

INVITED REVIEW

Contemporary methods of body composition measurement

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Summary

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Reliable and valid body composition assessment is important in both clinical and research settings. A multitude of methods and techniques for body composition measurement exist, all with inherent problems, whether in measurement methodology or in the assumptions upon which they are based. This review is focused on currently applied methods for in vivo measurement of body composition, including densitometry, bioimpedance analysis, dual-energy X-ray absorptiometry, computed tomography (CT), magnetic resonance techniques and anthropometry. Multicompartiment models including quantification of trace elements by in vivo neutron activation analysis, which are regarded as gold standard methods, are also summarized. The choice of a specific method or combination of methods for a particular study depends on various considerations including accuracy, precision, subject acceptability, convenience, cost and radiation exposure. The relative advantages and disadvantages of each method are discussed with these considerations in mind.

Introduction

Measurement of body composition is important in various physiological and pathological conditions. The clinical applications span from the evaluation of childhood obesity (Weber *et al.*, 2012) to the diagnosis of sarcopenia of elderly patients with chronic disease (Christensen *et al.*, 2012; Chung *et al.*, 2013). Furthermore, body composition measurement is frequently performed in sports and exercise settings for evaluating training programmes and optimizing nutrition for athletes. The multiplicity of conditions and subject characteristics each create different demands for the preferred method for quantifying body composition.

There is a multitude of established methods and techniques for in vivo estimation of body composition, ranging from simple field methods, for example skinfold measurement, to laboratory methods such as dual-energy X-ray absorptiometry (DXA), hydrostatic weighing and the more complex in vivo neutron activation analysis. Before selecting a method for measuring body composition in a given clinical situation or for a specific scientific protocol, various factors are to be considered as follows: availability of the equipment, financial costs, safety precautions regarding radiation dose, subject cooperability, etc. An essential requirement is that the selected method can provide valid and reproducible results given the actual subject characteristics.

Ideally, the methods for body composition measurement should be equally accurate in all subjects regardless of age, ethnicity, sex, health status, etc. Due to inherent assumptions of existing methods, this is not possible to achieve. Consequently, there can be large individual variations in the accuracy and precision of body composition measurement. In comparison studies, not only the mean bias between methods is of concern, the individual variation must also be considered (Altman, 1990). This aspect is important when selecting a method for body composition measurement, as the majority of contemporary methods can provide sufficiently accurate results for larger groups of subjects.

The reproducibility of a given method is essential to evaluate, especially in longitudinal studies. Many researchers report the reproducibility in terms of coefficient of variation (CV = standard deviation / mean), which is based on the expectation that the variance is proportional to the global mean. The CV is therefore highly dependent on the range of the measured variable. This issue can be compensated for using the standardized coefficient of variation (SCV) instead (Quan & Shih, 1996; Giraudeau *et al.*, 2003) or quantifying precision as standard error of the measurement (SEM).

A substantial number of studies have investigated methods of body composition measurement, and development of both new methods and techniques is still ongoing, resulting in a never-ending demand for between method comparisons.

The aim of this review is to provide a comprehensible summary of contemporary methods for body composition measurement in humans.

Models for body composition

A widely accepted five-level model for body composition research has been developed by (Wang et al., 1992). It divides the human body into different compartments at the following levels: atomic level, molecular level, cellular level, tissue-system level and whole body (Fig. 1). The five-level model provides a structural framework for explaining the relationships between the major body compartments.

At present, the most commonly applied model in studies of body composition is a two-compartment model, which partitions the body into fat mass (FM) and fat-free mass (FFM), where the latter is a heterogeneous compartment consisting of water, protein, carbohydrates and mineral (Fig. 2). Figure 2 also displays the differentiation in fat mass at the molecular level and adipose tissue at the tissue level (Shen et al., 2005). The terms fat and adipose tissue are often used interchangeably, but their differentiation must be considered, when measuring their mass and metabolic characteristics. Adipose tissue is anatomically defined and consists of adipocytes, nerves,

blood vessels and extracellular fluid. Fat is defined on the molecular level. In the human body, it is primarily found in adipose tissue, but triglyceride is also present in other kinds of tissues such as liver and skeletal muscle.

The estimation of FM and FFM *in vivo* is most often based on assumptions regarding the physical/chemical properties of the constituents that are not directly measured. If these assumptions are not met, the estimate of body composition will be inaccurate. Subsequently, to improve accuracy of body composition measurement, one often has to make use of combinations of modalities. No single method of body composition measurement is at present regarded as the gold standard to determine FM. Instead, multicompartment models have been proposed, where methods are combined to minimize the influence from assumptions regarding the constituents of FFM (Baumgartner et al., 1991; Wang et al., 1998).

Multicompartment models

Measurement of total body water (TBW) reduces the potential error in the classical two-compartment model, where constant hydration of FFM is assumed. This assumption has limited validity as hydration varies with age (Lohman, 1986; Hewitt et al., 1993), sex, nutritional status (Waki et al., 1991) and

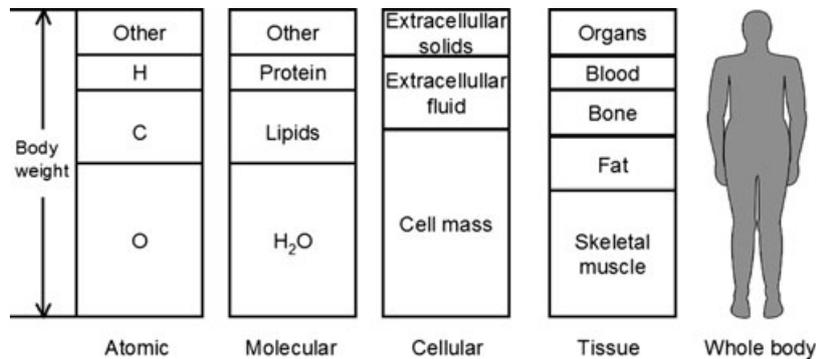


Figure 1 The five body composition levels. Adapted from Wang et al., 1992.

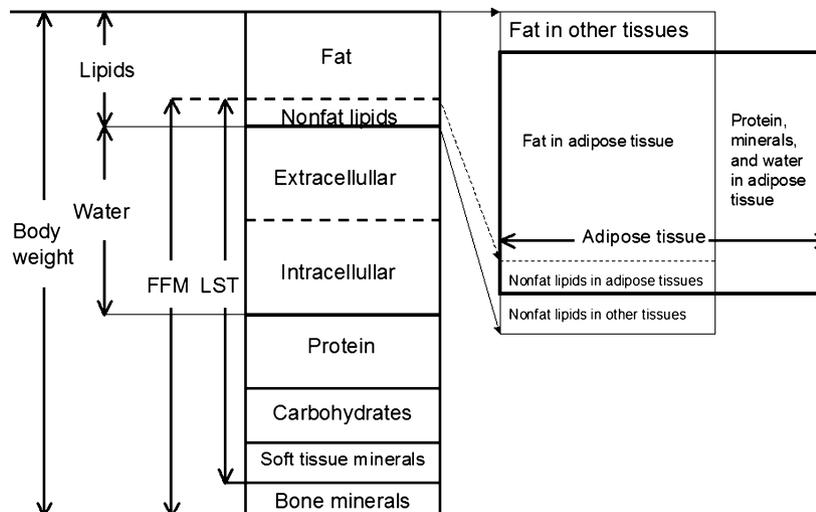


Figure 2 Main components of the molecular level of body composition and the relationship between lipid and fat (molecular level) and the tissue-organ-level component adipose tissue. Adapted from Shen et al., 2005.

certain diseases, such as renal disease, decompensated cirrhosis or congestive heart failure, that clearly result in alterations of the ratio between TBW/FFM.

The gold standard for determination of TBW is by dilution with tritiated water ($^3\text{H}_2\text{O}$), deuterium water ($^2\text{H}_2\text{O}$) or ^{18}O -labelled water (H_2^{18}O) administered orally. The labelled water is rapidly distributed within the body. Equilibrium requires 3–4 h, and analysis is based on samples of blood plasma or urine, in which the concentration of the stable element and the labelled one is measured (Schoeller, 2005). The volume of body water is equal to the amount of tracer added to the compartment divided by the concentration in the compartment. The isotopic tracers are not exclusively distributed in body water because of exchange with non-aqueous molecules. This causes an overestimation of TBW by approximately 3%, which should be corrected for (Racette et al., 1994). TBW determination by dilution is time-consuming and requires adequate laboratory facilities, which makes this method less applicable in large-scale studies or in field settings where the necessary equipment is unavailable.

The four-compartment model is based on further subdivision of FFM and controls for biological variability in both TBW and bone mineral mass (Mo) (Heymsfield et al., 1990; Wang et al., 2005). The four-compartment model can be represented as follows:

$$\frac{\text{FM}}{\rho_{\text{FM}}} + \frac{\text{TBW}}{\rho_{\text{H}_2\text{O}}} + \frac{\text{Mo}}{\rho_{\text{Mo}}} + \frac{\text{RM}}{\rho_{\text{RM}}} = \frac{1}{\rho_{\text{B}}} \quad (1)$$

This model assumes a constant density (ρ) for FM (ρ_{FM}), water ($\rho_{\text{H}_2\text{O}}$), residuals (ρ_{RM}) and bone mineral (ρ_{Mo}) at body temperature. The density of FM (triglycerides) has very small between-subject variability and can be assumed to be constant at $0.9007 \pm 0.00068 \text{ g cm}^{-3}$ (Fidanza et al., 1953). The density of residuals is more variable, due to an assumed distribution of protein, glycogen and soft tissue mineral. The density at body temperature is estimated to be 1.404 g cm^{-3} based on tissue removed from humans and mammals (Allen et al., 1959). The density of bone mineral has been estimated from animal bones to be 2.982 g cm^{-3} (Wang et al., 2005). Several equations have been developed for calculating total body fat by the four-compartment model, for example (Withers et al., 1992):

$$\text{FM} = 2.513 \times \text{BV} - 0.739 \times \text{TBW} + 0.947 \times \text{Mo} - 1.79 \times \text{BM} \quad (2)$$

Where BM = body mass, body volume (BV) can be determined by densitometry, and Mo is measured by DXA. The four-compartment method as described has been validated against multicompartment models involving *in vivo* neutron activation analysis (standard error of the estimate (SEE) in FM determination of healthy subjects $\pm 1.47 \text{ kg}$, $r = 0.98$) (Heymsfield et al., 1990).

The four-compartment model does not take into account the mineral content of soft tissue (Mst), which consists of soluble minerals and electrolytes in both intracellular and

extracellular compartments of soft tissue. Although the mass of Mst in the human body is small, the contribution of Mst can be considerable as the density of this component is high (3.317 g cm^{-3}) compared with other constituents (Wang et al., 2005). Wang et al. validated that Mst can be estimated from TBW in healthy individuals due to the relatively constant body fluid osmolality (Wang et al., 2002). A suggested equation for the calculation of FM based on the five-compartment model is as follows:

$$\text{FM} = 2.748 \times \text{BV} - 0.715 \times \text{TBW} + 1.129 \times \text{Mo} + 1.222 \times \text{Mst} - 2.051 \times \text{BM} \quad (3)$$

The body content of Mst can be measured by *in vivo* neutron activation analysis and whole-body counting. These techniques can provide quantification of selected atomic constituents of the body, from which the molecular-level components can be calculated. The reconstruction of body composition from the atomic level minimizes the assumptions related to tissue density, hydration and/or structure, but relies on assumptions regarding fixed relationships between elemental composition and the molecular structure of tissues (Ellis, 2000). A classical example is total body potassium (TBK) to estimate FFM, which is based on a constant relation between TBK and FFM. This relation, however, is known to be dependent on sex, ethnicity and age (Pierson et al., 1984; Ellis, 2005). The naturally occurring radioactive ^{40}K emits a 1.46 MeV gamma photon that can be assessed by whole-body counting. TBK is calculated from the constant ratio between ^{40}K and total potassium (Hendel et al., 1996).

In vivo neutron activation is performed by placing a subject in a neutron field, by which there is a possibility that atoms in the body will undergo a nuclear reaction, depending on the energy of the neutrons. For example, thermal neutron capture in ^{14}N produces excited ^{15}N , which rapidly de-excites, releasing a 10.83 MeV gamma photon. The gamma emission must be measured during the neutron exposure by solid scintillation detectors (Cohn et al., 1974). Total body nitrogen (TBN) is calculated using the measured counts of the relevant photopeak and a specific calibration factor derived from phantom measurements with known nitrogen content (Ellis, 2005). Assuming a constant nitrogen–protein ratio and complete incorporation of TBN into protein, the total body protein (TBPro) can be estimated as follows:

$$\text{TBPro} = 6.25 \times \text{TBN} \quad (4)$$

The method has been validated by chemical analysis of cadavers (Knight et al., 1986). Besides nitrogen, *in vivo* neutron activation analysis can be used to quantify a number of elements in the body, including oxygen (O), hydrogen (H), carbon (C), sodium (Na), calcium (Ca), phosphorous (P) and chlorine (Cl) (Cohn et al., 1974; Chettle & Fremlin, 1984; Ryde et al., 1987, 1990). Once these elements are known, it is possible to calculate several components of the molecular composition level similar to calculation of total body protein from TBN.

Based on *in vivo* neutron activation analysis, Wang *et al.* developed a six-compartment model to calculate FM (Eq. 5) (Wang *et al.*, 1998). This model includes glycogen, which is the remaining constituent of FFM not estimated in the five-compartment model.

$$FM = BM - \left(\frac{TBW + 6.525 \times TBN + 2.709 \times TBCa + 2.76 \times TBK + TBNa + 1.43 \times TBCl}{\rho_B} \right) \quad (5)$$

The technology required to perform *in vivo* neutron activation analysis is only available at few facilities. Radiation exposure, financial cost and limited availability makes them generally unfeasible for other purposes than selected studies of body composition methodology.

It has been speculated to which extent the use of multicompartiment methods for body composition measurement enhances measurement error as the inherent error of each device used in the model is propagated. Calculation of the propagated error or total error of measurement (TEM) incorporates the SEM for each modality used in the multicompartiment methods (Wang *et al.*, 2005). For four-, five-compartment and six-compartment models, the reported TEM values for % body fat are approximately 0.59–0.89 % fat (Withers *et al.*, 1998; Wang *et al.*, 1998; Silva *et al.*, 2006; Moon *et al.*, 2009a).

A comparison study between a six-compartment method and other multicompartiment models (Baumgartner 4-C, Heymsfield 4-C, Cohn 4-C, Siri 3-C) in healthy adults found bias of <1% body fat (Wang *et al.*, 1998). Equally, in female athletes, three-compartment (Siri 3-C) and four-compartment (Wang 4-C) methods resulted in SEE < 1% body fat compared with a five-compartment method (Moon *et al.*, 2009a). This suggests that limited accuracy is gained by measurement of additional compartments to the three- or four-compartment model in healthy individuals. Whether this can be applied in subjects with pathological conditions or in various age groups is not certain, and more research in this area is needed.

Densitometry – hydrostatic weighing and air displacement plethysmography

Measurement of body density using either hydrostatic weighing (HW) or air displacement plethysmography to estimate body composition is based on the two-compartment model. HW is a classical method, but is still today considered the gold standard for measurement of BV. The body weight of the subject in water is measured during full submersion after maximal expiration. Residual volume in the lungs must be measured during submersion to correct the measured body volume accordingly (Girandola *et al.*, 1977; Wilmore *et al.*, 1980; Latin & Ruhling, 1986). Body density (ρ_B) is calculated from the measured BV and weight and can be used to estimate FM (% body fat) from equations proposed by Siri (Eq.6) (Siri, 1956) and Brozek (Eq. 7) (Brozek *et al.*, 1963)

$$\% \text{Body fat} = \left(\frac{4.95}{\rho_B} - 4.6 \right) \times 100 \quad (6)$$

$$\% \text{Body fat} = \left(\frac{4.57}{\rho_B} - 4.142 \right) \times 100 \quad (7)$$

Estimation of body composition by densitometry assumes a constant and known density of FM and FFM for all individuals at, respectively, 0.9007 g ml⁻¹ and 1.100 g ml⁻¹ (Brozek *et al.*, 1963). These densities are derived from chemical analysis of a few human cadavers (male aged 25–48 years) (Keys & Brozek, 1953). The validity of the assumed constant value of density for FFM is questionable. It has since been documented that vast interindividual variation in the density of FFM exists, of which the density varies with age (Lohman, 1986), sex (Visser *et al.*, 1997; Chung *et al.*, 2013) and ethnicity (Wagner & Heyward, 2000; Deurenberg-Yap *et al.*, 2001). Research in muscular individuals such as highly trained athletes from several sports disciplines has found lower density of FFM compared with non-athletes due to higher water content and lower mineral and protein fractions (Modlesky *et al.*, 1996; Millard-Stafford *et al.*, 2001b).

HW requires extensive equipment, and the method is not well tolerated by many subjects because of the complete submersion in water. The method is subsequently not suited for small children and subjects who are unable to hold their breath under water due to disability, pulmonary disease, etc. Air displacement plethysmography (ADP) was developed as an alternative to HW for the estimation of body density suitable for a wider range of individuals (Gnaedinger *et al.*, 1963). The ADP device consists of a measuring chamber, where the subject is seated, and a reference chamber linked by a flexible airtight diaphragm, which is oscillated to produce small, sinusoidal volume and pressure changes in both chambers (Dempster & Aitkens, 1995). Poisson's law can be applied under the assumption of adiabatic conditions (i.e. without exchange of heat of a system with its environment (Atkins, 1996)).

$$\frac{P_1}{P_2} = \left(\frac{V_2}{V_1} \right)^\gamma \quad (8)$$

where γ is the ratio of the specific heat of the gas at constant pressure to that at constant volume (McCrorry *et al.*, 1995). Air close to the skin, hair, clothes and in the airways and lungs of the subject will be thermally affected, which makes it more compressible than air under adiabatic conditions. Therefore, it is necessary to correct for body surface area and measured thoracic gas volume (Collins & McCarthy, 2003). The effects of clothing and hair are reduced by the subject wearing minimal clothing (bathing suit) and a tight-fitting swim cap (Fig. 3). Fat mass is calculated in the same manner as described above using Eq. 6–7 or similar.

The majority of comparisons between ADP and HW in measurement of body density have shown good agreement in normal weight adults, obese adults and children (10–15 years) (Levenhagen *et al.*, 1999; Vescovi *et al.*, 2001; Demerath *et al.*,

2002; Ginde et al., 2005; Noreen & Lemon, 2006). The bias in determination of FM in healthy subjects ranges from -4.0 to 1.9% , with generally narrow limits of agreement (LOA). Millard-Stafford et al. found body density overestimated by ADP in a heterogeneous population of adults, but the authors concluded that ADP is sufficiently accurate for measurement of body density for estimating FM in a four-compartment model due to low bias and small individual error (Millard-Stafford et al., 2001a).

The validity of ADP measurement of body composition compared with multicompartiment methods in adults and children has shown bias of FM ranging from 2 to 4% and LOA at 2–7% (total error (TE) % body fat 2.7–6%) (Collins et al., 1999; Fields & Goran, 2000; Yee et al., 2001; Millard-Stafford et al., 2001a; Fields & Allison, 2012). Several studies have investigated the reproducibility of FM determination by ADP with CV% for adults ranging from 0.3 to 4.5% in sequential measurements (McCrorry et al., 1995; Iwaoka et al., 1998; Sardinha et al., 1998; Biaggi et al., 1999; Levenhagen et al., 1999; Miyatake et al., 1999; Vescovi et al., 2002; Demerath et al., 2002; Noreen & Lemon, 2006; Fields & Allison, 2012).

For most subjects/patients, ADP is a less troublesome method for measurement of body density compared with HW, which makes it more suitable for measurement of children, disabled patients, elderly, etc. Furthermore, a special ADP equipment for paediatric use (PEAPOD, Cosmed srl, Rome, Italy) has been developed, making measurement of infants possible (Ellis et al., 2007). This makes it an appealing alternative to HW in determining body density for use in multicompartiment models and for estimation of body composition of infants.

In general, for measurement of body composition, both HW and ADP are limited by the validity of the assumptions

that underlie conversion of ρ_B to composition. In healthy adults, the body composition measurement of densitometry-based two-compartment methods can be sufficiently accurate as found by Wang et al. (Wang et al., 1998) in a thorough study comparing 16 different methods to a six-compartment method. In other populations where the assumptions regarding hydration of FFM are violated, such as elderly subjects (Baumgartner et al., 1991; Goran et al., 1998), athletes (Moon et al., 2009a) or pregnant women (Hopkinson et al., 1997), the body composition measurement can be less accurate. Combining ρ_B and measurement of TBW in a three-compartment method improves accuracy significantly in several studies (Bergsma-Kadijk et al., 1996; Hopkinson et al., 1997; Withers et al., 1998; Wang et al., 1998; Clasey et al., 1999; Bosy-Westphal et al., 2003). In populations/subjects where it is unclear whether assumptions are met, densitometry-based methods are best used in combination with other methods in multi-compartment models.

Bioimpedance analysis

Bioimpedance analysis (BIA) is based on the electrical conductive properties of the human body. An electrical current will mainly pass through the compartment with the lowest resistance, which in the human body is the electrolyte-rich water. The conductivity will therefore be proportional to total body water (TBW) and to tissue with high water concentration (e.g. skeletal muscle). Impedance is the frequency-dependent resistance of a conductor to the flow of an alternating current.

In practice, BIA is performed by placing surface electrodes on the subject. This is primarily done in a tetrapolar arrangement, where drive electrodes are placed at the wrist and ipsilateral ankle (Kyle et al., 2004a). The potential drop – and hence the impedance, through the body – is measured by two measurement electrodes placed slightly proximally from the drive electrodes. By approximating the human body as a cylinder, the equation for the volume (V) of a conducting cylinder can be applied as follows:

$$V = \frac{\rho L^2}{R} \quad (9)$$

where R is the measured resistance, the length of the cylinder (L) (substituted by the height of the subject) and the specific resistivity of the tissue (ρ), which is an approximated weighted average of the resistivities of the tissues the current passes through (Geddes & Baker, 1967; Rush et al., 2012).

Equation 9 is accurate for a cylindrical conductor with a uniform cross-sectional area and a homogenous composition. This is obviously not valid in the human body; thus, the equation cannot be used directly to calculate TBW or FFM. Instead, the impedance index (L^2/R) is used as an independent variable in statistical regression equations. These equations have been developed by regressing L^2/R measured for a large group of subjects against TBW and/or FFM determined from a reference method (Kyle et al., 2004a). Some prediction



Figure 3 Air displacement plethysmography system (BodPod). Courtesy of Cosmed srl, Rome, Italy.

equations use a combination of impedance data and anthropometry (Chumlea et al., 2002; Sun et al., 2003).

An alternative to the whole-body approach is performing segmental BIA by applying additional electrodes, the placement of which is dependent on the segments of interest (Lorenzo & Andreoli, 2003). Segmental BIA can be used to determine fluid shifts and fluid distribution in pathological states such as renal failure or ascites (Zillikens et al., 1992; Zhu et al., 1999, 2000) while it is questionable whether it improves whole-body composition measurement (Thomas et al., 2003).

When applying the before-mentioned tetrapolar arrangement of electrodes, the parts of the body with the smallest cross-sectional area will mainly determine the measured resistance. Segmental BIA has demonstrated that whole-body impedance is dominated by the resistance in the extremities compared with the trunk (Baumgartner et al., 1989). A study of cirrhotic patients with ascites before and after paracentesis has shown that BIA incorrectly determines the volume of fluid removed by the procedure (Pirlich et al., 2000). This implies that whole-body BIA has limited sensitivity in evaluating the trunk of the body and is therefore less suitable for patients with an uneven distribution of fat (e.g. extreme truncal obesity) (Swan & McConnell, 1999).

Measurement of body composition by BIA can be affected by a number of factors: previous exercise, body position, skin temperature and dietary intake (Kushner et al., 1996; Gudivaka et al., 1996). Recommendations regarding standardized measurement conditions and subject preparation are summarized in the review by Kyle (Kyle et al., 2004b). The validity of the body composition measurement by BIA is highly dependent on whether the examined subject matches the reference population from which the regression equations are obtained (Roubenoff et al., 1997; Ellis et al., 1999; Buchholz et al., 2004). The statistical relation between L^2/R and body composition varies with age, ethnicity, hydration, health status, etc. (Buchholz et al., 2004), which makes it imperative to select a suitable BIA equation for a given patient subcategory. Many proposed prediction equations are developed using a two-compartment method or

DXA as criterion method. As these methods to some extent are limited by their assumptions regarding hydration, the derived regression equation can be based upon erroneous estimations of FFM and FM. BIA equations based on multicompartment models as criterion method are listed in Table 1.

Bioelectrical impedance spectroscopy (BIS) is an alternative method to utilize the conductive properties of tissue to obtain body composition variables. By changing the frequency of the electrical current, two components of the impedance can be determined: capacitive impedance (reactance) and resistive impedance (resistance). The capacitance arises from cell membranes and the resistivity from extra- and intracellular water. At very low frequency, the current does not penetrate the cell membrane and therefore only passes through the extracellular water (ECW). At high frequencies, the measured R reflects the combined intra- and extracellular water. As application and measurement of current at zero or infinitely high frequencies is not possible in practice, the R-values are predicted using nonlinear, least-squares curve fitting (Cole-plot). Mixture theories incorporate the resistance values to predict fluid volumes (de Lorenzo et al., 1997; Matthie, 2005). Calculation of TBW additionally requires anthropometric parameters (weight, height), an assumed body density and empirically derived resistivity constants (Jaffrin & Morel, 2008).

BIS has been compared with gold standard methods (dilution) for estimation of TBW in several studies including healthy young adults (Armstrong et al., 1997; de Lorenzo et al., 1997; Moissl et al., 2006), healthy elderly (Sergi et al., 2006), haemodialysis patients (Cox-Reijven et al., 2001; Moissl et al., 2006), patients with congestive heart failure (Sergi et al., 2006), adolescent athletes (Quiterio et al., 2009), infants (Collins et al., 2013) and pregnant women (Lof & Forsum, 2004). Overall, these studies find small bias, but wide LOA (± 5 l), suggesting substantial individual variation in accuracy of the TBW estimate.

Body composition measurement by BIA compared with four-compartment methods has shown bias in determining % body fat of -10 to 5% , with LOA ranging up to $\pm 8\%$ in

Table 1 BIA equations derived from three or four-compartment methods for calculation of FFM (kg).

	Population	n	Equation (FFM)	Reference
Deurenberg et al.	Healthy adults	661	$-12.44 + 0.34 \times \text{Height}^2 / R_{50} + 0.1534 \times \text{Height} + 0.273 \times \text{weight} - 0.127 \times \text{age} + 4.56 \times \text{sex}$	(Deurenberg et al. 1991)
Sun et al.	Women 12–94 year	1095	$-9.529 + 0.696 \times \text{Height}^2 / R_{50} + 0.168 \times \text{weight} + 0.016 \times R_{50}$	(Sun et al., 2003)
Sun et al.	Men 12–94 year	734	$-10.678 + 0.652 \times \text{Height}^2 / R_{50} + 0.262 \times \text{weight} + 0.015 \times R_{50}$	(Sun et al., 2003)
Heitmann	Healthy adults 35–65 year	139	$-14.94 + 0.279 \times \text{Height}^2 / R_{50} + 0.181 \times \text{weight} + 0.231 \times \text{Height} + 0.064 \times \text{sex} - 0.077 \times \text{age}$	(Heitmann 1990)
Baumgartner et al.	Elderly 65–94 year	98	$-1.732 + 0.28 \times \text{Height}^2 / R_{50} + 0.27 \times \text{weight} + 4.5 \times \text{sex} + 0.31 \text{ thigh circumference}$	(Baumgartner et al., 1991)
Dey et al.	Elderly	106	$11.78 + 0.499 \times \text{Height}^2 / R_{50} + 0.134 \times \text{weight} + 3.449 \times \text{sex}$	(Dey et al. 2003)
Houtkooper et al.	Male, female 10–14 year	94	$1.31 + 0.61 \times \text{Height}^2 / R_{50} + 0.25 \times \text{weight}$	(Houtkooper et al., 1992)

R_{50} - Resistance 50 kHz. Height in cm. Weight in kg. Thigh circumference in cm. Sex: 1 = men, 0 = women.

healthy adults (TE FFM 1.7–6.9 kg) (Fuller et al., 1992; Wang et al., 1998; Jebb et al., 2000; Chouinard et al., 2007; Moon et al., 2013). Prediction equations developed for specific populations include obese adults, elderly and children. BIA prediction equations developed for paediatric use compared with a four-compartment model show bias in % body fat from -2.7 to 13.7% , with vast individual variation ($LOA \pm 12\%$) (Wells et al., 1999; Radley et al., 2009; Talma et al., 2013). The best agreement was found using the equation proposed by Houtkooper et al. (Houtkooper et al., 1989, 1992) with bias in % body fat of -2.7% and $LOA \pm 8\%$ (Wells et al., 1999). Results from BIA validation studies in the elderly population also show varying degree of accuracy. One study found that FM was progressively underestimated by BIA, especially for female subjects [Baumgartner equation (Baumgartner et al., 1991)] (Goran et al., 1997), while others have found acceptable accuracy in a group of elderly, but large individual variation dependent on the prediction equation used (Moon et al., 2013). No generalized BIA equations have yet been developed for athletes using a four-compartment criterion method (Moon, 2013).

Assessing longitudinal changes in FFM and FM is controversial when significant weight loss occurs, due to the concurrent change in volume and composition (and hence resistivity) of the conducting tissue. Clinical studies in various populations including obese adults (Evans et al., 1999; Minderico et al., 2008; Johnstone et al., 2014), athletes (Matias et al., 2012) and elderly healthy subjects (Moon et al., 2013) show that BIA has limited accuracy on the individual level to track longitudinal changes in FM and FFM compared with a four-compartment model.

BIA as a method of body composition measurement has several advantages in being a safe, observer-independent, inexpensive field method that is easy to perform. The validity is highly dependent on selecting a regression equation suitable for the subject category in question. Ideally, results from a BIA equation in a given population should be cross-validated in a random subsample against estimates from a criterion method (e.g. multicompartiment method). TBW can be estimated by BIS with acceptable accuracy for evaluation of a group mean, but has questionable validity in individuals.

Dual-energy X-ray absorptiometry

Dual-energy X-ray absorptiometry (DXA) was originally developed for diagnosing and monitoring osteoporosis, by measuring bone mineral density (BMD) (Pietrobelli et al., 1996; Bonnick, 2009). The technique is based on the attenuation of calibrated X-ray beams with dual photon energy. Each atomic element will have a characteristic mass attenuation coefficient for a given photon energy (Cherry et al., 2003). When photons at two different energies pass through an absorber, attenuation can be expressed as a ratio (R) of attenuation at the lower energy to attenuation observed at the higher energy (Heymsfield et al., 1989; Mazess et al., 1990). Measurement of

body composition by DXA is based on discrimination of three compartments, distinguished by their R-value; FM, lean soft tissue (LST) and bone mineral. The theoretical R-values for constituents of fat and lean soft tissue have been validated in both *in vivo* and *in vitro* studies (Pietrobelli et al., 1996).

In practice, a rectilinear scan is performed and the R-value is measured in each pixel. This enables the scanner software to separate the pixels of the scan in to those containing bone mineral + soft tissue and those with soft tissue only (Plank, 2005). In the pixels only containing soft tissue (ST), fat (F) and lean (L) fraction (f) can be calculated from the following principles (Gotfredsen et al., 1986):

$$R_{ST} = f_L \times R_L + f_F \times R_F \quad (10)$$

$$f_L + f_F = 1 \quad (11)$$

Soft tissue compartment in the pixels containing bone cannot be measured directly, as the dual-energy principle only allows discrimination of two compartments. Instead, the overlying soft tissue is estimated using complex algorithms based on anatomical site and distribution of soft tissue in adjacent pixels (Nord & Payne, 1995; Pietrobelli et al., 1996). The extent to which the composition in the excluded area differs from the measured area is a source of systematic error for the individual subject. The software approach to this problem is proprietary information and varies between manufacturers and software versions.

There are multiple commercially available versions of DXA scanners and software by various manufacturers. Studies have found substantial variations in body composition measurements comparing machines from different manufacturers (Soriano et al., 2004; Malouf et al., 2013), different models from the same manufacturer (Mazess & Barden, 2000; Hull et al., 2009; Malouf et al., 2013) or the same scanner model (Economos et al., 1997; Lantz et al., 1999; Guo et al., 2004). Additionally, software upgrades can produce inconsistent results in the same scanner (Van et al., 1995). Both inter- and intramanufacturer comparisons and cross-calibrations are important for investigators, particularly during the course of longitudinal studies and in the context of multicentre trials.

It has previously been questioned to which degree the DXA measurement of body composition is affected by soft tissue hydration status. Pietrobelli et al. found in an experimental study that DXA fat estimation errors occur as a function of added fluid, but conclude that the error in fat estimation is relatively small ($<1\%$) and should not affect the accuracy of the body composition measurement substantially (Pietrobelli et al., 1998). In accordance, clinical studies have shown fat mass measurement on haemodialysis patients to be unaffected by fluid changes (Formica et al., 1993) and paracentesis of ascites did not alter fat mass measurement by DXA (Haderslev et al., 1999). A study comparing DXA and a four-compartment model including measurement of TBW found no correlation between hydration of FFM and difference in measured BF (LaForgia et al., 2009).

Other sources of potential errors in DXA measurement involve inaccurate positioning of the subject, especially for regional composition analysis (Libber *et al.*, 2012), presence of metallic implants (Henderson *et al.*, 2001), antecedent administration of radioactive tracer to the patient (Fosbol *et al.*, 2012) or the limitation that some subjects are too wide or too tall for the scan field. The latter can be compensated for in tall subjects by scanning the head and trunk + limbs in two separate scans (Evans *et al.*, 2005a; Santos *et al.*, 2012). Alternatively, the scan can be performed with the subject adopting a knee-bent position (Silva *et al.*, 2013). Obese subjects too wide for the scan field can be measured by performing a half-body scan, which is validated in studies using Lunar densitometers (Tataranni & Ravussin, 1995; Rothney *et al.*, 2009; Breithaupt *et al.*, 2011).

Body composition measurements by DXA compared with four-compartment models have shown good correlation between the two approaches in adult subjects. The majority of studies show bias in determination of % body fat from -3.8 to 2.8 % (Fuller *et al.*, 1992; Bergsma-Kadijk *et al.* 1996; Prior *et al.*, 1997; Withers *et al.*, 1998; Clasey *et al.*, 1999; Gallagher *et al.*, 2000; Arngrimsson *et al.*, 2000; Deurenberg-Yap *et al.*, 2001; Van Der Ploeg *et al.*, 2003b; Williams *et al.*, 2006; Santos *et al.*, 2010). Large individual differences in % body fat (LOA ranging up to $\pm 10\%$) were found in some studies of healthy normal weight subjects (Van Der Ploeg *et al.*, 2003b) and athletes (Arngrimsson *et al.*, 2000). In general, there was a tendency that DXA progressively underestimated FM in lean individuals (Withers *et al.*, 1998; Gallagher *et al.*, 2000; Arngrimsson *et al.*, 2000; Van Der Ploeg *et al.*, 2003b; Sopher *et al.*, 2004). This error is most likely due to X-ray beam hardening that causes the measured R-value to increase with decreasing tissue thickness (Goodsitt, 1992), which will result in overestimation of FFM. Other researchers have found an overestimation of FM compared with four-compartment methods (Williams *et al.*, 2006; Santos *et al.*, 2010). These conflicting results may arise from variations in DXA scanner hardware and/or different software algorithms to compensate for beam hardening, although no systematic bias between manufacturers has been found in the mentioned studies.

The precision of DXA determination of whole-body FM expressed as CV ranges from 0.8 to 2.7% (SEM 0.39–0.5 kg) and for lean soft tissue 0.4–1.3 % (SEM 0.35–0.54 kg) in sequential measurements (Johnson & Dawson-Hughes, 1991; Tothill *et al.*, 1994; Kiebzak *et al.*, 2000; Cordero-MacIntyre *et al.*, 2002; Genton *et al.*, 2006; Hind *et al.*, 2011; Fosbol *et al.*, 2012). Precision of regional body composition measurements has been reported to be poorer than whole-body measurements, particularly for fat mass of the trunk (Johnson & Dawson-Hughes, 1991; Tothill *et al.*, 1994; Kiebzak *et al.*, 2000; Cordero-MacIntyre *et al.*, 2002; Hind *et al.*, 2011).

A newer software application for DXA scanners provides the possibility to estimate visceral adipose tissue (VAT). The technique is based on identification of subcutaneous adipose tissue

(SAT) in the abdominal (android) region by measuring the width of SAT along the lateral extent of the abdomen. Using empirically derived geometric constants, the abdominal SAT is calculated. VAT is calculated by subtracting SAT from the total fat mass in the abdominal region (Kaul *et al.*, 2012). The method has been validated against CT determination of VAT, which is considered gold standard. Studies have shown a tendency for DXA to overestimate VAT, which seems to be increasing with body weight. A study of a heterogeneous population of normal weight and obese men and women found substantial bias and large limits of agreement in determination of VAT volume (bias 56 cm³ and LOA -355 to 468 cm³) (Kaul *et al.*, 2012; Bredella *et al.*, 2013). CV in short-term repeat measurement has been found to be 7.3–9.8% (Ergun *et al.*, 2012). Further development of the method is necessary before DXA measurement of VAT can be considered an alternative to CT or MRI.

Total body skeletal muscle mass (SM) can be estimated using DXA measurement of the appendicular lean soft tissue (ALST). Regression equations to calculate SM from ALST have been derived from skeletal muscle mass measured by CT or MRI. Validated equations exist for calculation of SM for adults, adolescents and children of diverse ethnicity (Kim *et al.*, 2002, 2004, 2006). Whether the method can be used for tracking longitudinal changes in SM is not fully disclosed, as a study of obese adolescents has shown systemic bias where DXA tended to overestimate SM gain (Lee & Kuk, 2013).

The advantages of DXA as a method of body composition measurement include observer independence, excellent precision for whole-body measurements, modest demands on the cooperability of the patient and relatively low financial cost once the equipment is installed. Additionally, reproducible measurement of regional body composition can be performed with DXA (Gjorup *et al.*, 2010) with low radiation dose compared with computed tomography [5–10 μ Sv for a DXA whole-body scan (Blake *et al.*, 2006)]. For paediatric measurement of body composition, DXA has the advantage of not being dependent on assumptions regarding bone density as the densitometry methods (i.e. HW and ADP). Scan time is relatively short (approximately 5 min), and in most cases, sedation of the child is not necessary.

The validity of DXA measurement of body composition is reduced in very lean or highly obese subjects, as several researchers have shown a progressive underestimation of FM in lean individuals and corresponding overestimation in obese subjects. Whether DXA measurement of body composition is affected by hydration status of the subject is not fully elucidated. The theoretical R-value of LST is based on the assumption of constant hydration, which could limit the applicability of DXA in subjects with altered hydration, but this has not been the case in clinical and experimental studies.

Another limitation of DXA is the variation in body composition measurement between different scanners and software versions. Cross-calibration is therefore always warranted following software updates and/or acquisition of new scanner

equipment as recommended by the International Society of Clinical Densitometry (Hangartner et al., 2013).

Imaging – CT and MRI

Imaging methods, such as computed tomography (CT) and magnetic resonance imaging (MRI), are considered the most accurate methods for *in vivo* quantification of body composition on the tissue level. Measures obtained using CT or MRI may be classified as total adipose tissue (TAT), subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT) and interstitial adipose tissue (IAT). Skeletal muscle can be compartmentalized into individual muscles or muscle groups. This level of specificity in tissue composition is only possible with CT or MRI (Ross & Janssen, 2005).

The basic CT system consists of an X-ray tube and detector that rotate in a perpendicular plane to the subject. The X-ray beam is attenuated as it passes through tissue, and the images are reconstructed with mathematical techniques. Each pixel of the CT image has a Hounsfield unit (HU) number as a measure of the attenuation relative to water (HU water = 0, HU air \approx -1000). Physical density is the main determinant of attenuation or HU-value. The HU-value for adipose tissue is between -190 and -30 and skeletal muscle 30–100 HU. The cross-sectional area (cm²) for the different tissues on each image can be determined by either manual or computerized segmentation discriminated by HU number (Kvist et al., 1986; Chowdhury et al., 1994). Total volume can be calculated by integrating data from consecutive slices. The different geometric models for estimating volume of adipose tissue are described in detail by Shen et al. (Shen et al., 2003).

The validity and accuracy of body composition measurement from CT have been validated by studies of human cadavers in measurement of abdominal adipose tissue (Rossner et al., 1990), thigh muscle mass (Engstrom et al., 1991) and AT of extremities (Mitsiopoulos et al., 1998). The studies compared the cross-sectional area (CSA) of the tissue of interest defined on CT images with the corresponding area measured on the cadaver. The accuracy of calculation of tissue volume has not been validated by cadaver studies. Reproducibility of sequential measurements of VAT expressed as CV ranges from 0.6 to 12.3% (Kvist et al., 1986; Thaete et al., 1995; Figueroa-Colon et al., 1998; Maurovich-Horvat et al., 2007; Yoon et al., 2008).

The radiation dose when using CT for whole-body composition is substantial, which especially is a concern if several measurements are planned during a course of treatment or clinical trial. MRI does not involve exposure to ionizing radiation and is based on the interaction between the hydrogen nuclei in the human body. When placed in a powerful magnetic field, the hydrogen nuclei will align themselves with the magnetic field in a known direction. A radio frequency (RF) pulse is applied causing the hydrogen nuclei to absorb energy. This energy is released as the nuclei return to the aligned state

in form of a RF signal. This signal is used to generate the magnetic resonance images (Edelman et al., 2006).

As MRI does not provide information on tissue density (as HU in CT images), segmentation of different tissues can be performed by manual delineation or automatically segmented based on either signal intensity, semiautomated tagging AT ('Seed growing function') or morphology (Arif et al., 2007; Shen & Chen, 2008; Gray et al., 2011). Fully automated analysis methods are faster and more reproducible than methods dependent on manual delineation, but may introduce errors regarding accuracy of adipose tissue measurement (Arif et al., 2007). Most often a semiautomatic approach is applied taking advantage of time-saving and precise automated procedures, but allowing manual correction to achieve accuracy (Shen & Chen, 2008).

MRI measurement of body composition has been validated in phantoms, animals and human cadavers, showing good agreement with values produced by dissection and chemical analysis (Fowler et al., 1991; Ross et al., 1991; Abate et al., 1994; Mitsiopoulos et al., 1998). The degree of reproducibility is dependent on the segmentation method, with CV% varying from 0.3 to 1.7% in determination of subcutaneous adipose tissue and 3.5–9.4 % in visceral adipose tissue (Sohlstrom et al., 1993; Elbers et al., 1997; Kullberg et al., 2007; Arif et al., 2007; Wald et al., 2012).

MRI for whole-body composition measurement is time-consuming and costly, and most facilities have limited capacity making the method less feasible for large-scale studies. As an alternative, whole-body adipose tissue or skeletal muscle can be estimated from a preselected level of a CT or MR image, assuming a linear relationship between CSA and whole-body composition (Lee et al., 2004; Shen et al., 2004; MacDonald et al., 2011). This is an appealing option for patients undergoing a routine diagnostic CT or MRI, for example in cancer staging, to avoid additional examinations. For example, a single mid-thigh-level slice from MRI has been used for estimation of muscle mass, with acceptable correlation coefficients $r^2 = 0.84$ – 0.9 (Lee et al., 2004). Similarly, a linear relationship exists between a slice 5 cm above lumbar vertebra L4–L5 and whole-body skeletal muscle with $r^2 = 0.855$ and adipose tissue $r^2 = 0.927$ (Shen et al., 2004). This relationship was maintained over a wide range of body composition, as well as in cancer and sarcopenic obesity (Mourtzakis et al., 2008). The potential pitfalls in estimating whole-body composition from CSA include errors in patient positioning, slice selection and image interpretation, including erroneous identification of specific muscles or intestinal content identified as adipose tissue (Potretzke et al., 2004).

Over the recent years, another type of magnetic resonance technology – quantitative magnetic resonance (QMR), EchoMRI (Echo Medical Systems, LLC, Houston, TX, USA) – has shown promising results in accurate measurement of body composition (Taicher et al., 2003). QMR is not an imaging modality (as MRI), but uses the differences in the nuclear magnetic resonance properties of hydrogen nuclei in organic

and non-organic environments to fractionate signals originating from fat, lean tissue and free water (Napolitano et al., 2008). Compared with a four-compartment model in adults, QMR tends to underestimate fat mass and the deviation is enhanced with increasing fat mass ranging up to 15% underestimation (Napolitano et al., 2008; Swe et al., 2010). On the other hand, QMR overestimated fat mass by up to 10% in infants and children (Andres et al., 2011). Precision in repeated measurements stated as CV in determination of fat mass is reported as 0.44% for adults and 1.42% for children (Gallagher et al., 2010; Andres et al., 2011).

QMR has advantages in being observer-independent, fast (scan time < 3 min), with capacity to accommodate subjects up to 250 kg and reported excellent precision (Gallagher et al., 2010), but further research and development is needed to improve accuracy and validate its applicability in research and clinical practice.

Anthropometry and ultrasound

Anthropometry involves measurement of body dimensions – length, width, circumference and skinfold thickness. Skinfold thickness has for many years been an accepted predictor of body density and total body fat. More than 19 sites for measuring skinfold thickness have been described, and at least 50 prediction equations are frequently used to calculate fat or fat-free mass from skinfold measurement (Wang et al., 2000). The majority of the prediction equations include skinfold thickness from several sites as well as other anthropometric variables, such as height or weight (Bellisari & Roche, 2005).

The use of skinfold thickness to estimate % body fat is based on the implicit assumption that there is a fixed relationship between SAT in predefined anatomical locations and total body fat. This relationship is dependent on various factors such as age, sex and health status (Durnin & Womersley, 1974; Lohman, 1981; Baumgartner et al., 1991). Classical, but still frequently used regression equations to predict FM such as those proposed by Jackson & Pollock (Jackson & Pollock, 1978; Jackson et al., 1980) are derived from measurement of body density by hydrodensitometry and calculation of FM by the Siri equation (Eq.6) (Siri, 1956). As previously discussed in this paper, the densitometric approach has limitations regarding hydration and mineral content of FFM, which could introduce errors in calculation of FM. More recently developed skinfold regression equations are derived from four-compartment methods, which should provide theoretically improved accuracy of the body composition measurement (Peterson et al., 2003; Evans et al., 2005b).

Studies comparing % body fat estimated by skinfold measurements and four-compartment methods reveal acceptable mean difference between the results, but the majority of studies show vast individual variation and wide LOA ranging from –13 to 22% body fat (Goran et al., 1997; Beddoe & Samat, 1998; Peterson et al., 2003; Van Der Ploeg et al., 2003a; Gause-Nilsson & Dey, 2005; Bellisari & Roche, 2005). An

exception is a cross-validation study of the Evans model (Evans et al., 2005b) in female athletes with acceptable LOA at approximately –6 to 3.5% body fat compared with a four-compartment method (Moon et al., 2009b). However, the same skinfold regression equation was used to determine body composition changes in highly trained athletes (male judo athletes) and showed large individual variation in FM and FFM determination measured by a multicompartiment model (Silva et al., 2009).

The measurement of skinfold thickness may appear simple to perform, but substantial intra- and interobserver variability has been observed (Nagy et al., 2008). Reasons for this variability include use of different callipers, location of the anatomical sites for measurement and variation in technique of grasping the skinfold. Additionally, measurement of skinfold thickness has practical limitations regarding difficulties in raising skinfold for measurement at certain body locations, presence of oedema and measurement of very obese patients with skinfolds too thick for accurate calliper application (Bray et al., 1978; Himes, 2001).

Ultrasound is an alternative approach to measurement of tissue thickness of SAT, muscle and intra-abdominal depth. An advantage of ultrasound compared with skinfold measurement is the possibility to measure very obese subjects and at anatomical locations where callipers cannot be applied (Kuczmarski et al., 1987; Bazzocchi et al., 2011; Pereira et al., 2012; Muller et al., 2013). Measurements can be performed by A- and B-mode technology. A-mode (amplitude mode) ultrasound displays tissue discontinuity as spikes on a graph (with tissue depth on the x-axis and signal amplitude on the y-axis). Tissue boundaries are determined from the amplitude of the spikes (Wagner, 2013). The B-mode (brightness modulation) method, which is the most frequently applied ultrasound technique, is based on a linear array transducer (frequency 5–7.5 MHz), which produces a two-dimensional image. The tissue boundaries are determined visually on the monitor, and thickness is measured with the use of electronic callipers (Bellisari & Roche, 2005). The accuracy of the B-mode method is greatly dependent on selecting the correct location for measurement and placing the electronic callipers accurately. With a standardized protocol and operator training, ultrasonic measurement of SAT is reproducible in both healthy subjects (Toomey et al., 2011), obese adults (Bazzocchi et al., 2011) and athletes (Muller et al., 2013). Softwares to aid discrimination of tissue boundaries are emerging for both A- and B-mode ultrasound, which may further improve accuracy and reproducibility (Wagner, 2013; Muller et al., 2013).

Anthropometric methods are popular due to the fact that they are inexpensive, well-tolerated field methods. The required equipment for skinfold thickness is simple and seemingly easy to use. To achieve sufficient precision and minimize interobserver variability, it is important to make use of standardized protocols and operator training. Body composition measurement by skinfold thickness can be used for large population studies, but considerable interindividual variation makes it less suitable for other purposes.

Conclusion

This review supplies a brief overview of contemporary methods for body composition – their advantages, limitations and applicability in clinical practice. As no single method directly and adequately can quantify FM and FFM *in vivo*, all methods rely on assumptions, the accuracy of which varies with patient characteristics – age, sex, nutritional state, etc. These assumptions are important to consider, as violations may cause erroneous results. Some methods are based on the assumption of constant hydration of FFM such as the densitometric methods and BIA, which makes them less suitable for subjects with altered hydration due to illness or ageing, unless they are combined with measurement of TBW. Other methods rely on prediction equations, where the accuracy is largely dependent on the degree of which the subject matches the reference population. Therefore, no general recommendations can be made regarding the appropriate method for a given patient subcategory as many variables must be considered.

Another consideration in planning body composition studies is which level of body composition is relevant to quantify, cf. the five-level model previously described. CT or MRI can provide accurate regional estimates of body composition on the tissue level, such as visceral adipose tissue, but in most cases, these methods are less suited for whole-body fat mass determination. Other modalities, for example ADP, are limited to estimation of whole-body composition only.

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Precision of body composition measurement is dependent on several variables; inherent error of the equipment used for measurement, subject characteristics and observer error. It is recommended that precision studies are performed at each facility in a random subsample of the given population using the equipment in question. Hereby, the least significant change (LSC) in body composition variables can be calculated. In longitudinal studies, standardized measurement conditions are also of great importance, for example time of day, before/after exercise, after bladder emptying, etc. In addition, the results of body composition measurement using, for example, DXA will vary dependent on manufacturer and software type. This further complicates the comparison of methods of body composition measurement. As equipment and software for each modality are under continuous development, there is a constant need for each investigator to be updated, to make optimal decisions on which modality or combination of modalities to be used.

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Conflict of interest

The authors have no conflict of interest.

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