INSTITUTE OF PHYSICS PUBLISHING PHYSICS IN MEDICINE AND BIOLOGY

Phys. Med. Biol. **51** (2006) R203–R228 doi:10.1088/0031-9155/51/13/R13

REVIEW

Development of methods for body composition studies

Sören Mattsson[1] **and Brian J Thomas**[2]

[1] Department of Radiation Physics, Lund University, Malmö University Hospital, SE-205 02 Malmö, Sweden
[2] School of Physical and Chemical Sciences, Queensland University of Technology, Brisbane, QLD 4001, Australia

Received 21 February 2006, in final form 29 March 2006
Published 20 June 2006
Online at stacks.iop.org/PMB/51/R203

Abstract

This review is focused on experimental methods for determination of the composition of the human body, its organs and tissues. It summarizes the development and current status of fat determinations from body density, total body water determinations through the dilution technique, whole and partial body potassium measurements for body cell mass estimates, *in vivo* neutron activation analysis for body protein measurements, dual-energy absorptiometry (DEXA), computed tomography (CT) and magnetic resonance imaging (MRI, fMRI) and spectroscopy (MRS) for body composition studies on tissue and organ levels, as well as single- and multiple-frequency bioimpedance (BIA) and anthropometry as simple easily available methods. Methods for trace element analysis *in vivo* are also described. Using this wide range of measurement methods, together with gradually improved body composition models, it is now possible to quantify a number of body components and follow their changes in health and disease.

1. Introduction

Body composition research covers a wide range of sciences: physics and technology, biology, medicine and public health. Studies of body composition of living man require reliable measurements. In the early days, such measurements were limited to those of weight and length. From these two parameters, the body mass index (BMI, weight (kg)/height^2 (m^2)) can be calculated and is currently the most commonly used index of under- and over-nutrition, and may be used to roughly predict body composition. In more detailed studies, submersion in water could be used to get the volume, which together with the weight information gives the mean density of the body.

The discovery of x-rays and the continuous development of that technology gave totally new insights into the composition of the human body—information which earlier was exclusively for the post-mortem investigator. Through total body potassium determination by means of measurements of the rare and long-lived naturally occurring potassium isotope

^{40}K in background-shielded laboratories, it became possible to estimate the body cell mass. The rapid development in radiation measurement technology and in atomic and nuclear physics gave rise to a number of *in vivo* measurement technologies using nuclear reactions (Cohn and Parr 1985, Sutcliffe 1996) and x-ray fluorescence analysis (Börjesson and Mattsson 2004). In parallel, various more or less detailed models to describe the various compartments in the human body and their interactions were developed (Wang *et al* 1992).

A great step forward in *in vivo* body composition analyses is connected to the intense development of the medical imaging technology, first taking x-ray computed tomography (CT) (Sjöström *et al* 1986) and later nuclear magnetic imaging (MRI) (Foster *et al* 1984) and spectroscopy (MRS) (Boesch *et al* 1997) into use. This has given a possibility of refining and detailing the various body composition models. It has also given a possibility of revealing links between structures within the body and its functions, giving the area an increasing importance in clinical research and practice as well as in public health (Pierson 2003).

In parallel to this intensive development of advanced analytical methods, there has been a need to develop more simple equipment such as instruments for air displacement plethysmography (to measure body volume as an alternative to underwater weighing), 3D body volume scanning, bioimpedance analysis (BIA) as well as for stationary or portable dual-energy x-ray photon absorptiometry (DEXA).

The mechanisms and health implications of fat and its distribution represent an area of very intensive research in wealthy countries, as is the relation between physical activity (fitness) and health. The other side of the world's problem is a severe under-nutrition of large population groups and an important question is whether body composition measurement could be of any value in the management of this under-nutrition. Similar problems exist in clinical practice with regard to critically ill patients.

Ten years ago, Sutcliffe (1996) wrote an extensive and still very valuable review paper of the field for this journal. The aim of the present review is to shortly summarize 50 years of developments in the field of body composition studies with special reference to the physics and technology used and also to give the readers an update of available measurement methods for total body and regional body composition studies.

2. Models for body composition

The human body can be described in various—more or less detailed—ways. The total body mass can be seen as a sum of all atoms, or all molecules, or all cells, or all tissues/organs in the body, etc (Wang *et al* 1992, Shen *et al* 2005). This is schematically described in tables 1 and 2(a).

The development of various models to describe the human body composition has been closely linked to the possibility for measurements. The earliest model (molecular level) divided the body into fat mass (FM) and fat-free mass (FFM). The mean density of any subject (measured e.g. by underwater weighing) could be used to determine the FM and FFM proportions of the body after correction for the lung volume. The introduction of shielded low-background laboratories with possibilities for total body potassium-40 measurements opened a possibility of estimating the FFM on the basis that potassium is mainly present in FFM. Later, various three-, four-, five- and six-compartment models were developed as schematically shown in tables 1 and 2(a).

Table 2(a) gives an overview of the most often used compartment models used depending on the base for the description of the body (in terms of atoms, molecules, organ/tissues, etc). In table 2(b), the various measurement methods, which are used to determine the size of the various components, are indicated.

Table 1. Simplified descriptions of the human body, in terms of atoms, molecules, cells, tissues, etc (after Wang *et al* 1992, 2005b, Ryde *et al* 1993).

Level	Model components	Number of compartments
Atomic	BM = O + C + H + N + Ca + P + S + K + Na + Cl + Mg + ⋯	>11
Molecular	BM = FM + FFM	2
	BM = FM + Mo + residual	3
	BM = FM + TBW + nonfat solids	3
	BM = FM + TBW + TBPro + M	4
	BM = FM + TBW + TBPro + M + G	5
	BM = FM + TBW + TBPro + Mo + Ms + CHO	6
Cellular	BM = cells + ECF + ECS	3
	BM = FM + BCM + ECF + ECS	4
Tissue/organ	BM = AT + SM + bone + visceral organs + other tissues	5
Whole body	BM = head + trunk + appendages	3

AT = adipose tissue; BCM = body cell mass; BM = body mass; CHO = carbohydrates; ECF = extracellular fluid; ECS = extracellular solids; FFM = fat-free mass; FM = fat mass; G = glycogen (main carbohydrate); M = minerals; Mo = bone minerals; Ms = soft tissue minerals; SM = skeletal muscle; TBPro = total body protein; TBW = total body water.

2.1. Atomic level

More than 96% of body mass is made up by the elements oxygen (61%), carbon (23%), hydrogen (10%) and nitrogen (2.5%). Other major elements are calcium (1.4%), phosphorus (1.1%), sulphur (0.20%), potassium (0.20%), sodium (0.14%), chlorine (0.14%) and magnesium (0.03%) (Emsley 1998).

Potassium can be measured *in vivo* by whole-body counting of ^{40}K and a number of the other elements (N, H, Ca, Cl, Na, P) can be analysed by neutron activation analysis *in vivo* (Morgan 2000). Total body potassium, nitrogen and carbon are used to estimate the total body cell mass, protein and fat, respectively (Sutcliffe 1996). Less frequent elements (trace elements) such as lead, cadmium, mercury and iodine can be measured *in vivo* by x-ray fluorescence analysis (Börjesson and Mattsson 2004) and in the case of cadmium also by neutron activation analysis (Krauel *et al* 1980).

2.2. Molecular level

The six major types of molecules constituting the human body are water, lipids, proteins, carbohydrates, bone minerals and soft tissue minerals.

To describe the body in terms of different molecules, various models are used (tables 1 and 2(a)). The simplest is a two-component model, which consists of FM and FFM. The three- and four-component models further divide the FFM into new components that can be measured *in vivo*. There is a specific three-component DEXA model, separating FFM into lean soft tissue and bone mineral. The widely used four-component model divides FFM into water, protein and mineral. The meaning of lipid and fat differ somewhat. Lipid includes all of the biological matter extracted with lipid solvents (e.g. by the chloroform–methanol extraction method). This means triglycerides, phospholipids and structural lipids. In contrast, fat refers to the specific family of lipids consisting of triglycerides. Approximately 90% of total body lipid in healthy adults is triglyceride (Shen *et al* 2005).

Table 2. (a) Description level for body composition (after Wang *et al* 1992, 2005b). (b) Measurement methods to determine the various components.

(a)

Atomic	Molecular	Cellular	Tissue/organ	Whole body
N, Ca, P, S, K, Na, Cl, ...	Minerals (bone, soft tissue), CHO	Extracellular solids (ECS)	Other tissues (bone, …)	Lower limbs
H	Protein		Visceral organs	
C	Lipid	Extracellular fluid (ECF)	Bone	Trunk
O	Water	Cells (Adipocytes)	Skeletal muscle	Upper limbs
			Adipose tissue (AT)	Neck
				Head

(b)

Atomic	Molecular	Cellular	Tissue/organ	Whole body
TBK, TBK and TBN	KxFFM, TBK/FFM, DEXA	3-methyl histidine, creatinine	CT, MRI	'Skinfold', Ultrasound, BIA

BIA = bioelectrical impedance analysis; creatinine = a skeletal muscle metabolite; CT = computed tomography; FFM = fat-free mass; K = potassium; TBK = total body potassium; TBN = total body nitrogen; 3-methyl histidine = a skeletal muscle metabolite; MRI = magnetic resonance imaging; 'skinfold': see section 3.8.2.

2.3. Cellular level

At the cellular level, the body is often divided into three components: cells, extracellular fluids and extracellular solids. The cells can be partitioned into two components: body cell mass and fat, of which the former is the metabolically active component.

2.4. Tissue/organ level

The major components are adipose tissue, skeletal muscle, visceral organs and bone as well as single solid organs. In the field of body composition, adipose tissue is different from fat although fat is found primarily in adipose tissue; intracellular fat (triglyceride) pools are also present in liver, skeletal muscle and other organs. There are also small circulating pools of fat mainly in the form of lipoproteins. Adipose tissue consists of adipocytes, extracellular fluid, nerves and blood vessels. These tissue components seem to be closely connected to health-related conditions (obesity, diabetes).

3. Methods for body composition studies

Since 1986, seven international conferences have provided updates on methodology as well as applications of various methods for body composition studies (Alpsten and Mattsson 1998, De Lorenzo and Mohamed 2003, Elia and Stratton 2005, Ellis and Eastman 1993, Ellis *et al* 1987, Yasumura *et al* 1990, 2000). There are also a number of extensive monographs to which the interested reader is referred (Forbes 1987, Heymsfield *et al* 2005, Kreitzman and Howard 1993).

3.1. Body fat from body density

Body fat or fat mass (FM) is one of the compartments in the two-compartment model of body composition. The other compartment being the fat-free mass (FFM). In principle (assuming a constant and known density for each of the FM and FFM components), FM may be determined if the density of the body (ρ_B) is known since in the two-compartment model

$$\frac{1}{\rho_B} = \frac{F_{FM}}{\rho_{FM}} + \frac{F_{FFM}}{\rho_{FFM}}, \tag{1}$$

where F_{FM} and F_{FFM} are the fractions of the fat and fat-free mass compartments and ρ_{FM} and ρ_{FFM} their respective densities.

Total body density can be obtained from a measure of body volume and weight. Whilst it is simple to obtain an accurate measure of body weight, the measurement of volume is demanding.

3.1.1. Hydrodensitometry (HD). Underwater weighing, or hydrodensitometry, has long been accepted as the 'gold' standard technique for determining total body volume. In this technique, the weight of the body is determined in air and again when the subject is fully submerged. Application of Archimedes's principle provides the body volume and, following correction for the residual lung volume and air in the GI tract, the body density is obtained. The correction for air in the GI tract is relatively small and the most common approach is to assume that it is a constant value of 100 ml (Buskirk 1961). Correction for residual lung volume is far more significant and may be subject to considerable error. The residual volume may be measured whilst submerged or outside the tank. Several techniques have been used for the purpose including oxygen dilution and nitrogen washout (Wilmore *et al* 1980). The residual volume may also be predicted from equations derived by a number of authors; however, the uncertainty in the estimation is large (Hackney and Deutsch 1985).

The '% body fat' can be estimated from the body density using one of a number of equations developed by various authors. Equations below were proposed

$$\text{by Siri (1956)} \qquad \%\ \text{body fat} = \left(\frac{4.95}{\rho_B} - 4.6\right) \times 100 \tag{2}$$

and

$$\text{by Brozek } \textit{et al} \text{ (1963)} \qquad \%\ \text{body fat} = \left(\frac{4.57}{\rho_B} - 4.142\right) \times 100. \tag{3}$$

Both of these equations assume a constant value for the density of FM and FFM. The use of a constant value for the density of fat is well accepted. However, the validity of using a constant value for the FFM has been questioned. Factors such as age, gender and race have been suggested as affecting the density of the FFM. Lohman (1989) demonstrated that children have a lower mineral content of the fat-free body than adults

and that body density and fat-free density are significantly affected by the lower bone mineral content. A lower density of the FFM in older white women was noted by Visser *et al* (1997) but not in black women nor black or white men. Variations from mean FFM densities have also been noted for athletes (Adams *et al* 1982, Millard-Stafford *et al* 2001) and elderly subjects with osteoporosis (Werdein and Kyle 1960).

The affect of race on the density of FFM has yet to be fully elucidated. Differences in the density of the FFM between different racial groups have been reported (Schutte *et al* 1984, Werkman *et al* 2000). However, other studies have not observed these differences (Visser *et al* 1997, Millard-Stafford *et al* 2001).

The limitations and uncertainties of the underwater weighing technique have been considered by many authors (see for example Ellis (2000)).

Notwithstanding the concerns with the accuracy of hydrodensitometry, the technique has been extensively used to estimate % body fat. However, its application is limited to subjects/populations for whom underwater weighing is possible. Generally, it may be difficult or impossible for the elderly, children and some patient groups. A relatively recent technique of air displacement plethysmography for measurement of body density obviates the need for underwater weighing.

3.1.2. Air displacement plethysmography (ADP). An alternative to underwater weighing for the estimation of body volume and hence body fat is the technique of air displacement plethysmography (ADP). A relatively new device (Bod Pod; Life Measurement Instruments) utilizes this technique. The device is comprised of a 'test' chamber and a 'reference' chamber, which are separated by a diaphragm. The subject is seated in the test chamber and the diaphragm oscillated to produce a slight change in volume and pressure in each chamber. These changes take place under adiabatic conditions and the relationship between pressure and volume is given by Poisson's law

$$\frac{\mathbf{P}_1}{\mathbf{P}_2} = \left(\frac{\mathbf{V}_2}{\mathbf{V}_1}\right)^{\gamma}, \tag{4}$$

where γ is the ratio of specific heat of the gas at constant pressure to that at constant volume.

This technique overcomes the difficulties associated with underwater weighing, but retains the uncertainties of the hydrodensitometry technique outlined above. An additional consideration of the air displacement technique is the requirement for adiabatic conditions. To minimize the effects of possibly non-adiabatic expansion/contraction of air trapped in the hair or in clothing, the subject is required to wear a bathing suit and swim cap. A correction based on body surface area is used to adjust for non-adiabatic changes of air in close contact with the skin.

The use of ADP to estimate body fat is much better tolerated than underwater weighing. Various studies have been conducted to validate the technique and to compare body fat estimates obtained from ADP with those from hydrodensitometry. The results have been somewhat conflicting.

A comparison of the % body fat in 30 healthy adults using two ADP instruments resulted in relatively large 95% limits of agreement (−3.67 to +2.5%) but with only a small bias (−0.59%) (Collins *et al* 2004). However, in a study involving 50 adults, Ball (2005) concluded that 'the interdevice variability of ADP has minimal impact on % body fat estimates'.

In an early study (McCrory *et al* 1995) involving 68 adults, no difference was found between % body fat determined by ADP and hydrodensitometry. Demerath *et al* (2002) compared the % body fat in 87 adults and 39 children by ADP and hydrodensitometry. They showed that mean % body fat from ADP was higher than from hydrodensitometry in the

adults (29.3% versus 27.7%, $p < 0.05$) but not in children. Biaggi *et al* (1999) did not observe a difference in adults (23 men and 24 women), but noted an effect of gender with ADP underestimating % body fat compared with hydrodensitometry in men and overestimating it in women. Ellis *et al* (2001) also did not observe a difference between % body fat measured by the two techniques for adults, but concluded that 'additional refinements may be needed to achieve interchangeability between ADP and HD for children'. Obviously, there is a need for further studies to fully validate ADP.

3.1.3. Three-dimensional body scanning. 3D body scanning and digital photographic anthropometry are relatively new techniques which can be used to measure body volume and hence body fat. The advantages of these new techniques are that they are fast and likely to be tolerated by most subjects. However, they have not at this stage been fully validated for body composition measurement and corrections for residual lung volume are still required.

There are a number of lasers or light-based 3D body scanners available. Hamamatsu (Japan) has developed a 3D laser based photonic scanner and claims an accuracy of ±0.5% in the measurement of a 100 cm circumference. Wells *et al* (2000) compared body volume measurements using a Hamamatsu 3D photonic scanner with ADP and hydrodensitometry in 22 adults and concluded that photonic scanning could not measure body fat with sufficient accuracy (scan precision was 0.57 l, or 4.1% body fat). Another approach uses digital photographs to estimate body volume (Mikat 2002).

Although relatively new, these techniques offer great potential to the assessment of body composition. Further development is needed to obtain the accuracy and precision required for estimation of body fat.

3.2. Dilution technique

3.2.1. Total body water. Water is present in all organs and tissues, in all cells. The water molecule is the most abundant molecule in the body. At a tissue or anatomical level, water can be viewed as being present in five compartments. These compartments are (1) intracellular water (in the cytoplasm and cell nucleus), (2) plasma water, (3) interstitial water (in the lymphatic system), (4) dense connective tissue water (in bone, cartilage, etc) and (5) transcellular water (excretory extracellular fluids such as bile, GI secretions, mucuses, cerebrospinal fluids, etc). The total amount of water in the body and its distribution is influenced by many physiological and patho-physiological conditions.

Total body water (TBW) can be determined using the dilution principle. According to that principle, the volume of the compartment is equal to the amount of tracer added to the compartment divided by the concentration in that compartment (Edelman *et al* 1952). The tracers used for determination of TBW can either be tritiated water (THO), deuterium water (DHO) or ^{18}O-labelled water ($H_2{}^{18}O$). The labelled water is rapidly distributed within the body. Equilibrium after an oral administration requires 3–4 h and the analysis can be based on samples of blood plasma or urine, in which the concentration of the stable element or molecule and the labelled one is measured. Corrections are needed for excretion and exchange with nonaqueous hydrogen or oxygen. In addition, physiological samples that undergo a chemical or physiological change may require correction for isotope fractionation. With careful attention to details, total body water can be measured with an accuracy of 1–2% and even better.

The use of isotope-labelled water is time consuming, as it needs multiple samples and access to specialized equipment and laboratory capacity. Consequently, isotopic methods to measure TBW have remained research tools, with clinicians relying on equations derived from normal population data (Watson *et al* 1980). While this approach works reasonably well for

individuals with close to normal body composition, the error may be large for malnourished, obese or fluid-loaded patients.

In parallel, tracers other than labelled water have also been used. Examples are antipyrine, ethanol and urea. Their volume of distribution has been taken as an estimate of that of TBW. These tracers are however all inferior to isotope-labelled water (Schoeller 2005), which is considered as the gold standard for TBW.

The ability to measure total body water accurately, non-invasively and rapidly, with results that are immediately available, would represent an important advance in body composition research. To meet such demands, Davies *et al* (2001) have developed a method to measure total body water, which enables immediate measurements of deuterium content in breath water from a single exhalation and measurements using flowing afterglow mass spectrometry (FA-MS) (Smith and Spanel 2001) after oral administration of D_2O.

3.2.2. Intracellular and extracellular water. Total body water can be subdivided into intracellular (ICW) and extracellular water (ECW). The ICW cannot be readily measured, but for ECW a number of tracers have been proposed and tested, including bromide, chloride, thiocyanate, thiosulphate, sulphate, insulin, sucrose and mannitol. Bromide and chloride dilution come the closest to approximating the extracellular space and with the advent of improved analytical techniques, bromine has become the most commonly used tracer. It is normally administered as an intravenous infusion of sodium bromide in isotonic saline. It is expected that essentially all the infused bromide would have equilibrated within the ECW by 4 h and would be retained within the ECW for at least 24 h (Vaisman *et al* 1987). The formula used to calculate the ECW is as follows: ECW (l) = (bromine in infusate (mmol) − bromine in urine (mmol)) × 0.804 bromine concentration in serum (mmol l^{-1}). The factor 0.804 corrects the result for the limited movement of bromine outside the extracellular water (e.g. entry into red cells), equilibrium effects and the average water content of serum (Bell *et al* 1984).

Chlorine is found mostly in extracellular fluid, but around 25% is bound into the skeleton. Since there is no suitable radioisotope of chlorine, bromine as sodium bromide or ^{35}S as sulphate may be employed as a tracer to determine the extracellular fluid, considering these anions do not cross the cell membranes significantly. Further, it is necessary to assume that over the period of equilibration after injection or ingestion of the tracer, no exchange with skeletal chlorine occurs.

The introduction of *in vivo* neutron activation analysis provided another method for the measurement of ECW, because TBC1 could be measured. Because chloride is distributed with 90% in the extracellular space, ECW is calculated as follows: ECW (l) = 0.9 (TBCl (meq)/plasma C1 (meq l^{-1})).

ICW is calculated as the difference between TBW and ECW.

3.2.3. Total body sodium and potassium. $^{22}Na^-$ or $^{24}Na^-$ may be used as a tracer for body sodium and $^{42}K^-$ for body potassium.

After injection, urine is collected over an equilibration time of 24 h and plasma concentration of radioactive and stable sodium (or potassium) measured. With correction for excretion through urine, the exchangeable mass of sodium (potassium) can be calculated.

3.3. Whole-body counting and neutron activation analysis

3.3.1. Total body potassium (TBK) measurements. Whole-body counting is a widely used method to measure total body potassium (TBK). Potassium is a unique element in the body

having a naturally occurring radioisotope ^{40}K, (0.0118% of all potassium) which decays with a half-life of 1.28×10^9 years emitting a 1.46 MeV γ-photon. Using full or partial radiation background shielding, total body ^{40}K can be estimated in 2–20 min depending on the type and size of the detector used. When the amount of ^{40}K in the subject is known, total body potassium (in millimole or kg) can be calculated as TBK = ^{40}K/0.000 118 (1 mmol = 39.102 mg).

It is worth mentioning that this method does not expose the subject to any extra radiation as CT and DEXA do.

Since potassium is found almost entirely (97%) within the cells, it provides a good estimate of the body cell mass (BCM). Moore *et al* (1963) first reported a ratio of TBK to BCM of 120 mmol kg^{-1}, which means that BCM (g) = 8.33 × TBK (mmol) for adults. Later studies have come up with a BCM/TBK ratio of 9.18 ± 0.09 for healthy adults. Recent studies have shown that this ratio can also be used for healthy children and adolescents (Wang *et al* 2005a).

The measurement of TBK has also been used to estimate the fat-free mass (FFM) in the body assuming that TBK occurs at a constant concentration in the FFM. The classical ratio between TBK and FFM was set to 68.1 mmol kg^{-1} FFM. An average for a number of studies gives 64.8 ± 2.1 mmol kg^{-1} for males and 59.6 ± 4.2 mmol kg^{-1} for females and 59–62 mmol kg^{-1} for boys and 54–59 mmol kg^{-1} for girls (Ellis 2005).

In contrast, the determination of the serum K levels in blood samples has been shown to be an unreliable index of the changes in the TBK concentration.

The ^{40}K technique can also be used to measure the K content in specific organs and tissues. Wielopolski *et al* (2003) have quantified the potassium content in the human brain at the level of about 4 g, with a potential for further improvements. Although the above relationship was derived for whole body, isolating the brain K component (about 4 g out of the whole body of 140 g) provides information on brain cell mass. Combined with a measurement of intracranial water, this technique would allow one to test the hypothesis of whether cytotoxic oedema (reflecting cell injury) is a component of the CNS injury process in MS patients.

3.3.2. In vivo neutron activation analysis (IVNA). When a subject is placed in a neutron field, there is a small probability that atoms in the body will undergo some type of nuclear reaction depending on the energy of the neutrons. In this way, neutron activation analysis can quantify a number of elements in the body, including hydrogen, carbon, nitrogen, oxygen, sodium, calcium, phosphorous and chlorine (Chettle and Fremlin 1984, Ellis 2005, Ryde *et al* 1987, Sutcliffe 1996). Fast neutrons are optimal with respect to tissue penetration. However, most IVNA reactions occur with thermal neutrons. Neutron activation systems therefore include a fast neutron source and rely on neutron thermalization with tissue interactions. Transuranic radionuclide sources such as ^{238}Pu–Be, ^{241}Am–Be and ^{252}Cf neutron sources have been frequently used. Increasing difficulties with supply and security for these neutron sources have created a renewed interest for accelerator-produced neutrons (Shypailo and Ellis 2005). The use of high-output, miniature D–T neutron generators will lessen the storage, increase the security and eliminate transport problems associated with transuranium sources.

Scans are typically carried out from shoulder to knee and require about 0.5–1 h for completion. Precision and accuracy are around 5%.

Prompt-gamma neutron activation. When a neutron is captured by a nucleus, the resulting nucleus, stable or radioactive, is usually not in its ground state. When the nucleus returns to its ground state, gamma rays are often promptly emitted. 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 rays must be measured during the neutron exposure. Large

sodium iodide detectors are used to quantify the 10.83 MeV gamma rays produced. The primary application of the TBN measurements is that it is directly proportional to body protein mass (protein = 6.25 × TBN). This method has become the gold standard for the measurement of total body nitrogen (protein). Measurements on cadavers have shown that the method yields compositional information similar to that of chemical analysis (Knight *et al* 1986).

Delayed-gamma neutron activation. Some thermal neutron capture reactions produce radionuclides with relatively short half-lives. This induced activity can be measured after the neutron irradiation, preferentially in a background-shielded whole-body counter. The technique is favourable for the measurement of total body calcium, phosphorous, sodium and chlorine.

Inelastic neutron scattering activation. The methods described above give no clear option to measure carbon in the body. Carbon can however be measured using fast neutrons (e.g. from a D–T neutron generator) having energies above 4.44 MeV. Fast neutrons that interact with matter by inelastic collisions result in prompt nuclear de-excitation with gamma-ray release $(n, n'\gamma)$ preferentially measured in large volume neutron-tolerant BGO detectors. Total body carbon (which is used as a measure of the body fat content) is derived by measuring the 4.44 MeV photons from ^{12}C nuclei (Kyere *et al* 1982) and the total body oxygen by measuring the 6.13 MeV gamma ray from inelastic scattering in oxygen. The carbon/oxygen ratio offers a technique to measure the axial distribution of fat because of the dramatic difference in C/O ratio between fat and fat-free tissues (Kehayias *et al* 1991, Kehayias and Zhuang 1993). The development of DEXA, CT and MRI methods for determination of body fat and its distribution has however reduced the interest for the neutron inelastic scattering method.

Radiation exposure and radiation protection problems. The drawback with the neutron activation technique is that it involves neutron radiation exposure. A total body nitrogen (protein) determination leads to a total body exposure of around 0.25–1 mSv and delayed neutron activation for Ca analysis leads to 5–10 mSv, while the corresponding number for the inelastic neutron scattering method is 0.5–1 mSv. For these reasons, neutron activation methods should be avoided for children and pregnant women.

3.4. Dual-energy x-ray absorptiometry

3.4.1. DEXA. Differential absorption of x-rays by bone and soft tissue was the basis for the original development of single photon absorptiometry to measure bone mineral density (BMD). The early applications used ^{125}I (27 keV) or ^{241}Am (59 keV) as the source of photons and the measurement was, in general, limited to the radius and ulna. A significant advance was associated with the introduction of dual photon absorptiometry (DPA) where the ^{125}I or ^{241}Am source was replaced with ^{153}Gd (44 and 100 keV). This had the advantage of greater precision and allowed measurement of the BMD of bony structures situated deep in soft tissue such as the lumbar vertebrae or femur. Further development of the technique permitted the measurement of whole-body BMD.

DPA was limited in application by the relatively low photon flux available from the ^{153}Gd isotopic sources used. This problem was overcome with the introduction of dual-energy x-ray absorptiometry (DEXA) which employs an x-ray tube and filters to produce one low and one high pseudo monoenergetic beam. The x-ray tube may be operated at constant potential (GE Lunar and Stratec models) or switched between two potentials (Hologic models). The radiation effective doses involved in whole-body BMD measurements using DEXA are small ($\leqslant 1.5 \times 10^{-2}$ mSv) making the technique widely applicable.

The greater photon flux also allows the DEXA technique to be applied to the measurement of soft tissue composition (fat and lean tissue). Either whole-body or regional measures of the three components (fat, lean tissue and bone mineral content) may be made.

The precision of a particular DEXA device for assessment of whole-body composition is generally good with coefficients of variation of about 1% for bone mineral content and 2–3% for total body fat. The usefulness of these quantities in diagnosis or monitoring the progress of osteoporosis and nutritional status has resulted in the widespread use of the DEXA technique. However, the technique has some well-documented limitations resulting mainly from the failure of assumptions made in the DEXA method (Genton *et al* 2002). The result is significant variations in measurements made on the same subjects using DEXA devices supplied by different manufacturers. Differences of 10% in fat mass (FM), 6% in lean tissue mass (Kistorp and Svendsen 1997) and 2.6–6.3% in % body fat (Tothill *et al* 1994) are typical of the differences reported. Indeed, even using different models from the same manufacturer may result in significant differences. Ellis and Shypailo (1998) observed a reasonably close agreement in bone mineral content (mean differences of −1.0 to 1.4%) but a larger difference in body fat mass of 6.4% when considering the cross calibration of a pencil-beam and a fan-beam absorptiometer from the same manufacturer. Oldroyd *et al* (1998) reported significant differences between % body fat (2%) and total body lean tissue (1 kg) when comparing two absorptiometers from the same company. These differences were reduced significantly (0.6% and 0.4 kg, respectively) following hardware changes.

One of the assumptions of DEXA is that the photon attenuation is similar in the soft tissue overlying and adjacent to the measured bone. Hakulinen *et al* (2003) investigated the effect of spatial variations of soft tissue composition over the calcaneus and reported a large inaccuracy in BMD determination. They also investigated a relatively new approach (DXL Calscan) in which DEXA is combined with laser definition of the measurement area and concluded that, in theory, elimination of the effect of non-uniform soft tissue on the measurement of BMD is possible.

The DXL Calscan device is a portable machine, which is an advantage when considering screening of populations removed from major centres. However, the efficacy of measurement at a peripheral site has been questioned, particularly in the diagnosis of osteoporosis.

3.4.2. CT. As in DEXA, dual-energy techniques can also be used in CT. For both single- and dual-energy CT methods, a very careful calibration of the CT unit must be undertaken. For dual-energy CT, the measurements involve two scans at different kVp. The advantage of the CT method is that the results will be measured only in the bone tissue of interest (the mechanical strength of the vertebrae is mainly dependent on the amount of trabecular bone in the vertebral body). Another method involves measurement of the amount of radiation from a monoenergetic gamma-ray source, that is coherently and incoherently scattered by the bone tissue. The coherent/incoherent ratio is very sensitive to the bone mineral density and using a well-defined collimation of the radiation source and detector, the investigated volume can be well defined and positioned in the trabecular part of the bone being investigated.

3.5. Computed tomography and magnetic resonance imaging

The application of computed tomography (CT) as well as magnetic resonance imaging (MRI) and spectroscopy (MRS) represents important advances in human body composition studies. These methods have been used to significantly increase our knowledge of the complex relationships between the body composition and various diseases (Ross 2003). They are

the methods of choice for validation of 'field' methods (e.g. bioelectric impedance). They are also the only methods available for detailed measurement in internal tissues and organs. These applications are now also used to measure the quality of various tissues including bone, skeletal muscle and hepatic tissue (Ross and Janssen 2005).

The first use of CT for body composition studies goes back to the 1970s when Heymsfield *et al* (1979) quantified the cross-sectional area of arm muscle using a single axial image and Borkan *et al* (1982) used CT to evaluate adipose tissue (AT) in the abdomen. Sjöström *et al* (1986) introduced whole-body imaging and multicomponent analysis to quantify total body and regional AT, skeletal muscle, bone and other organ/tissue volumes. Application of magnetic resonance imaging (MRI) as a method of body composition measurement was first reported by Foster *et al* (1984) and Hayes *et al* (1988) who quantified subcutaneous AT using MRI. Subsequently, several groups demonstrated the feasibility of measuring whole-body and regional AT and skeletal muscle mass using multiple MRI protocols (Ross *et al* 1992, Thomas *et al* 1998).

The application of CT and MRI in body composition is growing very fast. CT and MRI are now considered the most accurate methods available for *in vivo* quantification of total and regional adipose and skeletal muscle tissue.

CT and MRI are used to better describe the human body composition and to better understand the links between body composition and disease risk, as will be described below.

3.5.1. CT. CT images for body composition studies are built up of pixels (usually 1 mm × 1 mm). Each of the pixels has a CT or HU number. The CT number is a measure of the attenuation (mainly related to electron density) relative to water ((Hounsfield unit (HU) = 0) and air (HU = −1000). The CT number for AT is between −190 and −30 and skeletal muscle (free from AT) 30–100 HU. The tissue area (cm^2) for the different tissues (AT, skeletal muscle, bone, visceral organs, brain) on each cross-sectional CT image can be determined by either manual or computerized 'segmentation'. There are also various types of interactive software programs for this application. Tissue volumes are calculated by integrating the cross-sectional area data from consecutive slices.

CT is capable of distinguishing between different tissue types on the basis of their attenuation characteristics, which are a function of tissue density and chemical composition. Skeletal muscle attenuation is determined by measuring the mean attenuation value from all pixels within the range of 0–100 HU. The lower the HU values, the lower the density and the larger the fat content within the muscle. As an additional method of analysis, the distribution of attenuation values is described. Early studies that employed CT to measure muscle composition found associations between reduced skeletal muscle attenuation and aging, muscular dystrophy and other myopathies. More recently, Goodpaster *et al* (2000) observed that the mean muscle attenuation in skeletal muscle is reduced in obesity and type 2 *diabetes mellitus*, and that weight loss increases the mean attenuation value of muscle. Although some investigators have failed to observe a relationship between muscle attenuation and insulin resistance, CT-measured muscle composition *in vivo* represents a major advance with numerous applications in body composition studies.

In a manner similar to that used to determine skeletal muscle density, CT has also been employed to determine the density of liver tissue. Due to the normally higher density of the liver compared to the spleen, a lower mean liver attenuation value relative to that of the spleen indicates fatty infiltration of the liver as originally described by Piekarski *et al* (1980).

Recently, CT has been employed to measure the quality of various tissues, in particular skeletal muscle tissue. The focus has been to establish the lipid content since altered fat

deposition in skeletal muscle is linked to reduced insulin-stimulated glucose uptake. An increased muscle lipid content has also been noted in older persons and is associated with muscle wasting diseases.

A problem with multiple CT images over the whole body is the high radiation absorbed dose, resulting in effective doses of the order of 10 mSv per investigation. To reduce the doses, special low-dose technology has been developed in some laboratories (Starck *et al* 1998).

3.5.2. MRI and MRS. An important development in the field of body composition studies is the use of magnetic resonance imaging (MRI). To date, the principal application of MRI in human body composition research has been to characterize the quantity and distribution of AT and skeletal muscle and also subcutaneous adipose tissue, visceral adipose tissue, intramuscular adipose tissue, oedema and various organs (liver, kidneys, heart, spleen, pancreas). As there is no exposure to ionizing radiation, MRI is also used for body composition studies in children and adolescents.

MRI is used for quantitative as well as qualitative measurements. As with CT, recent evidence suggests that MRI may also be employed to measure the quality of *in vivo* skeletal muscle. However, because proton MRI integrates rather than separates the signals from distinct protons within the image voxel, conventional MRI is not useful for determining, for example, the concentration of lipid or water in skeletal muscle. Obtaining basic information within the tissue volume of interest requires application of 'chemical shift' imaging techniques. Several chemical shift methods have been developed which separate the water and fat signals in the region of interest, creating the potential to determine water and fat contents of skeletal muscle.

MRI has been used to measure AT and lean tissue in foetuses, children, normal-weight males and females, obese males and females, and diabetic and elderly populations. While the aforementioned studies base their observations largely on a single MR image, it is also possible to acquire whole-body MRI data in about 30 min. The acquisition of whole-body MRI data offers distinct advantages in assessing the influence of weight loss on body composition. For example, weight reduction scenarios may induce regional changes in AT or muscle. Thus, if an increase in skeletal muscle in one anatomical region is masked by a loss of skeletal muscle in another, only MRI studies could discover it. Indeed, whole-body MRI protocols have been employed to make important observations with respect to the effects of various disturbances in the total and regional AT and skeletal muscle distribution. Whole-body MRI has also been used to describe age-related muscle loss. Using fewer images, several investigations have employed MRI to assess the influence of weight loss, inactivity and resistance training on region-specific changes in skeletal muscle. More recently, Gallagher *et al* (2000) used an MRI protocol to measure the mass of several internal organs in the abdomen.

Intramyocellular lipids (IMCL) play an important role in the study of metabolism *in vivo*. Recent data indicate for example that IMCL are important in the pathogenesis of insulin resistance. Boesch *et al* (1997) showed that ^{1}H-MRS could be used to determine IMCL levels nonivasively and to separate the MR signal of extramyocellular lipids (EMCL) and IMCL.

MRS studies of IMCL are usually performed with clinical 1.5 T MRI systems and have employed the single-voxel MRS technique.

3.6. Bioimpedance analysis

Bioimpedance analysis (BIA) at a single frequency (usually 50 kHz) or at multiple frequencies is one of the more common techniques applied to the measurement of body composition. It is relatively inexpensive, portable and does not require extensive operator training. However,

care needs to be taken with its application and an understanding of its limitations is necessary if the results are to be correctly interpreted.

3.6.1. Principle of BIA. There are many papers, which describe in detail the principle of BIA (e.g. Kushner (1992), Lukaski and Bolunchuk (1988)). In brief, the underlying principle is that when an electrical current passes through the body it will mainly pass through water-containing tissues since bone and fat have a large impedance and do not conduct significant current. For a cylindrical conductor, the resistance, R, is given by

$$R = \rho L/A, \tag{5}$$

where ρ is the resistivity of the tissue, L is the length of the cylinder and A is the cross-sectional area of the cylinder.

Re-arrangement of equation (5) leads to the equation for the volume, V, of the conducting cylinder:

$$V = \rho L^2/R. \tag{6}$$

Although not the same quantity, the impedance (Z) is often used interchangeably with R in these two equations.

The human body exhibits both a resistive and a reactive component of impedance to an electrical current. The capacitive effect of the cell membrane results in the reactive component and the intracellular and extracellular water spaces are resistive. The simplest electrical analogue is therefore a parallel arrangement of the intracellular and extracellular pathways. The extracellular pathway is purely resistive and the intracellular pathway a series combination of a resistor and a capacitor. Bioimpedance measurements use a low-level alternating current, which passes through both the intracellular and extracellular pathways in a proportion which is frequency dependent. At zero frequency (dc), the current flows entirely through the extracellular pathway whilst at higher frequencies it passes through both the intracellular and extracellular pathways.

3.6.2. Wrist-to-ankle or segmental methodology? The body is reasonably well approximated by five interconnecting cylinders—two arms, two legs and the trunk. In the most common approach to BIA, the impedance is measured between the right wrist and ipsilateral ankle. A tetrapolar arrangement—two drive and two measurement electrodes—is employed. The tetrapolar arrangement is necessary because the skin–electrode contact impedance inhibits the use of a simple bipolar technique. A constant ac current is passed between the two drive electrodes and the potential drop, and hence impedance, is measured between the two measurement electrodes. One of the measurement electrodes is placed on the dorsal surface of the right wrist at the level of the process of the radial and ulnar bones and the other on the anterior surface of the right ankle between protruding portions of the tibial and fibular bones. The drive electrodes are placed approximately 5 cm distally from the measurement electrodes, one on the dorsal surface of the third metatarsal bone of the right foot and the other on the dorsal surface of the third metacarpal bone of the right hand (Lukaski *et al* 1985, Scheltinga 1992). The volume of the conducting tissues is estimated using equation (6) with the height of the subject being substituted for the length of the cylinder. Values for the resistivity (the constant of equation (6)) of the various organs may be found in the literature. However the resistivities of various water-containing organs are markedly different and, in practice, the constant of equation (6) needs to be a weighted average of the resistivities of the tissues, which makes calculation difficult. Hence, it is more common for the constant to be determined experimentally by regressing L^2/Z (or L^2/R) measured for a large group of

subjects against the total body water volume determined for those subjects from a reference method.

Many authors have noted that the impedance between the wrist and ankle is primarily due to the arm and leg with the trunk contributing only a small amount of the total measured impedance whereas the major component of the conducting tissues is in the trunk (Foster and Lukaski 1996, Thomas *et al* 1992). This anomaly is generally overlooked and indeed good correlations of volumes obtained using equation (6) and those by isotope dilution are obtained for control subjects (Houtkooper *et al* 1996). However, much poorer correlations are obtained when the equations obtained from a control population are applied to a different ethnic group or to a patient group (Ellis *et al* 1999, Buchholz *et al* 2004). This raises concerns with the general applicability of the equations and the underlying assumptions of a single cylindrical conducting volume. Kyle *et al* (2004a) have provided an extensive list of BIA prediction equations for healthy subjects published since 1990 for fat-free mass, body fat, ECW, ICW and body cell mass. The same authors (Kyle *et al* 2004b) also discuss the use and limitations of the prediction equations in patient groups.

In an attempt to overcome the concerns with the assumption of the body as approximated by a single cylinder, some authors have used a 'segmental' approach for the determination of the conducting volume (Organ *et al* 1994, Bracco *et al* 1996). In this approach, the impedances of the arm(s), leg(s) and trunk are determined separately together with the length of these segments. The total volume is then determined as

$$V = 2[\rho l^2/Z]_{\text{arm}} + 2[\rho l^2/Z]_{\text{leg}} + [\rho l^2/Z]_{\text{trunk}}. \tag{7}$$

In many studies, the resistivity of the segments is assumed to be a constant; however, Zhu *et al* (2006) have noted that segment-specific resistivity improves the estimate of body fluid volume in haemodialysis patients.

The extra complexity (six electrodes compared with four) in performing the segmental measurements and data analysis may not warrant the potentially marginal improvement obtained in healthy subjects but in disease states the segmental approach offers the potential for improved prediction of body water and body composition components. A practical improvement in the segmental approach is the placement of the two additional electrodes on the contralateral hand and foot. This allows the measurement of the impedance of the arm and leg without the necessity of placing electrodes on the shoulder or groin region and is less subject to errors associated with misplacement of these electrodes (Cornish *et al* 1999).

3.6.3. Multiple-frequency BIA. As mentioned above, the proportion of the ac current which flows through the intracellular and extracellular pathways is frequency dependent. This suggests that the technique may be used to measure both the extracellular and intracellular fluid volumes. Several of the early groups working in the field used measurements made at two discrete frequencies, one low and one high (usually 50 kHz) for this purpose (Espejo *et al* 1989, Segal *et al* 1991). However, theory indicates that it is the impedance at zero frequency (dc) which should be the best predictor of extracellular water (ECW). Measurement of the impedance at zero frequency is not possible because of the high skin impedance and hence the present approach is to measure the impedance at many frequencies between about 10 kHz and 1 MHz and then plot resistance against reactance at these frequencies. This produces a semi-circular plot generally referred to as a Cole–Cole plot (Cole and Cole 1941). Extrapolation of this plot to obtain the impedance at zero frequency is then possible and provides the best predictor of ECW both theoretically and experimentally (Cornish *et al* 1992). Extrapolation to obtain the impedance at infinite frequency is also possible, although less accurate, and this can

then be used with the resistance at zero frequency to obtain the impedance of the intracellular water (ICW) and hence the volume of ICW.

3.6.4. Localized measurements. BIA, either single or multiple frequency, may be applied to a portion or segment of the body. Cornish *et al* (1996) have demonstrated its utility in detection of lymphoedema of the arm and Scharfetter *et al* (2001) demonstrated its use in measuring local abdominal fat mass. Other applications where a localized measurement may be more sensitive than a whole-body measurement are the measurement of ascites fluid volumes (Schloerb *et al* 1996, Zillikens *et al* 1992).

3.6.5. Priorities. Multifrequency bioimpedance (spectroscopy) and the segmental methodology are becoming the (bioimpedance) methods of choice for assessment of total body water and its components. It is likely to become more accepted as manufacturers provide instruments designed for the purpose of segmental measurements and software specifically tailored to the segmental methodology. There is a need for adoption of a standard protocol, including electrode placement, so that results from different studies can be directly compared. Care also needs to be exercised in the selection of the prediction equation to ensure that they are applicable to the characteristics of the cohort under study.

3.7. Anthropometry

Many anthropometric measures have been proposed and used for assessment of total or regional body composition. This section deals only with two of the more common anthropometric methods—body mass index (BMI) and the skinfold measurement. A more complete description of other anthropometric techniques of assessing body composition may be found elsewhere (see for example Heyward and Wagner (2004)).

3.7.1. Body mass index. Body mass index (body weight in kg/height in metres squared) is primarily used to identify subjects as underweight, normal, overweight or obese. The World Health Organization provides a classification of overweight/obesity based on BMI. For adults, a BMI > 25 kg m^{-2} is classified as overweight and a BMI > 30 kg m^{-2} as obese. The BMI can also be used to classify normal and underweight subjects with a BMI < 18.5 kg m^{-2} being underweight and 18.5–25 kg m^{-2} being the normal range.

Both body weight and height (and hence BMI) may be measured, for most individuals, with a high degree of accuracy. However, care needs to be taken in classification of an individual based upon BMI alone. Factors which need to be considered when classifying an individual as underweight, obese, etc are age, gender, ethnicity and body build. For this reason, many authors have questioned the classification of obesity based upon BMI alone.

A number of studies have investigated the relationship between BMI and % body fat. Gallagher *et al* (1996) investigated the influence of age, gender and ethnicity on the relationship between % body fat and BMI. Their study included men and women of two ethnic groups (black and white) who resided in or near New York City. They concluded that the relationship was affected by age and gender but was ethnicity independent. Other authors have drawn similar conclusions regarding the influence of age and gender, but have also noted an effect of ethnicity. There has been some progress towards establishing age and gender specific definitions of obesity based upon BMI. For example, classification based on age and gender has been developed for children and adolescents to age 20 (Hammer *et al* 1991, Pietrobelli *et al* 1998, Cole *et al* 2000). However, for children it is their BMI percentile that is more

important. A BMI above the 95th percentile is considered as overweight and between the 85th and 95th percentiles at risk of becoming overweight. Considerable further work needs to be performed before cut-offs are universally accepted.

Maynard *et al* (2001) measured BMI and total body fat and fat-free mass (determined from hydrodensitometry) in 387 healthy white children. Where possible, the measurements were performed annually on each child between the age of 8 and 18 years. The aim of the study was to provide 'an insight into the meaning, significance and limitations of BMI as an index of adiposity during childhood'. BMI increased with age with mean values being similar for boys and girls except between at ages of 12–13 years. The increase in BMI with age was associated with changes in the fat-free mass rather than the fat. The question of the relationship of BMI with adiposity in children and adolescents has received considerable attention in recent years (Neovius *et al* 2004, Taylor *et al* 2002, Horlick 2001, Lindsay *et al* 2001). Overweight and obesity in adults have been shown to be related to their BMI in childhood (Guo *et al* 2000, Guo and Chumlea 1999). Childhood and adolescent overweight have also been shown to be related to cardiovascular risk factors (Freedman *et al* 1999, Must *et al* 1992, Lindsay *et al* 2001).

3.7.2. Skinfold measurements. The measurement of skinfold thickness is used to assess body density and hence % body fat. Skinfold thicknesses at specific sites provide reasonable correlation with % body fat. However, variations in the distribution of subcutaneous fat between individuals mean that multiple sites need to be sampled in order to obtain an accurate predictor of % body fat. The variability in the ratio of subcutaneous fat to total body fat also needs to be considered. There have been many predictive equations developed which incorporate various skinfold measures and other factors such as age, gender and ethnicity.

Although the measurement of skinfold thickness is relatively simple, the necessary skills need to be developed and practiced if accurate and precise measures are to be made. Considerable variability between operators has been noted. Reasons for this variability include

- the callipers used—different callipers may use different pressures resulting in a systematic difference,
- differences in location of the anatomical sites, and
- technique of grasping the skinfold.

Some authors recommend that the estimation of % body fat from skinfold measures should not be used in very obese subjects.

Further variability is associated with the use of an inappropriate prediction equation, which needs to be selected based on the characteristics of the cohort to be measured. Beddoe and Samat (1998) noted that for a given individual one cannot know if this condition holds or not. They also noted that biological variations rather than measurement errors are the major cause of error in estimating % body fat from skinfold measures. Using data from Durnin and Womersley (1974) to predict body density from skinfold measures and Siri's equation (Siri 1956) to calculate % body fat from the predicted body density, Beddoe and Samat showed that significant uncertainty (approximately $\pm$3–4% in the estimate of % body fat) was associated with the potential variability in the body density. Given the range of % body fat of subjects in their study (17–36%), this translated to an error of 14–22%. Beddoe and Samat further argued that a method based upon *in vivo* neutron activation analysis and tritiated water dilution would provide a better reference than densitometry for the measurement of total body fat. Beddoe (1995) compared the precision of several methods of measuring total body fat. He concluded that skinfold anthropometry was the least precise of the five techniques considered and that dual-energy x-ray absorptiometry provided the most precise technique. However, because of

its simplicity and low cost, the measurement of skinfold thickness remains one of the most commonly used techniques for assessing 'fatness'.

4. Analysis of trace elements *in vivo*

Many trace elements are essential for the function of the human body. Others become toxic over a certain concentration level. There is thus a need to control their levels in human organs and tissues. This is of special concern for occupationally and environmentally highly exposed persons. Monitoring and basic occupational/environmental research rely on measurements directly in humans as well as of samples taken from humans (urine, blood, faeces, etc) and from the environment. The distribution of trace elements within the body is often very non-uniform and found in specific organs (iodine in thyroid, lead in bone, cadmium and mercury in kidney cortex, etc). The two main non-invasive *in vivo* methods for determination of trace element concentration *in vivo* are x-ray fluorescence analysis (XRF) and neutron activation analysis (NAA) (Chettle *et al* 1987, 1999).

4.1. XRF and NAA in vivo

Reviews of *in vivo* XRF can be found in the literature (Börjesson *et al* 1998, Bradley and Farquharson 1999, Mattsson and Scott 1990, McNeill and O'Meara 1999) as can reviews of *in vivo* NAA (Sutcliffe 1996) for trace element applications.

In XRF, the part of the body to be studied is irradiated with photons of energy in excess of the binding energies for the K-electrons (or exceptionally L-electrons) of the element of interest. A high-resolution Ge or Si spectrometer counts the characteristic x-ray photons generated from that element.

The first *in vivo* XRF application was the non-invasive measurements of natural *iodine* ($\approx$400 μg g^{-1}) in the thyroid (Hoffer *et al* 1968). Later, a technique to measure iodine from x-ray contrast agents in blood pools *in vivo* was developed and used for quantitative kidney function determinations after urography (Grönberg *et al* 1983). Measurements of *lead in vivo* began in 1971 (Ahlgren *et al* 1976, Ahlgren and Mattsson 1979) using 122 and 136 keV photons from ^{57}Co for excitation of the Pb-K x-rays. Since then, a number of alternative XRF techniques have been developed (Somervaille *et al* 1985, Wielopolski *et al* 1989, Gordon *et al* 1993, Nie *et al* 1991). Long-term measurements on occupationally exposed persons have given unique information on the slow turnover of lead in the skeleton and the usefulness of *in vivo* skeletal lead measurements as long-term exposure index. The importance of bone as a source of 'endogenous' lead exposure has also been clearly illustrated (Nilsson *et al* 1991).

In addition to that, *in vivo* applications have been developed for measurements of *cadmium*, *mercury*, *gold*, *platinum*, *uranium* and a number of other elements through the years (Christoffersson and Mattsson 1983, Jonson *et al* 1988, Börjesson *et al* 1993, Shakeshaft and Lillicrap 1993, Börjesson *et al* 1995, O'Meara *et al* 1997). Table 3 summarizes some elements, which have been subjected to *in vivo* XRF.

Neutron activation analysis is possible for elements, which have a high cross section for capture of thermal neutrons. The technique has, as mentioned in section 3.3.2, proved useful for *in vivo* measurements of nitrogen for the determination of the whole-body content of protein. NAA can also be used to determine cadmium. Cadmium has a high cross section for capture of thermal neutrons followed by a prompt emission of a 559 keV gamma ray. Levels down to 1.7 mg in a kidney and down to a concentration of 3.3 μg g^{-1} in liver can be detected (Grinyer *et al* 2005). Levels of 40 μg g^{-1} in the liver are associated with kidney damage.

Table 3. Application, principal measurement site(s) for XRF and NAA analysis of trace elements *in vivo*. For XRF, the K absorption energy and characteristic x-ray energies are given (Börjesson and Mattsson 2004). For NAA, the type of reaction and the energy of the prompt or delayed photon to be measured are also given.

X-ray fluorescence analysis				
Element (Z)	Origin	Main *in vivo* measurement site(s)	K absorption energy (keV)	K_α x-ray energies (keV)
Cadmium (48)	Occupational, environmental	Kidneys, liver	26.71	22.98, 23.17
Iodine (53)	Natural abundance, medical (x-ray contrast)	Thyroid, blood	33.17	28.32, 28.62
Barium (56)	Medical (x-ray contrast)	Lungs	37.44	31.82, 32.19
Platinum (78)	Medical (cytotoxic agent)	Kidneys, liver, tumours	78.40	65.12, 66.83
Gold (79)	Medical (anti-rheumatic agent)	Kidneys, liver, bone joints	80.73	66.99, 68.81
Mercury (80)	Occupational, environmental	Kidneys, thyroid	83.10	68.89, 70.82
Lead (82)	Occupational, environmental	Bone	88.00	72.80, 74.97
Uranium (92)	Nuclear fuel cycle, nuclear weapons, ammunition	Bone, lungs	115.61	94.66, 98.44

Neutron activation analysis				
Element (Z)	Origin	Main *in vivo* measurement site(s)	Neutron reaction	γ-energy
Aluminium	Occupational, environmental	Bone	Activation	1.17 MeV
Manganese	Occupational, environmental	Bone	Activation	847 keV
Cadmium	Occupational, environmental	Kidneys, liver	Prompt γ	559 keV

Cadmium in kidneys and liver can also be determined by XRF at a considerably lower radiation dose than with neutron activation, but with lower uniformity of the sensitivity over the organs. The detection limit for cadmium in kidney cortex using the XRF technique is now so low that most people in the public can be analysed.

Neutron activation analysis has also been developed and to a limited extent used to measure *aluminium* in bone *in vivo* using the ^{27}Al(n, γ)^{28}Al reaction, giving a 1.78 MeV gamma ray. Due to the risk for disturbances from the reaction ^{31}P(n, α)^{28}Al, the neutron energy must be kept under 2.0 MeV using a low-energy neutron beam from an accelerator (Pejovic-Milic *et al* 2005).

Another example of the current developments is the work by Arnold *et al* (1999, 2002), who investigate the possibility of analysing *manganese* in liver and bone by neutron activation analysis also using an accelerator-based technique. They have not yet come down to normal levels, but see a potential for improvements.

5. Methods under development

There are a number of techniques, which have not been treated in this paper, for which the development is also very rapid, e.g. ultrasound.

Body composition methods have developed from being available only in highly specialized laboratories with possibilities for underwater weighing, whole-body counting and *in vivo* neutron activation analysis to being widely available due to the development of instrumentation such as air displacement plethysmographs, DEXA, CT and NMR, which now are widely available for researchers and practitioners at many hospitals. Together with the interest

to describe the major body components, mainly fat and fat-free mass, we can foresee an increasing interest for studies of in *vivo* metabolism, energy utilization, oxygen uptake, blood flow, blood volume, etc as well as for the assessment of the quality of organs and tissues, which makes it interesting to adopt the new clinical equipment such as PET/CT, high field MRI, fMRI and MRS to an increasing degree. As an example of a new method under development, the work by Stone and Robinson (2004) could be mentioned. They have recently demonstrated a method for determining total body water using a resonant cavity perturbation approach.

In parallel to the development of advanced physics and technology, there is an increasing need for field equipment such as BIA and air displacement plethysmography (Bod Pod) as well as for instruments for 3D body scanning. There is still an interest for K-40 measurements for estimation of fat-free mass.

In the future, we can also foresee an increased interest in methods and instruments which can be applied to animals. In principle, many methods described in the preceding sections can be applied to animals. However, those requiring compliance are obviously not applicable, e.g. hydrodensitometry.

6. Summary

Based on a wide range of measurable properties, analytical methods and known body composition models, clinicians and scientists can now quantify a number of body components and follow their changes in health and disease. Mass, volume and distribution of the various components (fat and fat-free mass, protein, water, (bone) minerals and fat) are the primary current measures in the field. The development of new technology such as positron emission tomography, functional MRI (fMRI) and magnetic resonance spectroscopy (MRS) will enable studies of the conditions of tissues and organs, their composition, nutrient and energy utilization, oxygen uptake, blood flow and other functional properties. This opens new possibilities of studying relations between body structure and composition on the one hand and the function of the body on the other during various periods of life. Concerning 'microbody composition', it will be of great interest to continue to study how intramyocellular fat (through single- and multivoxel MRS) is related to obesity and diabetes.

Internationally, there are huge problems with obesity as well as with malnutrition—at the same time. It is a great challenge to try to correlate the results of functional body composition measurements during lifetime to major epidemiological findings and to parallel investigations of molecular biology parameters.

For common medical and health care, there will be a great need for reliable and efficient, simple and relatively cheap instruments such as BIA, hand-held DEXA, etc.

References

Adams J, Mottola M, Bagnall K M and McFadden K D 1982 Total body fat content in a group of professional football players *Can. J. Appl. Sport Sci.* **7** 36–40

Ahlgren L, Lidén K, Mattsson S and Tejning S 1976 X-ray fluorescence analysis of lead in human skeleton *in vivo* *Scand. J. Work Environ. Health* **2** 82–6

Ahlgren L and Mattsson S 1979 An x-ray fluorescence technique for *in vivo* determination of lead concentration in a bone matrix *Phys. Med. Biol.* **24** 136–45

Alpsten M and Mattsson S 1998 International symposium on *in vivo* body composition studies *Appl. Radiat. Isot.* **49** 429–752

Arnold M L, McNeill F E and Chettle D R 1999 The feasibility of measuring manganese concentrations in human liver using neutron activation analysis *Neurotoxicology* **20** 407–12

Arnold M L, McNeill F E, Stronach I M, Waker A, Pejovic-Milic A and Chettle D R 2002 An accelerator based system for *in vivo* neutron activation analysis measurements of manganese in human hand bones *Med. Phys.* **29** 2718–24

Ball S D 2005 Interdevice variability in percent fat estimates using the BOD POD *Eur. J. Clin. Nutr.* **59** 996–1001

Beddoe A H 1995 Body composition—what is measurable? *Asia Pac. J. Clin. Nutr.* **4** 7–10

Beddoe A H and Samat S B 1998 Body fat prediction from skinfold anthropometry referenced to a new gold standard: *in vivo* neutron activation analysis and tritium dilution *Physiol. Meas.* **19** 393–403

Bell E F, Ziegler E E and Forbes G B 1984 Corrected bromide space *Pediatr. Res.* **18** 392–3

Biaggi R R, Vollman M W, Nies M A, Brener C E, Flakoll P J and Levenhagen D K 1999 Comparison of air-displacement plethysmography with hydrostatic weighing and bioelectrical impedance analysis for the assessment of body composition in healthy adults *Am. J. Clin. Nutr.* **69** 898–903

Boesch C, Slotboom J, Hoppeler H and Kreis R 1997 *In vivo* determination of intra-myocellular lipids in human muscle by means of localized ^{1}H-MR-spectroscopy *Magn. Reson. Med.* **37** 484–93

Börjesson J, Alpsten M, Huang S, Jonson R, Mattsson S and Thornberg C 1993 *In vivo* x-ray fluorescence analysis with applications to platinum, gold and mercury in man—experiments, improvements and patient measurements *Human Body Composition. In vivo Methods, Models, and Assessment* ed K J Ellis and J Eastman (New York: Plenum) pp 275–80

Börjesson J, Barregård L, Sällsten G, Schütz A, Jonson R, Alpsten M and Mattsson S 1995 *In vivo* XRF analysis of mercury: the relation between concentrations in the kidney and the urine *Phys. Med. Biol.* **40** 413–26

Börjesson J and Mattsson S 2004 X-ray fluorescence analysis in medical sciences *X-Ray Spectrometry: Recent Technological Advances* ed K Tsuji, J Injuk and R Van Grieken (Chichester: Wiley) pp 487–515

Börjesson J, Mattsson S and Alpsten M 1998 Trace element concentrations studied *in vivo* using x-ray fluorescence analysis *Appl. Radiat. Isot.* **49** 437–45

Borkan G A, Gerzof S G, Robbins A H, Hults D E, Silbert C K and Silbert J E 1982 Assessment of abdominal fat content by computed tomography *Am. J. Clin. Nutr.* **36** 172–7

Bracco D, Thiebaud D, Chiolero R L, Landry M, Burckhardt P and Schutz Y 1996 Segmental body composition assessed by bioelectrical impedance and DEXA in humans *J. Appl. Physiol.* **81** 2580–7

Bradley D A and Farquharson M J 1999 XRF and the *in vivo* evaluation of toxicological metals *X-Ray Spectrom.* **28** 270–4

Brozek J, Grande F, Anderson T and Keys A 1963 Densitometric analysis of body composition: revisions of some quantitative assumptions. *Ann. New York Acad. Sci.* **110** 113–40

Buchholz A C, Bartok C and Schoeller D A 2004 The validity of bioelectrical impedance models in clinical populations *Nutr. Clin. Pract.* **19** 433–46

Buskirk E R 1961 Underwater weighing and body density: a review of procedures *Techniques for Measuring Body Composition* ed J Brozek and A Henschel (Washington, DC: National Academy of Sciences)

Chettle D R, Armstrong R, Todd A C, Franklin D M, Scott M C and Somervaille L J 1999 Measurements of trace elements *in vivo Advances in in vivo Body Composition Studies. Recent Advances* ed S Yasumura *et al* (New York: Plenum) pp 247–257

Chettle D R and Fremlin J H 1984 Techniques of *in vivo* neutron activation analysis *Phys. Med. Biol.* **29** 1011–43

Chettle D R, Scott M C, Ellis K J and Morgan W D 1987 *In vivo* monitoring of trace elements in medicine and research *In vivo Body Composition Studies* ed K J Ellis, S Yasumura and W D Morgan (London: The Institute of Physical Sciences in Medicine) pp 300−-10

Christoffersson J-O and Mattsson S 1983 Polarised x-rays in XRF-analysis for improved *in vivo* detectability of cadmium in man *Phys. Med. Biol.* **28** 1135–44

Cohn S H and Parr R M (ed) 1985 Nuclear based techniques for the *in vivo* study of human body composition *Clin. Phys. Phys. Meas.* **6** 275–301

Cole T J, Bellizzi M C, Flegal K M and Dietz W H 2000 Establishing a standard definition for child overweight and obesity worldwide: international survey *Br. Med. J.* **320** 1240–3

Cole K S and Cole R H 1941 Dispersion and absorption in dielectrics: I. Alternating current characteristics *J. Chem. Phys.* **9** 341–51

Collins A L, Saunders S, McCarthy H D, Williams J E and Fuller N J 2004 Within- and between-laboratory precision in the measurement of body volume using air displacement plethysmography and its effect on body composition assessment *Int. J. Obes.* **28** 80–90

Cornish B H, Bunce I H, Ward L C, Jones L C and Thomas B J 1996 Bioelectrical impedance for monitoring the efficacy of lymphoedema treatment programmes *Breast Cancer Res. Treat.* **38** 69–76

Cornish B H, Jacobs A, Thomas B J and Ward L C 1999 Optimising electrode sites for segmental bioimpedance measurements *Physiol. Meas.* **20** 241–50

Cornish H, Ward L C and Thomas B J 1992 Measurement of extracellular and total body water in rats using multiple frequency bioelectrical impedance analysis *Nutr. Res.* **12** 657

Davies S, Spanel P and Smith D 2001 Rapid measurement of deuterium content of breath following oral ingestion to determine body water *Physiol. Meas.* **22** 651–9

De Lorenzo A and Mohamed E I (ed) 2003 Sixth international symposium on *in vivo* body composition studies *Acta Diabetol.* (Suppl. 1) **40** 1–319

Demerath E W, Guo S S, Chumlea W C, Towne B, Roche A F and Siervogel R M 2002 Comparison of percent body fat estimates using air displacement plethysmography and hydrodensitometry in adults and children *Int. J. Obes. Relat. Metab. Disord.* **26** 389–97

Durnin J V G A and Womersley J 1974 Body fat assessed from total density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years *Br. J. Clin. Nutr.* **32** 77–97

Edelman I S, Olney J M, James A H, Brooks L and Moore F D 1952 Body composition: studies in the human being by the dilution principle *Science* **115** 447–54

Elia M and Stratton R 2005 Abstracts 7th international symposium *in vivo* body composition studies *Int. J. Body Composition Res.* **3** 77–128

Ellis K J 2000 Human body composition: *in vivo* methods *Physiol. Rev.* **80** 649–80

Ellis K J 2005 Whole body counting and neutron activation analysis *Human Body Composition* 2nd edn ed S B Heymsfield *et al* (Champaign, IL: Human Kinetics) pp 51–62

Ellis K J, Bell S J, Chertow G M, Chumlea W C, Knox T A, Kotler D P, Lukaski H C and Schoeller D A 1999 Bioelectrical impedance methods in clinical research: a follow-up to the NIH technology assessment conference *Nutrition* **15** 874–80

Ellis K J and Eastman J (ed) 1993 *Human Body Composition. In vivo Methods, Models and Assessment* (New York: Plenum)

Ellis K J, Pratt J, Puyau M, Shypailo R and Butte N F 2001 Can air-displacement plethysmography replace hydrodensitometry for body composition analysis in children and adults? *Fed. Am. Soc. Exp. Biol. J.* **15** 1004 (abstract only)

Ellis K J and Shypailo R J 1998 Bone mineral and body composition measurements: cross-calibration of pencil-beam and fan-beam dual-energy x-ray absorptiometers *J. Bone Miner. Res.* **13** 1613–8

Ellis K J, Yasumura S and Morgan W D 1987 *In vivo Body Composition Studies* (London: The Institute of Physical Sciences in Medicine)

Emsley J 1998 *The Elements* 3rd edn (Oxford: Clarendon)

Espejo M G A, Neu J, Hamilton L, Eitzman B, Gimotty P G and Bucciarelli R L 1989 Determination of extracellular fluid volume using impedance measurements *Crit. Care Med.* **17** 360–3

Forbes G B 1987 *Human Body Composition. Growth, Aging, Nutrition, and Activity* (New York: Springer) pp 1–341

Foster M A, Hutchison J M S, Mallard J R and Fuller M 1984 Nuclear magnetic resonance pulse sequence and discrimination of high- and low-fat tissues *Magn. Res. Imaging* **2** 187–92

Foster K R and Lukaski H C 1996 Whole-body impedance—what does it measure? *Am. J. Clin. Nutr.* **64** 388–96

Freedman D S, Dietz W H, Srinivasan S R and Berenson G S 1999 The relation of overweight to cardiovascular risk factors among children and adolescents: the Bogalusa Heart Study *Pediatrics* **103** 1175–82

Gallagher D, Allen A, Wang Z, Heymsfield S B and Krasnow N 2000 Smaller organ tissue mass in the elderly fails to explain lower resting metabolic rate *Ann. New York Acad. Sci.* **904** 449–55

Gallagher D, Visser M, Sepulveda D, Pierson R N Jr, Harris T and Heymsfield S B 1996 How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? *Am. J. Epidemiol.* **143** 228–39

Genton L, Hans D, Kyle U G and Pichard C 2002 Dual-energy x-ray absorptiometry and body composition: differences between devices and comparison with reference methods *Nutrition* **18** 66–70

Goodpaster B H, Kelley D E, Thaete F L, Jing H E and Ross R 2000 Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content *J. Appl. Physiol.* **90** 104–10

Gordon C L, Chettle D R and Webber C E 1993 An upgraded ^{109}Cd K x-ray fluorescence bone lead measurement *Human Body Composition. In vivo Methods, Models, and Assessment* ed K J Ellis and J Eastman (New York: Plenum) pp 285–8

Grinyer J, Byun S H and Chettle D R 2005 *In vivo* prompt gamma neutron activation analysis of cadmium in the kidney and liver *Appl. Radiat. Isot.* **63** 475–9

Grönberg T, Sjöberg S, Almén T, Golman K and Mattsson S 1983 Non-invasive estimation of kidney function by x-ray fluorescence analysis: elimination rate and clearance of contrast media injected for urography in man *Invest. Radiol.* **18** 445–52

Guo S S and Chumlea W C 1999 Tracking of body mass index in children in relation to overweight in adulthood *Am. J. Clin. Nutr.* **70** 145–8

Guo S S, Huang C and Maynard L 2000 Body mass index during childhood, adolescence, and young adulthood in relation to adult overweight and adiposity: the Fels Longitudinal Study *Int. J. Obes.* **24** 628–35

Hackney A C and Deutsch D T 1985 Accuracy of residual volume prediction—effects on body composition estimation in pulmonary dysfunction *Can. J. Appl. Sport Sci.* **10** 88–93

Hakulinen M A, Saarakkala S, Töyräs J, Kröger H and Jurvelin J S 2003 Dual energy x-ray laser measurement of calcaneal bone mineral density *Phys. Med. Biol.* **48** 1741–52

Hammer L D, Kraemer H C, Wilson D M, Ritter P L and Dornbusch S M 1991 Standardized percentile curves of body-mass index for children and adolescents *Am. J. Dis. Child.* **145** 259–63

Hayes P A, Sowood P S, Belyanin A and Smith F W 1988 Subcutaneous fat thickness measured by magnetic resonance imaging, ultrasound and callipers *Med. Sci. Sports Exerc.* **20** 303–9

Heymsfield S B, Lohman T G, Wang Z and Going S B (ed) 2005 *Human Body Composition* 2nd edn (Champaign, IL: Human Kinetics) pp 1–522

Heymsfield S B, Olafson R P, Kutner M N and Nixon D W 1979 A radiographic method of quantifying protein-calorie undernourishment *Am. J. Clin. Nutr.* **32** 693–702

Heyward V H and Wagner D 2004 *Applied Body Composition Assessment* 2nd edn (Champaign, IL: Human Kinetics)

Hoffer P B, Jones W B, Crawford R B, Beck R and Gottschalk A 1968 Fluorescent thyroid scanning: a new method of imaging the thyroid *Radiology* **90** 342–4

Horlick M 2001 Body mass index in childhood—measuring a moving target *J. Clin. Endocrinol. Metab.* **86** 4059–60

Houtkooper L B, Lohman T G, Going S B and Howell W H 1996 Why bioelectrical impedance analysis should be used for estimating adiposity *Am. J. Clin. Nutr.* **64** 436–48

Jonson R, Mattsson S and Unsgaard B 1988 A method for *in vivo* analysis of platinum after chemotherapy with cisplatin *Phys. Med. Biol.* **33** 847–57

Kehayias J J, Heymsfield S B, LoMonte A F, Wang J and Pierson R N Jr 1991 *In vivo* determination of body fat by measuring total body carbon *Am. J. Clin. Nutr.* **53** 1339–44

Kehayias J J and Zhuang H 1993 Use of the Zetatron D-T neutron generator for the simultaneous measurement of carbon, oxygen, and hydrogen *in vivo* in humans *Nucl. Instrum. Methods* B **79** 555–9

Kistorp C N and Svendsen U L 1997 Body composition analysis by dual energy x-ray absorptiometry in female diabetics differ between manufacturers *Eur. J. Clin. Nutr.* **51** 449–54

Knight G S, Beddoe A H, Streat S J and Hill G L 1986 Body composition of two human cadavers by neutron activation and chemical analysis *Am. J. Physiol.* **250** E179–85

Krauel J B, Speed M A, Thomas B W, Baddeley H and Thomas B J 1980 *In vivo* measurement of organ tissue levels of cadmium *Int. J. Appl. Radiat. Isot.* **31** 101–6

Kreitzman S N and Howard A N (ed) 1993 *The Swansea Trial. Body Composition and Metabolic Studies With a Very-Low-Calorie Diet (VLCD)* (London: Smith-Gordon) pp 1–168

Kushner R F 1992 Bioelectrical impedance analysis: a review of principles and applications *J. Am. Coll. Nutr.* **11** 199–209

Kyere K, Oldroyd B, Oxby C B, Burkinshaw L, Ellis R E and Hill G L 1982 The feasibility of measuring total body carbon by counting neutron inelastic scatter gamma rays *Phys. Med. Biol.* **27** 805–17

Kyle U G *et al* 2004a Bioelectrical impedance analysis: part I. Review of principles and methods *Clin. Nutr.* **23** 1226–43

Kyle U G *et al* 2004b Bioelectrical impedance analysis: part II. Utilization in clinical practice *Clin. Nutr.* **23** 1430–53

Lindsay R S, Hanson R L, Roumain J, Ravussin E, Knowler W C and Tataranni P A 2001 Body mass index as a measure of adiposity in children and adolescents: relationship to adiposity by dual energy x-ray absorptiometry and to cardiovascular risk factors *J. Clin. Endocrinol. Metab.* **86** 4061–7

Lohman T G 1989 Assessment of body composition in children *Pediatr. Exerc. Sci.* **1** 19–30

Lukaski H C and Bolunchuk W W 1988 Estimation of body fluid volumes using tetrapolar bioelectrical impedance measurements *Aviat. Space Environ. Med.* **59** 1163–9

Lukaski H C, Johnson P E, Bolunchuk W W and Lykken G I 1985 Assessment of fat free mass using bioelectrical measurements of the human body *Am. J. Clin. Nutr.* **41** 810–7

Mattsson S and Scott M C 1990 *In vivo* elemental analysis using x-ray fluorescence: techniques and applications *XRF and PIXE Applications in Life Science* ed R Moro and R Cesareo (Singapore: World Scientific) pp 193–206

Maynard L M, Wisemandle W, Roche A F, Chumlea W C, Guo S S and Siervogel R M 2001 Childhood body composition in relation to body mass index *Pediatrics* **107** 344–50

McCrory M A, Gomez T D, Bernauer E M and Mole P A 1995 Evaluation of a new air displacement plethysmography for measuring human body composition *Med. Sci. Sports Exerc.* **27** 1686–91

McNeill F E and O'Meara J M 1999 The *in vivo* measurement of trace heavy metals by K x-ray fluorescence *Adv. X-Ray Anal.* **41** 910–21

Mikat R P 2002 Effect of lung-volume correction on total body volume assessment using dual digital photograph anthropometry *JEPonline* **5** 28–31

Millard-Stafford M L, Collins M A, Modlesky C M, Snow T K and Rosskopf L B 2001 Effect of race and resistance training status on the density of fat-free mass and percent fat estimates *J. Appl. Physiol.* **91** 1259–68

Moore F D, Olesen K H, McMurray J D, Parker H V, Ball M R and Boyden C M 1963 *The Body Cell Mass and Its Supporting Environment* (Philadelphia, PA: Saunders)

Morgan W D 2000 Of mermaids and mountains. Three decades of prompt activation *in vivo Ann New York Acad. Sci.* **904** 128–33

Must A, Jacques P, Dallal G, Bajema C and Dietz W 1992 Long-term morbidity and mortality of overweight adolescents: a follow-up of the Harvard Growth Study of 1922 to 1935 *N. Engl. J. Med.* **327** 1350–5

Neovius M G, Linne Y M, Barkeling B S and Rossner S O 2004 Sensitivity and specificity of classification systems for fatness in adolescents *Am. J. Clin. Nutr.* **80** 597–603

Nie H, Chettle D, Luo L and O'Mera J 2006 *In vivo* investigation of a new ^{109}Cd γ-ray induced K-XRF bone lead measurement system *Phys. Med. Biol.* **51** 351–60

Nilsson U, Attewell R, Christoffersson J-O, Schütz A, Ahlgren L, Skerfving S and Mattsson S 1991 Kinetics of lead in bone and blood after end of occupational exposure *Pharmacol. Toxicol.* **68** 477–84

Oldroyd B, Truscott J G, Woodrow G, Milner R, Stewart S P, Smith A H, Westmacott C F and Smith M A 1998 Comparison of *in vivo* body composition using two Lunar dual-energy x-ray absorptiometers *Eur. J Clin. Nutr.* **52** 180–5

O'Meara J M, Chettle D R, McNeill F E and Webber C E 1997 The feasibility of measuring bone uranium concentrations *in vivo* using source excited K x-ray fluorescence *Phys. Med. Biol.* **42** 1109–20

Organ L W, Bradham G B, Gore D T and Lozier S L 1994 Segmental bioimpedance analysis: theory and application of a new technique *J. Appl. Physiol.* **77** 98–112

Pejovic-Milic A, Byun S H, Comsa D C, McNeill F E, Prestwick W W and Chettle D R 2005 *In vivo* measurement of aluminium: recent developments *J. Inorg. Biochem.* **99** 1899–903

Piekarski J, Goldberg H I, Royal S A, Axel L and Moss A A 1980 Difference between liver and spleen CT numbers in the normal adult: its usefulness in predicting the presence of diffuse liver disease *Radiology* **137** 727–9

Pierson R N Jr 2003 A brief history of body composition—from F.D. Moore to the new Reference Man *Acta Diabetol.* **40** 114–6

Pietrobelli A, Faith M S, Allison D B, Gallagher D, Chiumello G and Heymsfield S B 1998 Body mass index as a measure of adiposity among children and adolescents: a validation study *J. Pediatr.* **132** 204–10

Ross R 2003 Advances in the application of imaging methods in applied and clinical physiology *Acta Diabetol.* **40** 45–50

Ross R and Janssen I 2005 Computed tomography and magnetic resonance imaging *Human Body Composition* 2nd edn ed S B Heymsfield *et al* (Champaign, IL: Human Kinetics)

Ross R, Leger L, Morris D, de Guise J and Guardo R 1992 Quantification of adipose tissue by MRI: relationship with anthropometric variables *J. Appl. Physiol.* **72** 787–95

Ryde S J S, Birks J L, Morgan W D, Evans C J and Dutton J 1993 A five-compartment model of body composition of healthy subjects assessed using *in vivo* neutron activation analysis *Eur. J. Clin. Nutr.* **47** 863–74

Ryde S J S, Morgan W D, Sivyer A, Evans C J and Dutton J 1987 A clinical instrument for multi-element *in vivo* analysis by prompt, delayed and cyclic neutron activation using ^{252}Cf *Phys. Med. Biol.* **32** 1257–71

Scharfetter H, Schlager T, Stollberger R, Felsberger R, Hutten H and Hinghofer-Szalkay H 2001 Assessing abdominal fatness with local bioimpedance analysis: basics and experimental findings *Int. J. Obes. Relat. Metab. Disord.* **25** 502–11

Scheltinga M 1992 *Bioelectrical Impedance Analysis (BIA): A Bedside Method for Fluid Measurement* (Amsterdam: VU University Press)

Schloerb P R, Forster J, Delcore R and Kindscher J D 1996 Bioelectrical impedance in the evaluation of liver disease *Am. J Clin. Nutr.* **64** 510–4

Schoeller D A 2005 Hydrometry *Human Body Composition* 2nd edn ed S B Heymsfield *et al* (Champaign, IL: Human Kinetics) pp 35–49

Schutte J E, Townsend E J, Hugg J, Shoup R F, Malina R M and Blomqvist C G 1984 Density of lean body mass is greater in blacks than in whites *J. Appl. Physiol.* **56** 1647–9

Segal K R, Burastero S, Chun A, Coronel P, Pierson R N Jr and Wang J 1991 Estimation of extracellular and total body water by multiple-frequency bioelectrical impedance measurement *Am. J. Clin. Nutr.* **54** 26–9

Shakeshaft J and Lillicrap S 1993 An x-ray fluorescence system for the determination of gold *in vivo* following chrysotherapy *Br. J. Radiol.* **66** 714–7

Shen W, St-Onge M-P, Wang Z and Heymsfield S B 2005 Study of body composition: an overview *Human Body Composition* 2nd edn ed S B Heymsfield, T G Lohman, Z Wang and S B Going (Champaign, IL: Human Kinetics) pp 1–14

Shypailo R J and Ellis K J 2005 Design considerations for a neutron generator-based total-body irradiator *J. Radioanal. Nucl. Chem.* **263** 759–65

Siri W E 1956 The gross composition of the body *Advances in Biological and Medical Physics* ed C A Tobias and J H Lawrence (New York: Academic)

Sjöström L, Kvist H, Cederblad A and Tylén U 1986 Determination of total adipose tissue and body fat in women by computed tomography, ^{40}K, and tritium *Am. J. Physiol.* **250** 736–45

Smith D and Spanel P 2001 On-line determination of the deuterium abundance in breath water vapour by flowing afterglow mass spectrometry with applications to measurements of total body water *Rapid Commun. Mass Spectrom.* **15** 25–32

Somervaille L J, Chettle D R and Scott M C 1985 *In vivo* measurement of lead in bone using x-ray fluorescence *Phys. Med. Biol.* **30** 929–43

Starck G, Lönn L, Cederblad Å, Alpsten M, Sjöström L and Ekholm S 1998 Radiation dose reduction in CT: application to tissue area and volume determination *Radiology* **209** 397–403

Stone D A and Robinson M P 2004 Total body water measurements using resonant cavity perturbation techniques *Phys. Med. Biol.* **49** 1773–88

Sutcliffe J F 1996 A review of *in vivo* experimental methods to determine the composition of the human body *Phys. Med. Biol.* **41** 791–833

Taylor R W, Jones I E, Williams S M and Goulding A 2002 Body fat percentages measured by dual-energy x-ray absorptiometry corresponding to recently recommended body mass index cutoffs for overweight and obesity in children and adolescents aged 3–18 y *Am. J. Clin. Nutr.* **76** 1416–21

Thomas B J, Cornish B H and Ward L C 1992 Bioelectrical impedance analysis for measurement of body fluid volumes: a review *J. Clin. Eng.* **17** 505–10

Thomas E L, Saeed N, Hajnal J V, Brynes A, Goldstone A P, Frost G and Bell J D 1998 Magnetic resonance imaging of total body fat *J. Appl. Physiol.* **85** 1778–85

Tothill P, Avenell A and Reid D M 1994 Precision and accuracy of measurements of whole-body mineral: comparisons between Hologic, Lunar and Norland dual-energy x-ray absorptiometers *Br. J Radiol.* **67** 1210–7

Vaisman N, Pencharz P B, Koren G and Johnson J K 1987 Comparison of oral and intravenous administration of sodium bromide for extracellular water measurements *Am. J. Clin. Nutr.* **46** 1–4

Wang Z M, Heshka S, Wang J and Heymsfield S B 2005a Body cell mass: validation of total body potassium prediction model in children and adolescents *Int. J. Body. Composition Res.* **3** 153–8

Wang Z M, Pierson R N Jr and Heymsfield S B 1992 The five level model: a new approach to organizing body composition research *Am. J. Clin. Nutr.* **56** 19–28

Wang Z M, Shen W, Withers R T and Heymsfield S B 2005b Multicomponent molecular-level models of body composition analysis *Human Body Composition* 2nd edn ed S B Heymsfield *et al* (Champaign, IL: Human Kinetics) pp 163–76

Watson P E, Watson I D and Bratt R D 1980 Total body water volume for adult males and females estimated from simple anthropometric measurements *Am. J. Clin. Nutr.* **33** 27–39

Wells J C K, Douros I, Fuller N J, Elia M and Dekker L 2000 Assessment of body volume using three-dimensional photonic scanning *Ann. New York Acad. Sci.* **904** 247–54

Werdein E J and Kyle L H 1960 Estimation of the constancy of the fat-free body *J. Clin. Invest.* **39** 626–9

Werkman A, Deurenberg-Yap M, Schmidt G and Deurenberg P 2000 A comparison between composition and density of the fat-free mass of young adult Singaporean Chinese and Dutch Caucasians *Ann. Nutr. Metab.* **44** 235–42

Wielopolski L, Ramirez L M, Coyle P, Heymsfield S and Zimman J 2003 Some aspects of measuring levels of potassium in the brain *Acta Diabetol.* **40** 73–5

Wielopolski L, Rosen J F, Slatkin D N, Zhang R, Kalef-Ezra J A, Rothman J C, Maryanski M and Jenks S T 1989 *In vivo* measurement of cortical bone lead using polarised x-rays *Med. Phys.* **16** 521−-8

Wilmore J H, Vodak P A, Parr R B, Girandola R N and Billing J E 1980 Further simplification of a method for determination of residual lung volume *Med. Sci. Sports Exerc.* **12** 216–8

Visser M, Gallagher D, Deurenberg P, Wang J, Pierson R N Jr and Heymsfield S B 1997 Density of fat-free body mass: relationship with race, age, and level of body fatness *Am. J. Physiol. Endocrinol. Metab.* **272** E781–7

Yasumura S, Harrison J E, McNeill K G, Woodhead A D and Dilmanian F A (ed) 1990 *In vivo Body Composition Studies* (New York: Plenum)

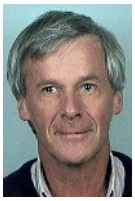
Yasumura S, Wang J and Pierson R N Jr (ed) 2000 *In vivo* body composition studies *Ann. New York Acad. Sci.* **904** 1–631

Zhu F *et al* 2006 Segment-specific resistivity improves body fluid volume estimates from bioimpedance spectroscopy in hemodialysis patients *J. Appl. Physiol.* **100** 717–24

Zillikens M C, van den Berg J W O, Wilson J H P and Swart G R 1992 Whole body and segmental bioelectrical impedance analysis in patients with cirrhosis of the liver: changes after treatment for ascites *Am. J. Clin. Nutr.* **55** 621–5

Biography

Sören Mattsson is head of the Department of Radiation Physics at Malmö University Hospital and professor of Medical Radiation Physics at Lund University, Sweden. He had earlier the same positions at Göteborg University/Sahlgrenska University Hospital. He has over 30 years experience of various areas of medical physics, including diagnostic imaging, *in vivo* body composition studies, heavy metal research, radioactive tracer studies, radiation therapy, radiation dosimetry and radiation protection.



Brian Thomas is Head of the School of Physical and Chemical Sciences at the Queensland University of Technology (QUT), Brisbane, Australia. He has more than 30 years experience of research in the area of body composition analysis moving into this area after completing a PhD in nuclear physics at the University of Western Australia. He held postdoctoral positions at the University of Toronto and University of Birmingham before moving to the QUT. His current research interest is primarily in the application of bioimpedance.