kg)	FM (%)
Siri (1961)	0.724	62.2	13.8	18.2
Van Raaj et al. (1988)	0.740	60.8	15.2	20.0
Fidanza (1987).	0.752	59.8	16.2	21.3
Catalano et al. (1995)	0.762	59.1	16.9	22.3

Table 14.4: Effect of adopting different hydration constants on fat mas (FM) and fat-free mass (FFM) calculated at 30 weeks gestation on women of normal weight (76kg) and total body water (45.0L). Data from Most et al. (2018).

Table 14.4 highlights the effect of adopting different hydration constants on the assessment of fat-free mass and fat mass in a women at 30wks gestation with a normal pre-gravid BMI (i.e., 24.8kg/m^2). Clearly, failure to consider the increase in hFFM in models based on total body water using isotope dilution would result in an overestimate of the actual fat-free mass in pregnant women, and thus underestimate fat mass when the 2-component model is applied.

Recently, reference charts for infants and children aged from 6wks to 5y by sex have been developed for percentiles for total body water measured by deuterium dilution, and fat-free mass calculated

from total body water using published hydration coefficients (Wells et al., 2020). These charts will help clinicians identify how specific diseases and their treatment impact on fat-free mass and fat mass in this age group.

The error associated with the measurement of total body water using these isotopic tracers is typically less than 1kg. This error equates to an uncertainty of about 10% (about 1.4kg) in the absolute fat mass of an average healthy adult or 2% in the estimate of percentage of fat. These relatively low errors have resulted in the isotope dilution method becoming the reference or gold-standard method in comparison with other measurement procedures for total body water (e.g., bioelectrical impedance: Section 14.8).

A 3-component model that partitions body weight into three major components can also be used to measure fat-free mass and fat mass:

$$\text{Body mass} = \text{fat} + \text{water} + \text{residual}$$

where residual (i.e., fat-free dry mass) is the sum of protein, bone mineral, and glycogen

This 3-component model partitions the body mass into fat, total body water, and the remaining fat-free dry mass, which is assumed to have a constant ratio of protein to mineral. This 3-component model avoids the assumption that the water content of fat-free mass is the same for all individuals of a given age and sex. Instead, the model provides an estimate of the hydration and density of fat-free mass. Three measurements are made: body weight (in kg), body volume (in liters), and total body water (in liters). Fat mass can be calculated from these three basic measurements by applying the 3-component model of Siri (1961) which includes both whole body density and total body water; see Wells et al. (1999) and Fuller et al. (1992) for further details. This version of the 3-component model provides improved practical estimates of both the fat mass and fat-free mass in children (Silva et al., 2013).

14.4 Multiple dilution methods

Multiple dilution can be used to estimate the volume of various body fluid compartments that, in turn, can be used to estimate two components of the fat-free mass: extracellular mass (ECM) and the body-cell mass (BCM). There is no way to quantify BCM directly, but it can be estimated after measurement of total body nitrogen by neutron activation analysis (Section 14.5) or total body potassium using ^{40}K (Section 14.2). However, neutron activation analysis exposes the subject to radiation, and both methods require a high degree of technical expertise. Alternatively, multiple dilution techniques can be used to estimate BCM.

Typically, multiple dilution involves determining both total body water (via isotope dilution; Section 14.3) and extracellular water (ECW), the latter using a tracer such as bromide that does not enter the intracellular space (Wong et al., 1989). The difference between these two measurements (i.e., TBW − ECW) reflects the intracellular water (ICW), which is more metabolically active than ECW and provides an estimate of BCM (Ribeiro & Kehayias, 2014).

To estimate BCM using this approach, a pre-dose blood or urine sample is taken followed by dosing with deuterium oxide and sodium bromide solution. After a 3-5h equilibration period, a final post-dose blood or urine sample is collected, although longer equilibration periods are needed for indi-

viduals with expanded ECW, including those with extreme obesity. Deuterium enrichment of the biological sample is described in Section 14.3, whereas bromide enrichment is determined by high-performance liquid chromatography or non-destructive liquid X-ray fluorescence. The reported precision for ICW calculated using this approach is about 2.5% provided standardized protocols are used (Earthman, 2015). The ratio of ECW/TBW can also calculated using this approach. In a study of nursing home elderly, the ratio ECW/TBW was significantly higher compared to the ratio in free-living elderly, prompting the suggestion that this ratio may have potential as a surrogate method for the clinical assessment of frailty (Kehayias et al., 2012).

14.4.1 Application of multiple dilution methods

The ECM is defined as the component of the fat-free mass which exists outside the cells. It consists of both fluid (e.g., extracellular fluids, plasma volume) and solid (e.g., skeleton, cartilage, tendons) components which are involved in transport and support and are not metabolically active.

In contrast, the BCM is the total mass of cells in the body that consume oxygen and produce work (i.e., the metabolically active, energy-exchanging mass of the body). These components are the nonfat cellular portion of tissues, primary the skeletal muscle, organ tissue mass, blood, and the brain (Wang et al., 2004). Nutritional status, physical activity level, and disease states alter BCM, which thus serves as a helpful biomarker.

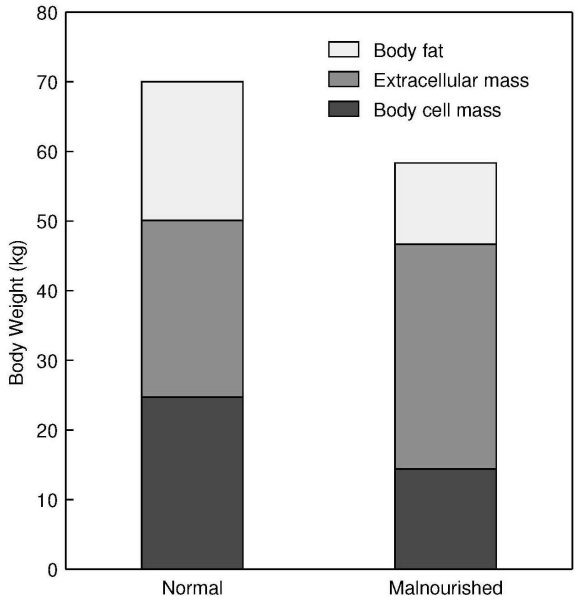

Figure 14.4. The mean body composition of 25 normally nourished healthy volunteers and 75 malnourished patients. From Shizgal (1981).

Measurements of BCM and ECM are especially critical in malnourished patients. Values for total fat-free mass in these patients may remain unchanged, but the composition of the fat-free mass is abnormal, with a reduced BCM, concomitant with an expansion of the ECM, as shown in Figure 14.4. Hence, any loss in body weight in such patients reflects a loss of body fat (Shizgal, 1987).

14.5 *In-vivo* activation analysis

A group of related techniques involving *in-vivo* neutron activation analysis (NAA) allow the direct estimation of the amount of a range of chemical elements in the living human body. Most other techniques used in body composition studies, with the exception of whole body counting for potassium, generate data on tissue density or volume, but not data on the amount of a component. As a result, multicomponent elemental models based on *in vivo* NAA have gradually become accepted as reference methods for the calibration of many of the other techniques described in this chapter.

Nearly all the major elements present in the body can be analyzed by *in vivo* NAA, including hydrogen, oxygen, carbon, nitrogen, calcium, phosphorus, sodium, and chlorine (Cohn et al., 1984). Of special interest is the application of NAA to measure the carbon-to-oxygen (C/O) ratio *in vivo*. This ratio can provide an independent, unbiased measure of the distribution of fat and fat-free mass, which is not

dependent on the assumptions about the composition of fat-free mass. Small changes in fat-free mass can be monitored, making the method appropriate for studying the depletion of fat-free mass with aging (Kehayias et al., 2000).

A major negative factor associated with *in vivo* NAA is that the subject is exposed to radiation. This and the associated risks must always be explained to the subject. *In vivo* NAA is most used in clinical medicine for the determination of total body nitrogen (TBN), total body calcium (TBCa), and bone mass as discussed below. TBN and TBCa are only determined in a relatively small number of laboratories worldwide — an indication of the expense involved and technical difficulties associated with the method.

14.5.1 Total body nitrogen by *in vivo* NAA

Nitrogen is normally determined by NAA by bombarding the patient, in a supine position, with a low neutron flux from a ^{238}PuBe source or from a cyclotron or neutron generator. During irradiation, a proportion of ^{14}N is converted to an excited state of ^{15}N, which decays almost immediately to its ground state, emitting a "prompt" γ-ray at 10.83MeV. This activity is counted by an array of sodium iodide detectors in a whole body counter (Figure 14.5). The detected γ-ray counts are proportional to the absolute mass of total body nitrogen (Beddoe & Hill, 1985). Measurement of the nitrogen content of the body gives a measure of total body protein because the mass of nitrogen bears a fixed ratio to the mass of protein (1g N = 6.25g protein). The radiation exposure using this method is about 0.3mSv. This compares with the national background radiation from cosmic and other sources of about 3.5mSv/y.

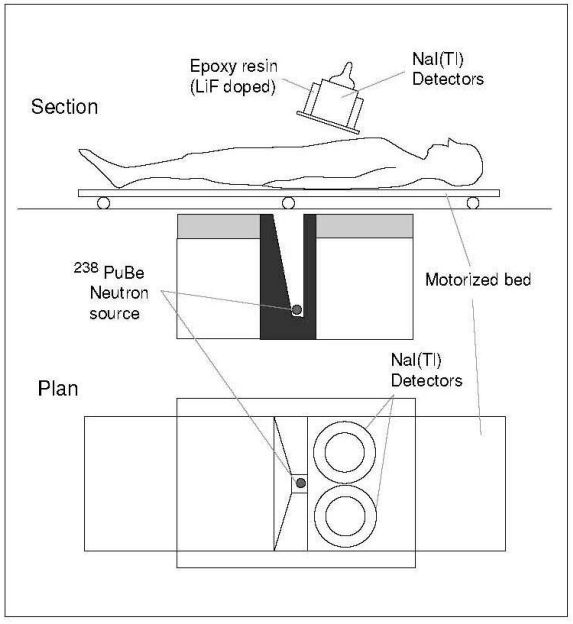

Figure 14.5. *In vivo* γ-neutron activation. Redrawn from Cohn et al. (1981b).

The 10.83MeV γ-ray is specific to nitrogen and is at an energy which is not affected by interference from other reactions. Extensive shielding, however, is necessary around the sodium iodide detectors to reduce the level of background radiation. Nevertheless, corrections to the γ-count must still be made for the background.

The calibration of the counter is critical and must consider the height, weight, and adiposity of the subject because of the varying neutron attenuation and internal absorption of the emitted γ-rays. Calibration is achieved using phantoms or by using hydrogen as an internal standard (Vartsky et al., 1979).

The accuracy of prompt γ-neutron activation for measuring total body nitrogen has been validated by comparing total body nitrogen in two human cadavers with results obtained by direct chemical analysis of nitrogen (Knight et al., 1986). Close agreement between the two techniques was found. This study also confirmed the use of the ratio 6.25 for the relationship between total body protein and total body nitrogen.

14.5.2 Application of body nitrogen by *in vivo* NAA

Changes in total body protein of hospital patients with diseases such as cancer, renal dysfunction, hypertension, chronic heart disease, and rheumatoid arthritis, and with severe trauma or sepsis have been studied using prompt γ-neutron activation (Beddoe & Hill, 1985). The results indicate that substantial losses of body protein may occur, even when conventionally adequate nutritional support has been provided for some of these patients. Depleted total body protein is also a consistent finding among acutely ill anorexic patients. In a longitudinal study, Haas et al. (2018) reported that after recovering from anorexia nervosa, depletion of body protein measured by *in vivo* INAA remained in adolescent patients after 7mos, even though body weight was restored. Clearly, further work is required to identify nutritional intervention procedures which minimize loss of body protein in hospital patients and those with anorexia nervosa.

14.5.3 Total body calcium by *in vivo* NAA

The method is based on the conversion of a proportion of the naturally occurring isotope ^{48}Ca in the body to ^{49}Ca (half-life = 8.8min) by exposing the patient to a low neutron flux. Immediately following irradiation, the patient is transferred to a whole-body counter, and the γ-rays emitted by the decay of ^{49}Ca are detected by an array of sodium iodide detectors.

The counting geometry is similar to that used for total body potassium, and again care must be taken in positioning the subject and correctly accounting for the varying height, weight, sex, and body mass index of subjects. In particular, for subjects with BMI > 30, the effects of neutron attenuation become significant, necessitating additional corrections or a special calibration (Ma et al., 2000). The reported accuracy and precision are from 1-2% (Cohn et al., 1974).

14.5.4 Application of total body calcium by *in vivo* NAA

The radiation exposure using this method varies from 2.5-25mSv, depending on the neutron source. This range is considerably higher than that experienced during dual X-ray absorptiometry (DXA) and limits the general applicability of the method. Consequently, the use of neutron activation analyses for the assessment of total body calcium has largely been replaced by DXA (Section 14.11). Instead, the method is now mainly used as a calibration tool for other techniques.

The assessment of total body calcium by *in-vivo* neutron activation analysis is also discussed in Chapter 23 under the assessment of calcium status.

14.6 Densitometry

Body density was one of the first measures of body composition to be made (Behnke et al., 1942). It is relatively easy to measure, and in the past, body density was the gold-standard method to determine percentage body fat using the 2-component model. Certain disease states characterized by excess fluid retention and under-mineralization decrease the density of fat-free mass. Consequently, densitometry is now often combined with other measures in a 4-component model of body composition so that the actual bone mineral content and hydration and density of fat-free mass are measured instead of applying assumed constants, thus providing a more accurate assessment (Silva er al., 2013).

Hydrostatic weighing was the initial densitometric method used to determine body volume, and hence whole body density. However, it is now being replaced by plethysmographic methods that are

more acceptable to subjects, particularly children (Fields & Goran, 2000; Wells & Fewtrell, 2006). All three methods are described below. A concluding section describes the calculation of body fat from body density (Section 14.6.4).

14.6.1 Hydrostatic weighing

The conventional method of directly measuring whole body density involves weighing the subject and then using Archimedes' principle to determine the volume of the subject. Thus, the subject is weighed first in air and then when completely submerged in water in a large tank. The subject is instructed to squeeze out any air bubbles trapped inside the bathing suit, and to expel as much air as possible from the lungs before immersion. The hydrostatic weight is recorded at the end of the forced expiration. Multiple readings should be taken using a continuous and sensitive recording of underwater mass, the heaviest corresponding to the most complete expiration. This method requires a high degree of water confidence and thus the method is not suitable for children younger than 8y, the elderly, obese, or unhealthy persons.

The body volume is then calculated from the apparent loss of weight in water (i.e., the difference between the weight of the person in air and his or her corresponding weight in water). Once total body mass and body volume have been determined, whole body density can be readily calculated, on the basis that density is mass per unit volume and the density of water is l.0kg/L at 4°C:

$$\text{Whole body density} = (\text{body weight in air (kg)}) \,/\, (\text{apparent loss in weight (kg)})$$

Three corrections must be applied:

- Hydrostatic weighing is usually performed in water at 30°C instead of 4°C. At this higher temperature, the density of water is 0.9957kg/L, and a water temperature correction factor must be applied.
- Air trapped in the lungs also contributes to the amount of water displaced by the subject under water. Residual air in the lungs can be measured while the subject is in the tank. Alternatively, the volume can be estimated using a nitrogen washout, helium dilution, or oxygen dilution (Brodie, 1988). This correction can also be estimated from empirical equations. The residual air volume is then subtracted from the body volume.
- The volume of the air trapped in the gastrointestinal tract also contributes to the total amount of water displaced. This volume is small and is never measured. It is often taken as 100mL.

The within-subject variability in gastrointestinal gas volume, however, can be quite large (0-500mL in adults), reducing the precision of the method. Even when the above corrections are carefully applied, considerable uncertainty remains. If the whole body density is used to calculate the percentage of body fat (see below), these uncertainties become errors in the percentage of body fat. The errors may be systematic rather than random in nature and may be large when the residual air volume is estimated. If the residual air volume is in error by 300-500mL, there will be a corresponding uncertainty in the percentage of body fat from 3-5%.

Hydrostatic weighing can give very reproducible results for whole body density, provided that the examiners and the subjects are well trained. For example, Durnin and Rahaman (1967) reported a

standard deviation of 0.008kg/L for serial measurements on three subjects over a 1y period.

14.6.2 Water-displacement plethysmography

The use of a plethysmograph eliminates the necessity for totally immersing the subject in water, a disadvantage of hydrostatic weighing (Section 14.6.1). For the measurement, the plethysmograph is zeroed and filled with water (Figure 14.6). The subject is then weighed, and a weight of water equal to the weight of the subject is removed from the plethysmograph. The subject then stands with water up to the neck only, and the head is covered by a clear-plastic dome. The volume of air surrounding the head of the subject, and in the lungs and gut, is then determined by measuring the pressure changes produced by a pump of known stroke volume (Garrow et al., 1979). This allows the total volume of the subject to be determined. The total time for the test, including three measurements on each subject, is about 20min.

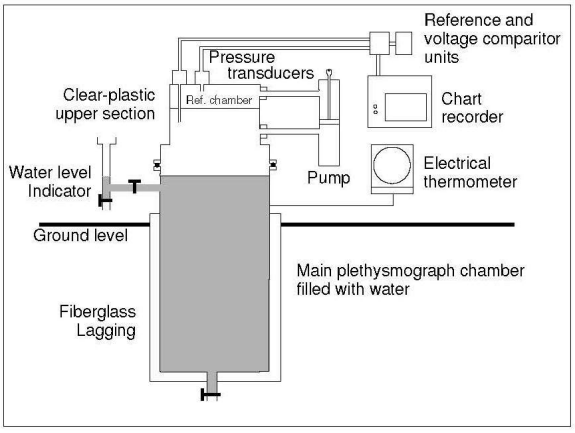

Figure 14.6. Measurement of body density using a plethysmograph. From Garrow et al. (1979)

The method has been used successfully to measure body density of obese adults (Garrow et al., 1979). Estimates of body fatness obtained compared favorably with those based on total body potassium.

Water-displacement plethysmography has now been replaced by air-displacement plethysmography (discussed below) for the measurement of total body volume. Nevertheless, water-displacement plethysmography has been adapted to measure leg volume, and is used by clinicians to study chronic venous insufficiency (CVI). For a review of the clinical use of water displacement leg volumetry and the potential errors that may occur, see Rabe et al. (2010).

14.6.3 Air-displacement plethysmography

The measurement of body volume has been significantly eased by the development of an air-displacement plethysmography device (Bod Pod). These devices determine the volume of a subject indirectly by measuring the volume of air displaced by the subject inside an enclosed chamber — plethysmograph or Bod Pod. The method is quick, comfortable, automated, non-invasive, safe, and does not require extensive technical training. For infants up to about 6mos of age, a device known as the “Pea Pod” is available and can accommodate infants up to about 8kg. For small children from 2-6y, the Bod Pod with a Pediatric Option can be used, whereas the standard Bod Pod can be used for older children (i.e., > 6y), adults, and the elderly.

The air-displacement plethysmograph consists of an ovoid fiberglass structure divided into two sections: a rear reference chamber and a front test chamber containing the seated subject. (Figure 14.7). The dividing wall between the two chambers encompasses a large diaphragm. This can be made to oscillate under computer control, generating complementary pressure changes in the test and reference chambers which are recorded. These changes and the application of the basic gas laws

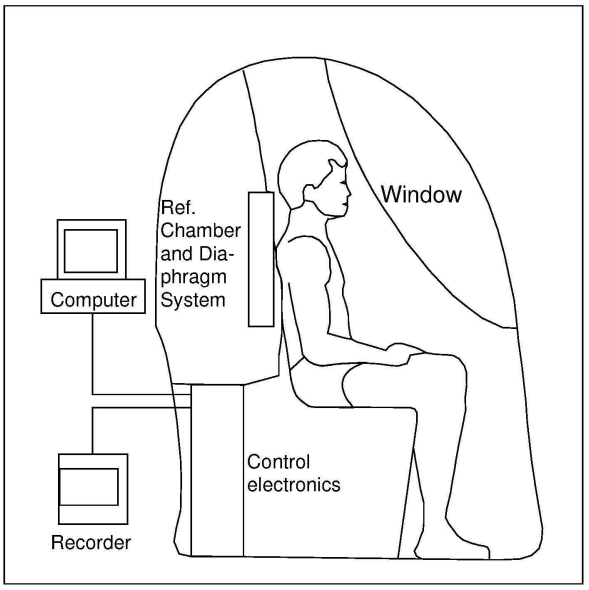

Figure 14.7. Measurement of body volume using an air-displacement plethysmograph. Modified from Dempster & Aitkens (1995).

allows the volume of the test chamber to be calculated. Measurements both with and without a seated subject provide information on the total body volume of the subject.

While they are seated in the test chamber, subjects should wear minimal clothing, often a swimsuit and a tightly fitting bathing cap to minimize the volume of air contained near the hair or skin, and in clothing. Measurements over a 1-min period usually suffice, and an average of two separate trials should be used to calculate body volume. For details on the methods and performance of the PEA POD system for measuring body composition in infants, see Ellis et al. (2007); for moderately premature infants, see Forsum et al. (2016), and for children aged 2-6y, see Fields et al. (2012).

A correction for the average volume of air in the lungs and thorax during normal breathing (VTG) should be applied. This can be measured while the subject is in the test chamber (Dempster & Aitkens, 1995), or predicted based on age, sex, and height (McCrory et al., 1998). Pregnancy must also be considered because thoracic gas volume declines throughout pregnancy by about 100mL per trimester. Hence, VTG needs to be adjusted for the trimester-specific decline in lung volume (Most et al., 2018). Once body volume has been measured, whole body density (D) can be calculated from measured body mass.

For a comparison of the components and capabilities of the three plethysmography techniques, see Fields et al. (2015).

14.6.4 Application of densitometry to calculate body fat

Once whole-body density has been measured by one of the methods outlined above, the percentage body fat can be calculated. This involves the selection of an empirical densitometric equation relating fat content to whole body density (D). Several empirical densitometric equations have been derived based on the classic two-component model for body composition in which body weight is divided into fat and fat-free mass, and relying on assumptions that ignore inter-individual variability in the composition of the fat-free mass.

All the classical densitometric equations shown below assume:

- The density of the fat-free mass is relatively constant.
- The density of fat for normal persons does not vary among individuals.
- The water content of the fat-free mass is constant.
- The proportion of bone mineral (i.e., skeleton) to muscle in the fat-free body is constant.

The different authors all assume the density of fat and the fat-free mass do not change with age and sex. There are two constants in each equation, C_1 and C_2, the different authors suggesting slightly different values for each:

$$\%\mathrm{F} = ((4.950/\mathrm{D}) - 4.500) \times 100\% \ (\mathrm{Eq.A})$$
$$\%\mathrm{F} = ((4.570^*/\mathrm{D}) - 4.142) \times 100\% \ (\mathrm{Eq.B})$$
$$\%\mathrm{F} = ((5.548^*/\mathrm{D}) - 5.044) \times 100\% \ (\mathrm{Eq.C})$$

The Siri (1961) equation (Eq.A) is most widely used and assumes that the densities of fat and fat-free mass are about 0.90 and 1.10kg/L respectively. Brožek et al. (1963) (Eq.B) and Rathbun and Pace (1945) (Eq.C) used the concept of a reference man of a specified density and composition; these equations avoid the requirement of estimat;ing the density of fat-free mass. The constants applied in all these equations came from the chemical analysis of a few adult cadavers dissections, animal data, and indirect estimates of fat-free mass in human subjects (Heymsfield et al., 1991; Silva et al., 2013). None of these classical empirical equations relating fat content to body density, however, are suitable for adult patients in clinical settings when the composition of their fat-free mass may be abnormal. This will include patients undergoing hyperalimentation with high-sodium fluids, or with congestive heart failure or liver disease, as total body water content as a fraction of fat-free mass may be markedly higher in these patients, thus violating the assumption that the water content of the fat-free mass is a constant (i.e., 73.2%). In these patients, the density of fat-free mass is decreased.

Not surprisingly, in patients with diseases associated with under-mineralization, the density of fat free mass is also decreased (Werdein & Kyle, 1960). Consequently, in all these patients, fatness will be overestimated when the 2-component model is applied (Wells & Fewtrell, 2006). More recent research has raised concerns over the assumption of constant properties for hydration and density of fat-free mass when these classical empirical equations are applied to assess body composition not only in patients with certain diseases, but also in healthy children and adolescents, the elderly, pregnant women and those with obesity, as noted earlier.

Although fat has relatively uniform properties throughout the life course (zero water and a density of 0.9007kg/L), in contrast fat-free mass has different properties in children compared to adults. This arises because of chemical maturation of the fat-free mass during growth which results in higher levels of water and lower levels of mineral and proteins, and thus changes in the hydration and density of the fat-free mass (Wells et al., 2010), as noted earlier. Nevertheless, the adult-derived values for the density and hydration of fat-free mass and applied in the classical equations shown above have often been used to study body composition in children (Silva et al., 2013).

In an effort to improve the accuracy in the estimates of percentage body fat based on pediatric densitometry (and hydrometry, Section 14.3), Wells and co-workers measured body composition via a 4-component model in a large, healthy sample of children and adolescents aged 4-23y. Use of this model overcomes the limitations associated with the assumptions of constant properties for hydration and density of fat-free mass applied in the Siri equation. Their results confirmed that the previously published adult values for the density and hydration of fat-free mass when applied to pediatric populations were inappropriate. They developed new empirical age- and sex-specific reference values for the hydration and density of fat-free

	Males				Females			
Age	**Hydration (%)**	**Density (kg/L)**	**C_1**	**C_2**	**Hydration (%)**	**Density (kg/L)**	**C_1**	**C_2**
5y	76.5	1.0827	5.36	4.95	76.7	1.0837	5.33	4.92

	Males				Females			
Age	Hydration (%)	Density (kg/L)	C_1	C_2	Hydration (%)	Density (kg/L)	C_1	C_2
6y	76.3	1.0844	5.32	4.90	76.1	1.0865	5.27	4.85
7y	76.1	1.0861	5.28	4.86	75.5	1.0887	5.22	4.79
8y	75.9	1.0877	5.24	4.82	75.2	1.0900	5.19	4.76
9y	75.7	1.0889	5.21	4.79	75.1	1.0909	5.17	4.74
10y	75.5	1.0900	5.19	4.76	75.0	1.0916	5.15	4.72
11y	75.3	1.0911	5.16	4.73	75.0	1.0924	5.13	4.70
12y	75.2	1.0917	5.15	4.72	74.9	1.0937	5.10	4.67
13y	75.0	1.0920	5.14	4.71	74.6	1.0954	5.07	4.63
14y	74.8	1.0927	5.13	4.69	74.4	1.0975	5.02	4.58
15y	74.4	1.0942	5.09	4.66	74.1	1.0996	4.98	4.53
16y	74.0	1.0960	5.05	4.61	73.8	1.1011	4.95	4.49
17y	73.7	1.0978	5.02	4.57	73.7	1.1020	4.93	4.47
18y	73.5	1.0991	4.99	4.54	73.6	1.1027	4.92	4.46
19y	73.4	1.1000	4.97	4.52	73.6	1.1031	4.91	4.45
20y	73.3	1.1006	4.96	4.51	73.6	1.1035	4.90	4.44

Table 14.5: Median values for hydration, density, and constants (C_1 and C_2) for the pediatric version of the equation of Siri (1961), obtained by using the LMS (lambda, mu, sigma) method. Data from Wells et al. (2010).

mass; these are shown in Table 14.5. In addition, they developed prediction equations of density and hydration for fat-free mass by age, sex, and body mass index (BMI) SD score; see Wells et al. (2010) for more details.

Note that the values for the C_1 and C_2 constants for the adult males (age 20y) shown in Table 14.5. are similar to the corresponding values shown in the Siri equation above (i.e., 4.95 for C_1 and 4.50 for C_2), whereas for adult females, the corresponding values are slightly lower: 4.90 for C_1 and 4.44 for C_2 at 20y. With the substitution of the age- and sex-specific C_1 and C_2 constants from Table 14.5 in place of the C_1 (4.95) and C_2 (4.50) constants in the Siri equation, the accuracy of the 2- component model for estimat;ing fat mass of a healthy pediatric population based on densitometry could be improved.

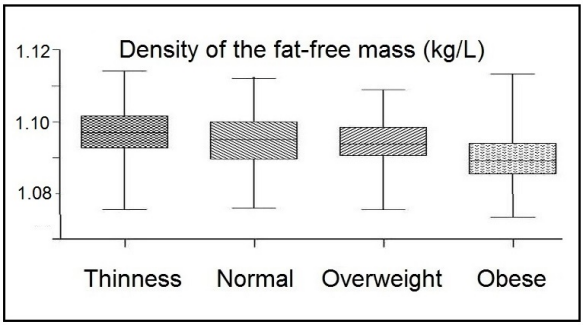

Figure 14.8. Distribution of values for density of fat-free mass based on the 4-component model and stratified by nutritional status grouped by BMI SD score for UK subjects 4-22y. Redrawn and abbreviated from Gutierrez-Marin et al. (2019).

More recent research has highlighted increasing values for hydration of fat-free mass (see Section 14.3 and Figure 14.3) but decreasing values for the density of fat-free mass in the children with a heavier body mass index (BMI) (Figure 14.8). This trend was observed earlier in children (Haroun et al., 2005) and adults (Waki et al., 1991). Gutierrez-Marin et al. (2021) have developed a predictive equation to estimate the density of fat-free mass for use when using a 2-component model to assess children and adolescents with different degrees of obesity. Application of the equation shown below improves the accuracy and precision of the density of the fat-free mass (DFFM) estimates in this population.

$$\mathrm{DFFM} = 1.0791 + (0.009 \times \mathrm{age} + 0.0021 \times \mathrm{gender}) - (0.0014 \times \mathrm{BMISDS})$$

Where age is in years; gender 1 (male), 2 (female); DFFM = density fat-free mass; BMISDS = body mass index Z-score

An excel file with all the steps in the calculation is available online as supplementary material in Gutierrez-Marin et al. (2021).

Similar trends in the values for the hydration and density of fat-free mass have been observed in studies of older Hispanic Americans (González-Arellanes et al., 2019) and obese Mexicans (González-Arellanes et al., 2021) all aged > 60y. Again values for fat-free mass density were lower, but higher for the hydration fraction suggesting that modifying the assumptions regarding both the density and hydration values for fat-free mass applied in the classical densitometric empirical equations may also be appropriate for the elderly and in conditions of obesity. Once percentage body fat has been calculated, then total body fat (TBF) and/or fat-free mass can be derived as follows:

$$\text{Total body fat (kg)} = \text{body wt (kg)} \times \%\ \text{body fat} \div 100$$
$$\text{Fat-free mass (kg)} = \text{body wt (kg)} - \text{total body fat (kg)}.$$

14.7 Total body electrical conductivity

Total body electrical conductivity (TOBEC) is measured by observing the changes induced by placing the subject in an electromagnetic field (Baumgartner, 1996). The extent of the change depends on the overall electrical conductivity of the body and, in particular, on the proportions of fat and the fat-free mass in the body: the fat-free mass, comprised largely of water with dissolved electrolytes, will readily conduct an applied electric current, whereas fat is anhydrous and a poor conductor. In more modern equipment, the subject lies supine on a motorized bed (Figure 14.9) that is passed in a series of steps progressively through a uniform solenoid coil. A 2.5MHz oscillating radiofrequency current is passed through the coil. This induces an electromagnetic field in the space enclosed by the coil, which, in turn, induces a current in the subject, the magnitude depending on the conductivity of the subject. A second reading is taken when the coil is empty; the difference represents the measurement. The use of Fourier analysis improves the assessment of the lean body mass (Van Loan & Koehler, 1990).

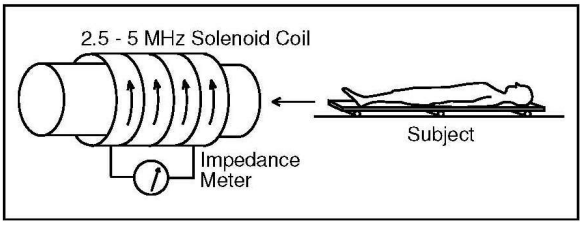

Figure 14.9. Measurement of body composition by total body electrical conductivity. Modified from van Itallie et al. (1985).

Measurement of TOBEC is simple, safe, noninvasive, fast, and causes no discomfort to the subjects. The technique can be used on individuals who cannot be weighed underwater. However, the instrument is expensive.

Careful consideration must be given to the positioning of the subject and corrections for body geometry and length applied. Treuth et al. (2001) used a variety of techniques, including TOBEC, to determine body composition in 8y prepubertal girls. The authors concluded that the determinations are highly method dependent and that results from all six methods studied (TOBEC, total body potassium, isotope dilution for total body water, bioelectrical impedance, anthropometry, and DXA) are not interchangeable. The results highlight how TOBEC measurements can be evaluated by comparison with other methods. Unfortunately, most of the other methods are also subject to error and bias.

14.7.1 Applications of total body electrical conductivity

To interpret TOBEC measurements in terms of the quantity of fat-free mass in the body, a calibration equation is applied generated by measuring the fat-free mass of a reference population using an alternative technique and relating this to the TOBEC value of each individual. A calibration equation has also been developed to measure fat-free mass and fat reliably and precisely in neonates through 1y of age (Fiorotto & Klish, 1991; Fiorotto et al., 1995).

TOBEC is relatively insensitive to shifts of fluid or electrolyte between the intracellular and the extracellular compartments and to variations in bone mineralization. For example, in a study of middle aged and elderly subjects (35-90y), higher values for fat free mass were reported when predicted from measurements based on TOBEC compared to those for fat-free mass predicted by using densitometry or hydrometry (Van Loan & Koehler, 1990). These findings led to the suggestion that the higher fat-free mass values arise because the TOBEC signal is unaffected by decreases in bone mineralization.

14.8 Bioelectrical impedance

The use of bioelectrical impedance (BIA) for the assessment of body composition is widespread, in large part due to the affordability, portability, and ease of use of the bioimpedance devices. None of the BIA devices measure body composition directly. They measure an electrical response of the body, resistance, when exposed to an electrical current. The resistance measured is transformed into a prediction of total body water by an algorithm, and from that the body fat mass is determined.

Three categories of BIA devices are available commercially: single-frequency (SF-BIA), multiple-frequency (MF-BIA), and bioimpedance spectroscopy (BIS); these are described below together with some new developments in BIA. Both the SF-BIA and MF-BIA devices rely upon population-specific prediction equations. In contrast, BIS uses biophysical modeling to estimate body compartments. All the algorithms are based on or validated against other body composition reference methods, which are not totally accurate and error-free. Each BIA device works with an inbuilt algorithm specific to the device and for the population for which the algorithm was developed so it is not possible to compare studies unless the same combination of device / equation / population is used (Ward, 2019; Sheean et al., 2020). Furthermore, the reference population on which the BIA algorithm was based must be appropriate for the target subject being measured (Lemos & Gallagher, 2017).

Many factors may influence the precision and accuracy of BIA techniques. They include factors associated both with the individual (e.g., degree of adiposity, fluid and electrolyte status, skin temperature) and with the environment (ambient temperature, proximity to metal surfaces and electronic devices), the assumptions underlying prediction or modeling equations, instrumentation, and variations in the protocols used for the BIA measurements. Box 14.2 presents recommendations for optimizing whole body BIA measurements.

Box 14.2. Recommendations for optimizing whole body BIA measurements in an adult. From Earthman (2015).

Preparing for the measurement

- **Food/beverage and activity.** Individual should fast (nil per os except water) and avoid alcohol, caffeine, and exercise at least 8 hours prior to measurement in the morning (research settings); shorter time frames and

other times of day may be acceptable in the clinical setting—note time of day for consistency in follow-up measures.

- **Void bladder**. Individual should void bladder prior to measurement.
- **Clean skin surface**. Clean skin surface well with alcohol; individual should not use lotion or oils on the skin prior to measurement; avoid placing electrodes on broken skin.
- **Device calibration**. Calibrate the bioimpedance device according to the manufacturer's recommendations prior to measurement.
- **Height and weight**. Obtain an accurate measure of height and weight.

Testing conditions and considerations

- **Device placement**. Place device on a nonmetal surface, at least 1m away from electronic or magnetic devices.
- **Ambient temperature**. Avoid excessively warm or cool ambient temperature.
- **Electrodes and leads**. Use electrodes with sufficient surface area ($\geq 4cm^2$); store electrodes in sealed bag away from heat; use device-specific leads provided by manufacturer.
- **Electrode placement**. Place electrodes at least 5cm apart, if possible; proximal electrodes should never be moved from standard anatomical site placement; if necessary, the distal electrodes may be moved to achieve at least 3cm of separation; the most important thing is to measure and record distance between electrodes to ensure placement consistency for follow-up measurements.
- **Side of body**. If using standard tetrapolar placement of electrodes, measure on the same side of the body as previous measures; in individuals with amputations, muscle atrophy, or other abnormal conditions, use the nonaffected side, if possible; be consistent on side of measurement for follow-up. Right-side measurements are commonly used in the literature.
- **Body positioning and limb separation**. Body position should be supine, except for stand-on scale devices, with arms separated ≥30° from the trunk and legs separated by about 45°; in individuals with overweight and obesity, separate arms from trunk and legs from each other using rolled cotton towels/blankets.
- **Fluid and electrolyte status**. Note if serum electrolytes are abnormal; it is best to conduct bioimpedance measurements only when serum electrolytes are normal. Note if edema is present; edema causes lower resistance values.
- **Menstrual cycle in women**. Note menstrual cycle; be consistent in terms of timing for follow-up measurements.
- **Timing of measurement**. If individual is ambulatory, individual should assume a supine position for 5-10min; standardize the timing for measurements by noting the time when the individual assumes the supine position and the time when you take the measurement (eg, at 10 minutes), and ensure consistency of timing for all followup measurements. Note if individual is confined to bed.

- **Repeat measurements**. Repeat measures recommended for research studies

The level of precision produced by both SF-BIA and MF-BIA devices is said to be good, with variability between repeat measures reported as 1%-2%. For BIS measurements, precision is more variable (i.e., 2%-3%) (Earthman, 2015).

In general, many BIA devices yield relatively valid estimates of fat-free mass and other body compartments in healthy normal-weight individuals and for large-scale epidemiological studies (i.e., mean level accuracy), but results in clinical settings, especially for individuals with fluid overload, have been mixed. None of the devices generate valid estimates for whole body composition in individuals with obesity (Earthman, 2015).

Like TOBEC (Section 14.7), BIA also depends on the differences in electrical conductivity of a weak alternating current which flows at various rates depending on the composition of the body. The current is well conducted by tissues with a high water and electrolyte content such as blood and skeletal muscle but is poorly conducted by adipose tissue, bone, and air-filled spaces which have a low water and electrolyte content. The voltage decrease of the current as it passes through the body is detected through current sensing surface electrodes, and the impedance data are recorded by the BIA device. See Kyle et al. (2004) for details of the theory of BIA.

The placement of the adhesive electrodes to the body is important and varies, depending on the device and whether whole-body or segmental measurements are sought. The standard placement is a wrist-ankle tetrapolar arrangement on the hand and foot of one side of the body. Alternatively, the electrodes can be placed on both sides of the body on both limbs using an 8-electrode arrangement, or on different segments of the body. For the stand-on BIA devices, direct contact with the electrodes is achieved at the feet and/or hands depending on the device.

The extrapolation from raw impedance data to volume depends on several key assumptions. For example, one of the assumptions is that stature is an acceptable surrogate for the unknown true length of the conductor, whereas another assumes the human body is comprised of five cylinders of uniform cross-sectional area. Unfortunately, these assumptions are often violated, especially those related to body differences in geometry in individuals with obesity and/or longer or shorter than average limbs. With the introduction of segmental and MF-BIA techniques, some of the limitations of the BIA method for these individuals have been overcome. For patients with clinical conditions in which hydration is altered (e.g., heart or liver failure), however, assuming a fixed hydration will yield body composition estimates that are inaccurate. For a detailed description of the assumptions, see Mulasi et al. (2015).

14.8.1 Single-frequency bioelectrical impedance (SF-BIA)

Single frequency BIA devices (SF-BIA) were the earliest devices developed. With these, the impedance of a weak electrical current (typically 500-800mA) and a single current frequency (i.e., typically 50kHz) is passed between two electrodes, usually located in the standard wrist-ankle tetrapolar arrangement on one side of the body, as shown in Figure 14.10, while the subject is supine in a horizontal position.

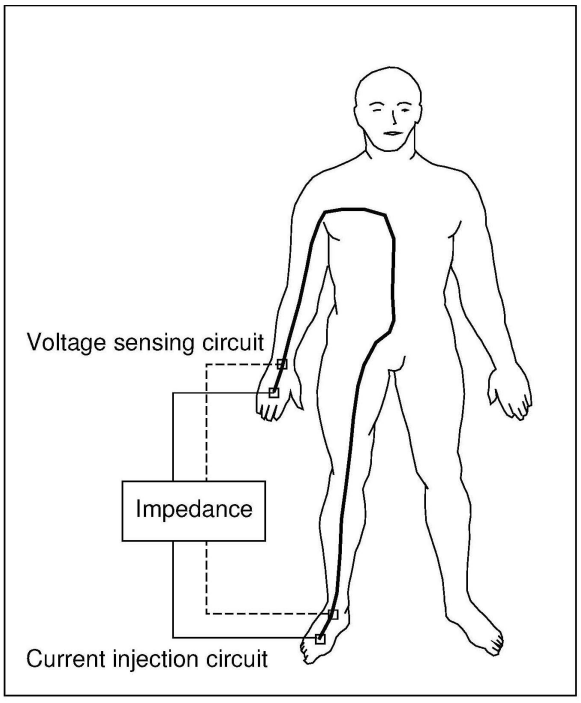

Figure 14.10. Measurement of whole-body bioelectrical impedance, showing diagrammatically the current path through the body and the positioning of the four electrodes

To derive fat-free mass, total body water (TBW) is predicted from raw impedance data by applying an appropriate regression equation and the use of the assumed hydration constant for fat-free mass (i.e., typically 0.732 for a healthy adult) (Section 14.3). However, as discussed earlier (see Sections 14.3 and 14.6.4), many factors influence the hydration of fat-free mass which have the potential to limit the accuracy of the estimates of fat-free mass from SF-BIA data.

Theoretically, the impedance measured at a single frequency (e.g., 50kHz) from a SF-BIA device is unable to differentiate between extracellular (ECW) and intracellular water (ICW) (or body cell mass, BCM). Many SF-BIA devices produce predictions of these outputs based on assumptions that may hold true in healthy individuals but not for those who are obese or sick.

14.8.2 Multiple-frequency bioelectrical impedance (MF-BIA)

Multiple-frequency BIA devices (MF-BIA) measure impedance at two or more frequencies, typically at 4 or 5 frequencies, including at least one low (most commonly 5kHz) and several higher ones (typically 50, 100, 200, and 500kHz). At lower frequencies the impedance to current flow allows for the determination of the extracellular water space (ECW), whereas at the higher frequencies (i.e., > 50kHz) the impedance to current allows for the determination of ECW and intracellular water space (ICW) (i.e., TBW). The volume of ICW, derived by subtracting ECW from TBW, can be used to estimate body cell mass (BCM) based on the assumption that cells are comprised of 70% water.

Several population-specific prediction equations are available to predict ECW, TBW, ICW, and BCM based on reference values derived from appropriate criterion methods. Nevertheless, again underlying assumptions may be violated in those individuals with obesity and in certain clinical populations, leading to errors, highlighting the importance of not accepting, without question, the output from a BIA device which uses the proprietary equations of the manufacturer (Earthman, 2015).

Sex-specific BIA prediction equations, validated earlier from MF-BIA resistance measures in the U.S NHANES III in 1988-1994 (Chumlea et al., 2002), have been used to compile ratios of fat mass to fat-free mass at 5th, 50th, and 95th percentiles by sex, age group, and BMI category (i.e., underweight, normal weight, overweight, class I/II and class III obesity) for non-Hispanic persons aged 18-90y. These fat/fat-free mass reference values that account for age, sex, and BMI can be used to identify individuals at risk for body composition abnormalities (Xiao et al., 2018).

Measurements from newer MF-BIA devices can now be made across body segments such as the limb and trunk, or across a small body region such as the muscle bed of the calf. Some MF-BIA devices can

be used in upright and supine positions so that they can be used for non-ambulatory or bed-bound persons.

14.8.3 Bioelectrical impedance spectroscopy (BIS)

Bioelectrical impedance spectroscopy (BIS) devices use biophysical modeling to estimate body compartments, as noted earlier; see Mulasi et al. (2015) for more details. These devices apply the electric current (typically ≤ 800μA) over a range of frequencies, from very low (e.g., 1 or 5kHz) to very high (e.g., 1000-1200kHz), measuring impedance data at ≥ 50 frequencies. This allows a more direct and individualized measure of ECW, ICW, and TBW compartments compared with those generated with SF-BIA and MF-BIA. Such measures are particularly useful in patients with altered fluid homeostasis (Mulasi et al., 2015). For those with obesity, the values for ECW and ICW can now be corrected using BMI as a surrogate for adiposity, thus allowing a more accurate assessment of body composition in individuals with obesity (Moissl et al., 2006).

Many validation studies of BIS conducted in healthy and clinical populations are available. Although good agreement at the population level can be achieved, agreement between reference methods and BIS at the individual level is poor, as noted for the measurements from SF-BIA and MF-BIA devices. This limits the assessment of whole body masses and fluid volumes in clinical settings.

14.8.4 Bioelectrical impedance vector analysis (BIVA)

Bioelectrical impedance vector analysis is a graphical procedure which uses the plot of resistance and reactance standardized for height to create a vector that can be compared with gender- and race-specific reference values from healthy population samples. BIVA can be generated from 50kHz data. It can provide information on hydration and body cell mass for patients in whom calculation of body composition is incorrect due to altered hydration (Mulasi et al., 2015). In a recent study of children with severe-acute undernutrition in Ethiopia, BIVA measurements successfully differentiated between those children who were dehydrated and those with edema (Girma et al., 2021). During treatment, edematous children lost fluid whereas non-edematous children gained small amounts of fat-free tissue. Moreover, BIVA parameters correlated with biomarkers of nutritional status (Girma et al., 2018).

14.8.5 New developments in bioelectrical impedance analysis

The questionable validity of BIA approaches for the assessment of whole body composition estimates in clinical populations, especially those with abnormal body geometry or altered fluid homeostasis, has led to the use of raw BIA measurements *per se* for bedside assessment of nutritional status and/or clinical outcomes. Raw BIA data are independent of the use of regression prediction models and assumptions of constant chemical composition of the fat-free mass, unlike the measurements from SF-BIA and MF-BIA devices. The raw BIA data used comprise single-frequency (50kHz) phase-sensitive measurements to determine: (i) phase angle, and (ii) the ratio of multifrequency impedance values.

Phase angle is estimated directly by a phase-sensitive BIA device without additional conversion to specific body compartments, followed by comparison with population-specific reference values. Phase angle is also independent of the body weight and height of an individual patient. The phase angle concept is based on changes in resistance and reactance as alternating currents pass through

evaluated tissues, providing information on hydration status and cell mass; for more details see Lukaski et al. (2017). Low phase angles values are typically related to more-severe illnesses and worse overall health outcomes. Currently, the major challenge of using phase angle for clinical assessment is the lack of consensus on the choice of cut-points to identify malnutrition (or poor clinical outcomes) (Mulasi et al., 2015).

The ratio of impedance measured at 200kHz to impedance measured at 5kHz — termed the impedance ratio or prediction marker — is said to reflect the ratio of ECW/TBW fluid distribution which is currently being explored as a potential indicator of nutritional status and/or clinical outcomes. However, as stated for phase angle, the lack of a consensus on the reference cut-points to use to identify malnutrition (or poor clinical outcomes) remains a major challenge for the use of the impedance ratio for clinical assessment (Mulasi et al., 2015). Investigations to date suggest that such reference cut-points may differ depending on the population under study and the BIA device used.

Of particular interest is whether both phase angle (PA) and the impedance ratio (IR) are useful for the diagnosis of sarcopenia (with and without the presence of obesity) and malnutrition in clinical settings (Mulasi et al., 2015). Reference cut-points for both phase angle and impedance ratios by sex, ethnicity, and age-decade have been compiled from U.S NHANES 1999-2004 based on BIS data. Validation with other reference measures (e.g. DXA) is needed to assess whether PA/IR are appropriate for the assessment of nutritional status in a clinical population (Kuchnia et al., 2017).

14.8.6 Applications of bioelectrical impedance

Several population-specific regression equations have been developed based on reference methods capable of predicting not only fat-free mass, but fat mass and other compartments from 50-kHz data. Examples of these reference methods include those based on a 4-component model or dual-energy X-ray absorptiometry (DXA); see Kyle et al. (2004) for more details. The regression equation chosen must be matched closely to the characteristics of the subject. However, unfortunately, many devices do not specify the equation programmed into their software so that the prediction equation used with the device may not be appropriate for the individual being measured.

Use of a fat-free mass index has also been explored for its potential to predict nutritional status and/or clinical outcomes. The index is generated from 50kHz data and is a height-corrected index of fat-free mass (i.e., fat-free mass/height squared) calculated by a standardized prediction equation and compared with reference data (Schutz et al., 2002). To date, challenges remain when applying fat-free mass index to assess nutritional status in clinical settings and more research is needed (Mulasi et al., 2015).

Interestingly, the American Society for Parenteral and Enteral Nutrition (ASPEN), does not support the use of BIA for the assessment of body composition in the clinical setting, based on their systematic review of 23 BIA studies. Their main objections included the scarcity of data on the validity of BIA in specific clinical populations, difficulties comparing studies using different BIA devices, variability in the body compartments estimated, and the proprietary nature of manufacture- specific BIA regression models to procure body composition data. For a summary and discussion of the studies reviewed which led to this conclusion, see Sheean et al. (2020).

14.9 Computerized tomography

Computerized tomography (CT) is a high-resolution imaging technique that is widely used in clinical settings to quantify *in vivo* body composition at the tissue-organ level. Total adipose tissue, subcutaneous adipose tissue, and visceral adipose tissue can be assessed. Computerized tomography can also measure skeletal muscle, individual muscle, or muscle groups, and evaluate the quality of muscle by identifying the infiltration of fat within the muscle, a condition known as myosteatosis. Computerized tomography is especially useful for investigating quantitative and qualitative changes in muscle and fat in the trunk area where the use of DXA is limited.

Computerized tomography (CT) is based on the relationship between the attenuation of an X-ray beam and the physical density of the tissues through which the beam has passed. The known differences in attenuation of X-rays between lean soft tissue and adipose tissue can be used to distinguish these tissues as well as to determine mixtures of them. From this relationship, a two-dimensional high-resolution radiographic image of the underlying anatomy of the scan area can be constructed.

The CT scanner is made up of two components: a collimated X-ray source and detectors, and a computer that processes the scan data and produces an X-ray image. The subject lies on a movable platform within the scanner gantry. The designated area to be scanned is a plane through the middle of the central aperture of the gantry and parallel to the gantry. The X-ray beam is made to rotate around the subject, generating a cross-sectional "slice" through the patient. As the X-rays pass through the tissue, the beam undergoes attenuation, and the intensity is recorded and stored in the scanner computer. The latter then processes the stored information by using a series of complex algorithms to construct a cross-sectional image (Figure 14.11). Multiple images generated following the movement of the patient through the scanner gantry are used to produce an integrated scan of the subject.

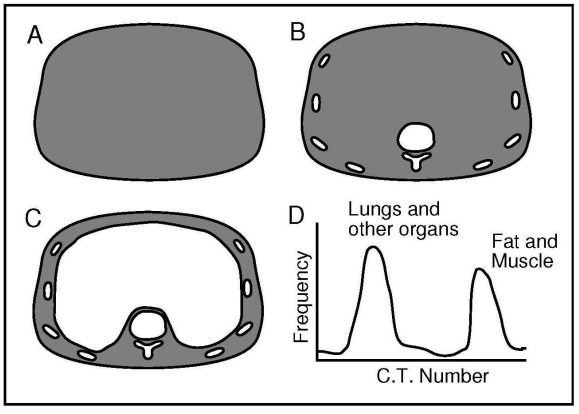

Figure 14.11. Body composition from a computerized tomography scan at the thorax level, showing three images resulting from different computer processings of the same scan. A: The air/skin interface. B: Major skeletal elements. C: Adjusted to show the interface between the lungs and other organs and the surrounding muscle and fat. D: Histogram of the pixel density from scan C.

The integrated scan shows varying degrees of shading according to the magnitude of the X-ray beam attenuation, which, in turn, depends on the physical density of the scanned tissues. Tissues with a greater density cause a greater absorption of X-ray energy and, consequently, a higher attenuation value. The demarcation between tissues of differing density — adipose tissue, skeletal muscle, bone, and visceral organs — can be very good.

The use of computerized tomography is limited by its high-dose radiation exposure and high cost. Efforts have been made to reduce the radiation dose by using a lower-dose protocol, essential when planning a CT scan in a child (Sorantin et al., 2013). This protocol has little deterioration in image quality (Kapur et al., 2021). Single-slice scanning can also be used to reduce the radiation dose.

A new technique with extremely low radiation exposure, shorter scan time, and relatively low cost compared to whole body CT, termed "peripheral quantitative computer tomography" (pQCT) has been developed. This new technique has been used primarily to investigate

bone mineral content and to assess bone fragility in older patients (Jiang et al., 2018). However, despite these efforts, radiation exposure is still high, restricting the use of CT images for clinical use during disease treatment rather than for body composition assessment.

14.9.1 Application of computerized tomography

The method has several uses. It can be used to assess changes in the visceral organ mass in under-nutrition and obesity, to portray the distribution of subcutaneous versus visceral adipose tissue, and to establish bone density in osteopenia (Heymsfield et al., 1987). New techniques with improved spatial resolution can be used to detect and measure fat in areas of the body where fat is not physiologically stored, such as the liver, pancreas, heart, and skeletal muscle. The fat deposited in these areas is termed visceral ectopic fat and is known to contribute to the development of coronary artery disease as well as other cardiovascular disorders (Neeland et al., 2019).

The region of special interest for the study of body composition using CT scans is the third lumbar vertebra (L3). This is the region where a single image of a cross- sectional area provides the best correlation of whole-body skeletal muscle volume. The validity and accuracy of body composition measurements by CT based on cross-sectional area of tissues have been validated by studies of human cadavers, although no validation of the calculation of tissue volume has been performed (Fosbøl & Zerahn, 2015).

Increasingly, CT scans are also being used to identify CT-defined sarcopenia, a condition associated with a decrease in muscle mass and function which, although originally described in the elderly, is also of concern among the chronically ill nonelderly (Peterson & Braunschweig, 2016). To date, however, there are no clear diagnostic cutoff values for CT to identify sarcopenia based on skeletal muscle mass, which has limited the application of CT for clinical use.

14.10 Magnetic resonance imaging

Unlike computer tomography or DXA, magnetic resonance imaging (MRI) does not use ionizing radiation so that the technique can be used on infants, and for long-term follow-up when multiple scans on the same person are required. The MRI technique is mainly used to evaluate the quantity and distribution of adipose tissues and skeletal muscle mass, although it can also detect changes in body composition, even in the presence of small body weight changes (Lemos & Gallagher, 2017). In older equipment, scanning times of 10min were necessary, but in more modern equipment, this has been reduced to under 2min. Nevertheless, the MRI technique requires technical expertise, is expensive, and the equipment is bulky (Prada & Heymsfield, 2014).

Magnetic resonance imaging uses differences in the nuclear magnetic resonance properties of hydrogen atoms in organic and non-organic environments to distinguish signals originating from fat, fat-free mass, and free water. The hydrogen protons behave slightly differently in adipose versus lean tissues. The differences are in the relaxation time that it takes for the nuclei to release the radio-frequency-induced energy and return to a random configuration. These differences can be used to map the distribution of adipose versus lean tissue in the body (Ross, 1996).

The imaging process involves placing the subject in a very strong magnetic field. Some of the nuclei in the body attempt to align themselves relative to the applied field. The effect is particularly marked for

^{1}H protons. Only a small fraction of the protons become aligned, but they are sufficiently numerous for the effect to be detectable when the field is removed or altered. It is then that the differences between the lean and adipose tissue become apparent.

14.10.1 Application of magnetic resonance imaging

Magnetic resonance imaging is often ued in clinical settings, when, instead of using whole body imaging which is time consuming and expensive, sectional body composition studies that often employ the L3 lumbar vertebra as the landmark, are used. This landmark has the highest correlation with whole body skeletal muscle and visceral fat volume. Alternatively, to identify sarcopenia in older adults, a single-slice at the mid-thigh level can be used as this provides a good estimate of skeletal muscle and fat volume in the thighs, and correlates with clinical criteria; see Lee et al. (2019) for more details.

14.11 Dual energy X-ray absorptiometry

Dual-energy X-ray absorptiometry (DXA) is a technique that can be used with individuals (except pregnant women) across the entire age range and at relatively low cost. DXA is widely used to assess body composition, with an overall precision that exceeds that of any other body composition method (Prada & Heymsfield, 2014).

With the latest generation of densitometers, body composition can be assessed with a single whole-body scan so that radiation exposure is low with a minimal acquisition time. In addition, the newer instruments enable individuals with extreme obesity to be scanned. Modern DXA scanners use an X-ray source, a detector, and an interface with a computer system for imaging the scanned area of interest. The source generates X-rays at two different energy levels — 40KeV and 70-100KeV. These pass through the body and are identified by the photon detector that measures the amount of photon energy absorbed at the two energy levels by the soft tissue and bone. Low-density material (i.e., soft tissue) allows more photons to pass through, so they attenuate the X-ray beam less than materials with a higher density, such as bone. An image is generated as the photon detector measures the differential attenuation (or absorption) of the low and high X-rays by the soft tissue and bone. Only in regions of the body where no bone is present can measurements of the relative proportions of fat and lean tissues be made; see Prada & Heymsfield (2014) for more details.

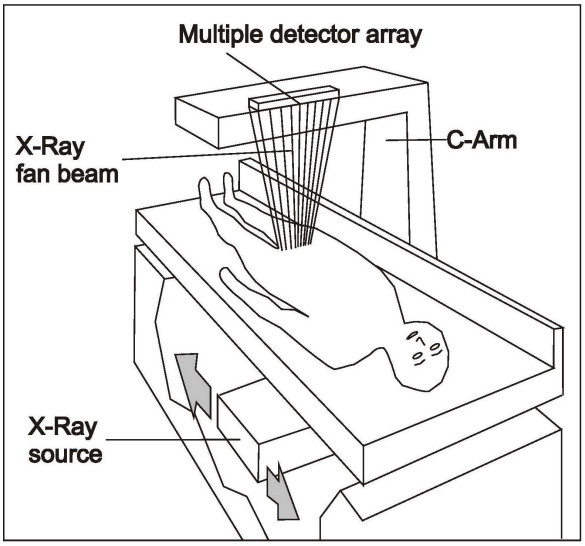

Figure 14.12. Dual-energy X-ray scanner with multiple detector array and X-ray fan beam.

During an investigation, the subject, on a movable bed, is scanned rectilinearly from head to toe. The X-ray source is situated beneath the scanning bed, and scans usually take from 5-20min depending on type of scan and software selected (Figure 14.12).

The accuracy of DXA results is influenced by both technical and biological factors; these are summarized in Figure 14.13. The technical factors include differences in machine calibration procedures, the

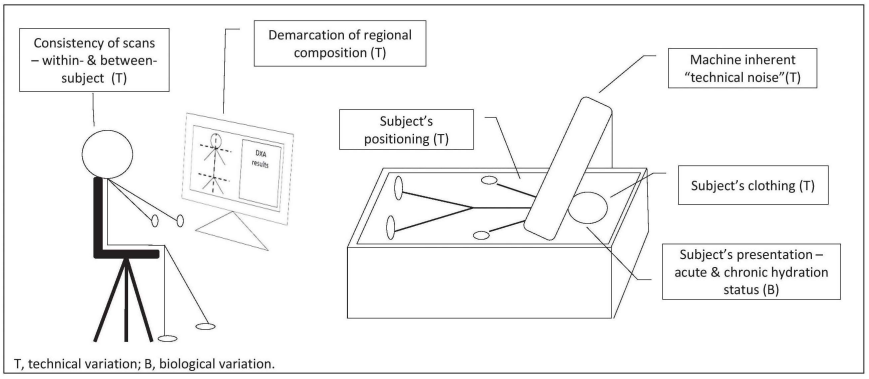

Figure 14.13. Sources of variability or error when carrying out a DXA scan to measure body composition. Modified from Nana et al. (2015).

protocols used in demarcating the regions (arms, legs and trunk) in estimates of body composition, the positioning of the subject on the scanning bed, and the effect of clothing. Sources of biological variability that can affect accuracy include hydration status because the hydration of fat-free mass is assumed to be constant (i.e., about 73%). Hence, when changes in hydration status are large (i.e., higher than 5%), as may occur in the presence of diseases with water retention (e.g., heart, kidney or liver failure), the fat-free mass is over-estimated (Nana et al., 2015). In older DXA models, obesity was also a factor limiting accuracy, but newer instruments allow larger and heavier individuals to be scanned. Finally, the ability to compare body composition estimates between DXA manufacturers is limited because of variations in the hardware and software packages used by manufacturers, highlighting the urgent need for universal calibration of DXA machines (Prada & Heymsfield, 2014).

Several investigators have tried to establish the absolute accuracy of gross measurements of body composition *in vivo* performed using DXA. Many of these studies have examined correlations between measurements made by DXA on pig cadavers and subsequent direct chemical analysis; results have been mixed (Jebb, 1997).

14.11.1 Applications of DXA

Dual-energy X-ray absorptiometryis now the primary method for generating accurate data on bone mineral content and density for the diagnosis of osteoporosis (see Chapter 23 for more details). DXA is also widely used to assess body composition, with an overall precision that exceeds that of any other body composition method (Prada & Heymsfield, 2014). Both total and regional estimates of the three body compartments — fat-free mass, body fat, and bone mineral content — can be determined. However, DXA does not have the ability to discriminate between visceral, subcutaneous, and ectopic fat present in organs such as liver or muscle.

Estimates of the appendicular skeletal muscle mass (ASM) can be obtained from DXA. These estimates are generated by summing the amount of lean soft tissue (LST) of the arms and legs, as they are composed mainly of muscle (except for a small amount of connective tissue and skin) (Prada & Heymsfield, 2014). From these estimates, the ASM index can be calculated by dividing the arm and leg lean soft tissue (LST) by height squared. Both the ASM and the ASM index values are considered the gold standard method for the diagnosis of sarcopenia. For published cutoff points to define sarcopenia based on DXA, see Earthman (2015).

In a systematic review based on eight DXA studies by the American Society for Parenteral and Enteral Nutrition (ASPEN), DXA was recommended for the assessment of fat mass in patients with a variety of disease states, but not for fat-free mass (Sheean et al., 2020). They stressed that validity of DXA for

assessment of fat-free mass in any clinical populations remains unknown. Clearly, additional research is needed to evaluate the validity of DXA for quantifying fat-free mass.

14.12 Ultrasound

Diagnostic ultrasound, also called sonography, is an imaging technique used in clinical settings to measure various tissue thicknesses, including muscle, bone, and subcutaneous and visceral adipose tissue. The device is portable, low cost, and capable of making fast and noninvasive regional estimates of body composition with no exposure to ionizing radiation. Consequently, this scanning technique can be used in children and pregnant women and is suitable for large epidemiological studies, although the results generated are highly dependent on the skill of the operator.

The ultrasound technique involves the use of high-frequency sound waves from a transducer. These penetrate the skin surface and pass through the adipose tissue until they reach the muscle tissue. At the adipose-muscle tissue interface, a proportion of the sound waves are reflected back to the transducer as echoes. Hence, the transducer both transmits and receives the ultrasound. The degree of reflection is dependent on the changes in acoustic impedance (i.e., the product of tissue density and acoustic velocity) between two tissue interfaces. The higher the acoustic impedance, the stronger the generated reflection, and thus, the better the quality of the image.

The acoustic impedance of fat and muscle are somewhat similar and much lower than bone which has a relatively high acoustic impedance; air has almost no impedance. Consequently, there is a weaker echo at the fat-muscle interface than at the muscle-bone interface. When the transducer receives the beam, it converts the echo into electric signals to form a two-dimensional image. The relative strength, or amplitude, of echoes is apparent from the brightness of the image on the computer screen, with strong reflections appearing white, weaker reflections grey, and no echoes black. As a result, a grey-scale image is produced with white borders for the skin-subcutaneous fat and muscle-bone interfaces and a visible, but less distinct border for the fat-muscle interface; see Wagner (2013) for more details.

However, the interpretation of ultrasound images is difficult and depends on the technical expertise of the operator who must identify the interfaces and measure the thickness of the tissue layer of interest using electronic calipers. Care is needed to identify and place the two caliper points at the boundaries of the tissue to be measured. An automated discrimination method is available to identify the tissue boundaries for some ultrasound devices, although studies comparing the automated method with the manual discrimination are limited (Wagner, 2013).

To use ultrasound, the measurement site is marked with a water-soluble transmission gel that provides acoustic contact without depression of the dermal surface. The high-resolution transducer is then placed without loss of contact with the skin so that the ultrasonic beam is perpendicular to the tissue interfaces at the marked site. A transducer receives the echoes and translates them into depth readings viewed on a computer screen. Thicknesses of 100mm or more can be measured, and density interfaces can be detected with an accuracy of 1mm. The tissue is not compressed, thereby eliminating errors associated with variations in the compressibility of skinfolds (Fanelli & Kuczmarski, 1984).

14.12.1 Applications of Ultrasound

Visualization of a fetus during a prenatal examination is the most familiar application of ultrasound. However, ultrasound is also used to measure the quality and quantity of skeletal muscle mass, an index of lean soft tissue, in the elderly, patients with cystic fibrosis, and patients confined to bed rest (Prada & Heymsfield, 2014). Alternatively, measurement of the thickness of subcutaneous fat can be used to monitor changes in body composition of hospital patients receiving nutritional support.

In general, the ultrasound method provides a reasonable estimate of adipose tissue thickness in humans, compared to total body electrical conductivity (TOBEC) and skinfold caliper techniques (Fanelli & Kuczmarski, 1984). For obese persons especially, ultrasound may be superior to skinfold caliper techniques for measuring subcutaneous fat (Kuczmarski et al., 1987). The method can also be used to measure thickness of other tissues such as muscle and bone, as noted earlier; for more details see Mayans et al. (2012) and Karjalainen et al. (2008).

An ultrasound system has also been designed specifically for body composition which could be the user-friendly ultrasound alternative to skinfolds and other field methods for estimat;ing percentage body fat (Wagner, 2013). However, currently the diverse technology across the commercially available devices and the lack of standardized measurement protocols makes it difficult to compare results across studies (Lee et al., 2019). The American Society for Parenteral and Enteral Nutrition (ASPEN) did not recommend the use of ultrasound to assess body composition in a clinical setting, based on their systematic review of seven studies (Sheean et al., 2021). More research is needed to develop consensus on the optimal method to conduct ultrasound measurements and to generate population-specific reference data so that the method can be used to assess lean tissue and diagnose malnutrition in a clinical setting (Earthman, 2015).

14.13 Summary - Method comparisons

Increasingly, clinicians are using the more robust and reproducible *in vivo* methods described in this chapter to measure body composition in view of the limitations of BMI as a proxy for adiposity and the recognition that differences in body composition are associated with an increased risk of diseases across the lifespan. Such diseases may include AIDS-associated wasting, diabetes, osteoporosis, sarcopenia, cardiovascular disease, and anorexia nervosa. Even during early infancy, changes in body composition (both fat and fat-free mass) can provide more understanding of the initiators and mediators of the developmental origins of adult cardiometabolic disease (McMillen et al., 2005).

However, no single *in vivo* method has the ability to track body composition accurately from infancy to adulthood; all have strengths and technological limitations. Fields and co-workers (2015) have compared selected *in vivo* methods for assessing whole-body composition across the life span; details are shown in Table 14.6. When compiling this table, the investigators considered the technological limitations of each method, and the following four criteria:

- Cost of the measurement / device

Method	Infancy (< 6mo)	Childhood (2-6y)	Adolescence (7-17y)	Adulthood (≥ 18y)
Hydrostatic weighing	NA	I	D|I	P
Air displacement plethysmography	P	Pa|D	P	P
Whole-body counting	UE|C	UE|C	UE|C	D|C
Dual-energy X-ray absorptiometry	D|R	D|R	R	P|R
Bioelectrical impedance	VA|NV	VA|NV	VA	P
Computed tomography	UE	UE	UE	UE
Isotope dilution	I	D|P	D|P	P

Method	Infancy (< 6mo)	Childhood (2-6y)	Adolescence (7-17y)	Adulthood (≥ 18y)
Magnetic resonance	D|C	D|I|C	D|I|C	P|C
Ultrasound	NV	NV	NV	NV

Table 14.6: Comparison of selected methods for determining whole-body composition across the life span.
C, cost limits widespread use; D, difficult to perform but still possible; I, impractical (maybe impossible) due to age- and compliance-related issues; NA, not applicable; NV, not valid or little data exist in determining validity; P, probative data support its validity, precision, and reliability in this population; PL, possible—limitations exist in interpretation to whole-body composition; R, radiation involved, marginally small although institutional review boards may limit its use; UE, may be unethical due to radiation; VA, validity is ambiguous or weak in this population. [a]gap exists between 6mos and 2y.

- Level of patient cooperation needed for accurate results
- Feasibility in obtaining an interpretable result given the patient's age
- Appreciation of the age of the patient relative to the assumption of the underlying principles in the chosen method.

Based on their review of the methods listed for whole-body assessment in Table 14.6, Fields et al. (2015) chose air displacement plethysmography (Section 14.6.3) as the method with the highest degree of accuracy and reliability and with the least degree of technical error across the life span. Currently a "suite" of air displacement plethysmographic devices are available which can be used to track and monitor body composition from birth, into adolescence and throughout adulthood; they are described in Section 14.6.3 and have also been reviewed by Fields et al. (2015).

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