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Chapter Two:

Laboratory methods of Body composition Analysis

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This chapter will cover:

- 2.1 Cadaver analysis
- 2.2 Underwater weighing
- 2.3 Air Displacement Plethysmography
- 2.4 Isotope Dilution methods
- 2.5 Medical imaging methods
- 2.6 Dual X-ray Absorptiometry
- 2.7 3D photonic scanning

2.1 Cadaver analysis

In the initial stages of body composition analysis, cadavers were analysed and later used as a criterion method for validating developing methods which could be used in live subjects. Cadavers have been analysed chemically and anatomically. The chemical approach has allowed the measurement of fat, protein and minerals while the anatomical approach has allowed the determination of the gross tissue weight for different parts of the body separated by dissection. Most information available today is based on analyses performed in the mid-1900s (Matiegka, 1921; Widdowson, McCance & Spray, 1951). This method represents the only truly accurate measurement of composition because of the capability for chemical assessment of dissectible tissue. The mass of these collectively equates that of the fresh cadaver after evaporation of body fluids during the dissection process.

The use of cadaver analysis has been essential for comparison and validation of indirect methods of assessing body composition. In 1984, in a collaboration between a Belgian and a Canadian group, an analysis of 25 cadavers was carried out to provide normative data on the weights and densities of the different body compartments (i.e. skin, adipose tissue, muscle, bone and vital organs) as well as validating indirect methods for the in-vivo estimation of body composition and to provide new models for the assessment of body composition (Clarys, Martin & Drinkwater, 1984). The outcomes for these validation studies (referred to as the Brussels cadaver study) resulted in the conclusion that there were no satisfactory methods for the in vivo estimation of muscle, bone and adipose tissue weights in humans, due to a very small amount of direct data available. In 1990, however, the

research group was able to develop acceptable prediction equations for estimating muscle mass based on cadaver analysis (Martin, Spenst, Drinkwater & Clarys, 1990).

Today we have access to a number of different laboratory methods, each with their advantages and limitations. The development of multiple compartment models are now considered to be the most accurate as they include measurements of body composition at several levels ranging from the atomic to the whole-body level. Multi-component methods, however, are costly and time consuming, hence the need for simpler, faster yet accurate methods of acceptable accuracy.

2.2 Underwater weighing (densitometry)

Underwater was long considered as a criterion method for estimating percent body fat. Modern hydrodensitometry systems consist of a scale within a large heated tank of water (commonly 35-37°C). The participant exhales maximally, while totally immersed and body weight is then recorded. This is repeated at least five times and the highest value is used directly, or alternatively the mean of the highest two or three readings.

Underwater weighing is based on the principles of Archimedes (287-212 BC) where the specific gravity of an object is hypothesised to be the ratio of its mass to the mass of an equal volume of water where:

$$\text{Specific gravity} = \frac{\text{Weight in air}}{(\text{weight in air} - \text{weight in water})}$$

This calculation can be applied for the determination of body volume and body density through water submersion where the loss of weight in water (weight in air-weight in water) equals the weight of displaced water. The water displaced represents a known volume because the density of water is known and constant at different temperatures, provided in table 2.1.

*** Table 2.1 near here ***

Theoretically, underwater weighing can be performed in any still water of adequate depth, for instance a swimming pool. In practice, the most common set up is a specialised small water tank and a seat suspended on cables attached to a load cell which allows the immersion of the participant into the tank. Prior to measuring, the participants are weighed in air wearing swimwear before entering the tank and dislodging air bubbles on the skin surface. Participants are required to expel as much air as possible from their lungs during complete submersion and are then weighed, as illustrated in figure 2.1.

*** Figure 2.1 near here ***

Residual lung volume is then calculated – most commonly with the participant in the tank with the head above the water level . Residual air trapped inside the body (lungs, gastro-intestinal tract) is highly buoyant, and is not part of the body, and therefore needs to be adjusted for. Hence the density equation needs to account for this:

$$\text{Body Density (D}_b\text{)} = \frac{\text{weight in air (kg)}}{[(\text{weight in air (kg)} - \text{weight in water (kg)}) / \text{Density of water}] - \text{trapped air (l)}}$$

(Trapped air comprises the sum of measured residual volume, and gut gas (most commonly assumed to be 0.1 l)

Body mass can be divided into its fat (f_{fat}) and fat-free (f_{FFM}) fractions such that

$$1/D_b = f_{\text{fat}}/D_{\text{fat}} = f_{\text{FFM}}/D_{\text{FFM}}$$

where D_{fat} and D_{FFM} are the densities for the fat and fat free mass compartments, respectively (Ellis, 2000).

Residual lung volume can be predicted from gender, age and stature, but can also be measured directly through oxygen dilution, helium dilution or nitrogen washout. For oxygen dilution, the individual takes 6 deep breaths of a known quantity of pure oxygen (commonly using an anaesthetic bag with exactly 3 l), beginning and ending with complete expiration (Wilmore, Vodak, Parr, Girandola, & Billing, 1980).

For Nitrogen washout:

$$RV = \frac{VO_2 * FEN_2}{0.798 - FEN_2} - DS * CF$$

(Where VO_2 is measured into an anaesthetic bag; FEN_2 is the fraction of N_2 at equilibrium $[100 - (\%O_2 + \%CO_2)]/100$ DS is dead space of mouthpiece and valve; CF is the BTPS correction factor.

Percentage body fat can be calculated using simple equations such as the Siri (1956) or the Brozek, Grande, Anderson & Keys (1963) equations.

$$\% \text{ fat (Siri)} = [(4.95 / D_b) - 4.50] \times 100$$

$$\% \text{ fat (Brozek et al.)} = [(4.57 / D_b) - 4.142] \times 100$$

The Siri and the Brozek *et al.* equations are based on the two-compartment model, suggesting that the body is composed of fat mass and fat free mass (bone, water, lean tissue etc). This is somewhat simplistic as fat free mass composition has been shown to vary considerably with age, gender, ethnicity and exercise. Nevertheless, this approach is highly reproducible ($\pm 1\%$) for individual persons. More complex equations can also be used for calculating percentage body fat from body density based on four compartment models, which take account of the variation in FFM constituents.

Underwater weighing methods were developed to determine body fat as a percentage of total body mass. Due to variations in body hydration, protein and mineral content, however, it has been estimated that a total cumulative error for 3-4% can result when determining the percentage body fat of an individual. Without a correction factor to account for this variation, it is suggested that densitometry should not be used as a reference method for heterogeneous populations (Ellis, 2000).

A number of assumptions need to be made for calculating body density and percentage body fat. It is assumed that the separate densities of the body components are cumulative, that the density of body constituents are constant between individuals; that the proportions of the constituents of the fat-free mass are constant from person to person; that a subject being measured differs from a reference body only in fat content; and that buoyant gas at the time of underwater weighing can be estimated. Unfortunately, none of these assumptions can be fully justified. It is further assumed that the density of fat remains constant, however, there is reason to believe that there may be variations of fat density in relation to gender, ethnicity, growth, sexual maturation, aging, physical activity, and disease (Ellis, 2000).

There are also a number of limitations with under water weighing. Firstly, the individual being measured needs to be comfortable to be immersed in the water while sustaining a maximal exhalation. In addition, for the measurements to be accurate, the individual has to have recovered from previous exercise, to be fasted, fully hydrated and voided. The water in the tank needs to be stirred/mixed to ensure that the water does not stratify and that temperature (and therefore density) of the water remains constant. In theory, underwater weighing is capable of providing body density estimates on very large individuals, however, not all obese patients are able to participate in this procedure which requires climbing steps, descending into a tank of water, hold their breath under water. However, the procedure can be performed in other settings such as swimming or hydrotherapy pools.

Although underwater weighing has historically been considered the gold standard body composition estimate, but may be impractical to implement in most clinical and research settings.

Underwater weighing is mostly used for comparative measures against which other methods of body composition can be validated as part of a multi-component model, or to ascertain the validity of more practical methods in a variety of groups such as children, obese, or the malnourished.

2.3 Air displacement plethysmography

Air displacement plethysmography (ADP) is a rapid, comfortable, non-invasive and safe way of measuring body volume and percentage body fat. Unlike several other methods for assessing body volume, ADP is also able to accommodate various groups of people, including children, obese, elderly and disabled persons.

ADP uses similar fundamental principles to hydrodensitometry where body volume is measured within a known capsule volume and the air pressure difference is detected following breathing. There is now a commercially available machine for ADP called the BodPod (COSMED, Rome, Italy). The system consists of two linked but separate chambers: one serving as a reference volume and the other for the participant. The individual is required to wear close-fitting clothing and a swim cap to minimise trapped air and to remove all jewellery. The participant is required to sit in the measuring chamber and to remain still during the measurement. Once the door is closed and sealed, the pressure increases slightly, and a diaphragm separating the two chambers oscillates which alters the pressure and volume in each. The ratio of the pressures is a measure of the test chamber volume, which is calibrated by a known volume of 50 litres before measuring an individual. The air in the chamber is allowed to compress and expand adiabatically making use of Poisson's Law which states that the ratio of the volumes of the test and reference chambers is equal to the ratio of the pressure amplitudes following perturbation. The relationship of pressure versus volume, at a fixed temperature, is used to calculate the volume of the participant in the measuring chamber as illustrated in figure 2.2.

*** Figure 2.2 near here ***

When carrying out the measurement, one needs to account for temperature change, gas composition (CO_2 and O_2) as well as isothermal versus adiabatic conditions. There is also a need to account for clothing, hair, body surface area and thoracic gas volume. Moisture on the skin as well as hair alters the correction for compressibility of air next to the body surface, leading to an underestimation of percentage body fat.

When measuring body volume by means of ADP, it is assumed that this is taking place in adiabatic conditions. There are, however, a number of potential sources of error due to the presence of isothermal air the volume of which can be compressed 40% more than are adiabatic air. The sources of isothermal air include air trapped in the hair, air in the lungs, air close to the skin and air trapped in clothing. These sources of isothermal air are minimised by ensuring that the individual being measured is wearing a swim cap, by measuring air in the lungs and correcting for it, and by ensuring participants either wear a tight fitting swim suit or lycra shorts. Air close to the skin is also corrected for automatically by the software by calculating a surface area artefact (SAA) based on an estimation of body surface area calculated from body mass and stature. Inaccuracies in these, whether from biological variation in stature and mass, or the formula used to predict surface area will thus contribute to error in the calculation of percentage body fat because of inappropriate estimates of SAA.

A failure to account and correct for sources of isothermal air results in an underestimation of body volume. The importance of wearing appropriate clothing during ADP using the BODPOD was investigated by Peeters & Claessens (2009). They investigated the potential for measuring individuals in a public setting (for example a gym) wearing sports apparel. Their findings demonstrated not only that this approach resulted in a significant underestimation of body volume, overestimation of body density, underestimation of body fat but that these measurements had poor test-retest reliability even if the individuals were wearing exactly the same clothing for their repeat measurements. There was also a significant trend of increasing error as body density increases. Interestingly, These researchers more recently demonstrated that even wearing different types of swim caps can result in significantly different body measurement (Peeters & Claessens, 2010). Their results demonstrated that lycra swim caps do not compress the scalp hair adequately when compared to a silicon cap, not fully eliminating the effect of isothermal air trapped in scalp hair and therefore resulting in and underestimation of body volume.

Other conditions that need to be met for the accurate use of the BodPod system are that the equipment should ideally be in a room of its own, in specific temperature and humidity ranges which do not alter substantially. Changes in pressure due to windows, doors shutting and other movements in the building may affect the validity of the measurements, although avoiding these may be hard to achieve in practice. It is also essential that the participant remains still and breathes regularly because irregular tidal breathing (e.g. yawning, throat clearing, coughing, laughing or breath-holding) will jeopardise the accuracy of the measurement.

Although the BodPod is a system that is straightforward to use properly and reliable, there remain some limitations in the use of this approach. Individuals who are either very tall or very large may find it difficult to fit in the BodPod. The equipment is still to be validated for these and other groups of

interest (e.g. sufferers of certain diseased conditions, disabled individuals or pregnant women). In addition in some instances individuals can experience claustrophobia while inside the BodPod. Despite these limitations, ADP is a promising method for percent body fat estimation and future work should be done to validate its use in different populations and with larger numbers of participants.

2.4 Isotope dilution methods

Isotope dilution can be used to assess body composition through the use of tracers. Their use is based on the assumption that the volume of a compartment is equal to the ratio of the dose of a tracer/concentration in that body compartment within a short time after the dose is administered (Ellis, 2000). The tracer can either be administered orally or intravenously. To measure the tracer, usually two body fluids are collected (e.g. saliva, urine or blood). An initial body fluid collection would be carried out followed by the administration of the tracer. This would provide a background level of the tracer before administration. The body fluid would then be collected again after a pre-determined time for the penetration of the tracer within the compartment of interest, and for equilibrium to be reached.

If a significant amount of tracer is excreted before equilibrium is reached, then a cumulative urine sample (up to 24 hours) may be collected to adjust dose estimate, or alternatively, several blood samples can be collected and the tracer content can be extrapolated to time zero.

When assessing body composition by means of a tracer, a number of assumptions are made. The first assumption is that the tracer is equally distributed only in the exchangeable pool (i.e. the body fluids in which the tracer is in a dynamic state). The second assumption is that the tracer is equally distributed within this pool. Thirdly, that the tracer is not metabolised during the equilibration time; and fourth, that the tracer equilibrium is achieved relatively rapidly. Any breach of these assumptions requires the adjustment of the ratio of administered dose of tracer to fluid concentration.

The measurement of total body water (TBW) allows for the determination of fat free mass (FFM) because of the differential hydration status of fat and FFM. Since FFM contains approximately 73% of water (although this varies with age) and fat mass contains very little water. With TBW and the hydration coefficient, FFM can be estimated. Isotopes of oxygen and hydrogen are introduced into the body as labelled water. The molecules diffuse through the body and equilibrate with the body water. The extent to which the isotope concentration has been diluted indicates the quantity of water in the body.

For TBW, the most direct in-vivo measurement is based on the dilution principle using tracer dose of labelled water (Tritium (^3H), deuterium (D_2O) or oxygen – 18 (^{18}O). This method requires the collection of two body fluids with a sampling pre-dose and the second collection after an equilibration time of 2-3 hours. A larger body water pool would be indicated by a lower isotope concentration.

For extracellular water (ECW) measurement, the basic dilution techniques are the same as for TBW. The difference, however, lies in the administration and collection. The tracer is added to the water and the body fluid usually sampled is plasma. Nonradioactive bromine is the most commonly used tracer. The tracer is administered orally with a second plasma sample collected approximately three to four hours later.

Intracellular water volume can be measured using the dilution of radioactive potassium (^{42}K), but this tracer is short lived and is no longer commercially available. Today, an oral dose of D_2O and bromine is more commonly used from which TBW and ECW are determined and intracellular water (ICW) can then be calculated by subtracting the ECW from the TBW. Thus, calculating ICW involves the cumulative error of the measurements for TBW and ECW.

The method of analysis is dependent on the choice of tracer. ^3H is measured by radioactive beta-counting; ^{18}O is measured by mass spectroscopy; D_2O is measured by infrared absorption, gas chromatography or mass spectroscopy and Br is measured by either high pressure liquid chromatography, x-ray fluorescence, spectrophotometry or mass spectrometry. When determining the tracer to be utilised, it is important to note that ^{18}O is very expensive, that ^3H is cheap but radioactive. Also, background levels are important when D_2O or ^{18}O are used because these occur naturally in the body, while natural levels of ^3H in the body are very low. The dose of the tracer injected is dependent on the mass of the participant and time of body fluid collection. This method of measuring percent body fat is now generally used as part of multi-compartment models against which cheaper and more practical methods of assessment can be compared (Ellis, 2000).

2.5 Medical imaging methods

There are a number of medical imaging techniques available. These include whole body potassium, computed tomography, magnetic resonance imaging and ultrasound. Medical imaging methods use different physical properties to determine internal dimensions and volumes. Most medical imaging methods are used for diagnostics, though can be adapted for body composition.

Total Body Potassium (TBK)

Potassium occurs naturally in the body, and because of this a background value needs to be established. The radioactive isotope ^{40}K makes up $\sim 0.012\%$ of natural potassium in the body and emits a high-energy wavelength γ (1.46 MeV). More than 50% of these will exit the body where they can be externally measured. To do this safely and accurately requires 1) γ -ray detectors with high energy resolution and efficiency; 2) adequate shielding around the subject and detectors to reduce the background levels; 3) a means of identifying and recording the 1.46MeV γ of the ^{40}K (Ellis, 2000). The subject is placed in a supine position between two NaI detectors within a room constructed of a steel shield. Steel itself has contained much higher levels of background activity since the nuclear events of Hiroshima and Nagasaki, and the subsequent nuclear testing in Pacific atolls in the 1950s. As a result, TBK shields are frequently made from radiation-free steel salvaged from ships built before world war II. ^{40}K is recorded as the supine participant slowly passes beneath the detectors to determine the total body potassium (TBK). Based on the assumption from previous cadaver work that the K fraction in fat free mass is 50-62 g/Kg (Boddy, King & Davies, 1973), TBK can be used to determine body cell mass as well as to estimate FFM. With the development of alternative methods, TBK has become less routine in body composition research, but is still used in occupational health settings to assess radiation exposure for workers in the nuclear industry.

In vivo neutron activation (IVNA)

Controlled neutron irradiation transforms atoms of a chemical element to the next nuclear state. In a fraction of a nano-second, the nucleus descends to its ground state, resulting in the release of gamma-rays which have element-specific energies. If, following neutron capture, the atom is altered to a radioactive isotope, the energy will decay over time with a known half-life. Controlled neutron beams can be of varying energies where rapidly-moving neutrons are optimal for tissue penetration, however, most in vivo neutron activation (IVNA) methods rely on low energy (or thermal) neutrons. The energies for a number of elements that occur in the body (i.e. H,C,N, O, Na, Ca, P, and Cl) can be quantified using this method although IVNA is most frequently used to measure total body calcium and total body nitrogen. Different elements require different radiation doses for quantification. For example, nitrogen measurement requires a relatively low dose (<0.3 mSv) while Ca measurements require a much larger dose (>3 mSv) (Ellis, 2000).

The main drawbacks of IVNA is primarily the radiation dose associated with it, and with a relative scarcity of facilities, there is also a lack of standardisation between different instrument designs for the measurement of IVNA.

Computed Tomography

Computed Axial tomography (CAT or CT) scans use a high dose of ionising radiation in the form of X-rays to image a variety of tissues in the body. The X-ray beam is passed through the body and

measured by detectors positioned on the far side of the subject. The X-ray source and detector assembly is rotated as a single unit around the body. Although CT is acquired in the axial plane, coronal and sagittal images can be produced by computer reconstruction. Radiocontrast agents are often used with CT for enhanced delineation of anatomical structures.

Both CT and MRI images are composed of pixels, which are usually 1mm x 1mm and have a third dimension relating to slice thickness. Volume elements are referred to as voxels which have a grey scale that provides image contrast and reflects tissue composition. CT images appear similar to those of MRI although the grayscale contrast indicating different tissues is reversed. CT is a highly accurate and precise method of measuring, but this comes at the expense of a high radiation dose (i.e. ~1-21 mSv per examination using a single-slice spiral CT depending on the site being assessed (Brix *et al.*, 2003).

Magnetic resonance Imaging (MRI)

Hydrogen nuclei (protons) are abundant in living tissues and have magnetic moments which result in them acting as small magnets. These magnetic moments are usually random and cancel each other out, but when subjected to the external magnet of a MRI scanner, the protons have a high affinity for alignment with the magnetic field. The frequency at which the nuclei will change its orientation (relative to the direction of the magnetic field) is called the Larmor frequency. When radiofrequency (RF) energy, at the Larmor frequency, is applied perpendicular to the direction of the magnetic field, the nuclei will absorb the energy and change alignment. When the applied RF signal is turned off, the stored energy is released as an induced RF signal when the nuclei resume their original positions. Parameters of this induced signal are manipulated by the MRI software to produce images characterising specific tissues

Since adipose and lean tissue do not vary greatly in hydrogen densities, to enhance the imaging contrast between lean and fat tissue, another property of the nuclei, the relaxation time (T1 in ms) is also used. Relaxation time refers to the time it takes for the nuclei to release the RF-induced energy and return to a random configuration. T1 for protons in fat is considerably shorter than that for protons in water (Ellis, 2000).

To obtain a whole body image by MRI, a series of multiple scans along the length of the body are required. This procedure can take 30min or longer, by acquiring blocks of images which are subsequently joined together. Figure 2.3 depicts an abdominal cross-section measured at the umbilicus. MRI has been validated by cadaver analysis and is very useful for body composition as it has the potential for measuring VAT and SAT compartments separately. Most of the literature on MRI scanning involves the supine body, but more recently, positional MRI enables the measurement to be

acquired in any orientation, which has provided valuable insight into structural adjustments arising from postural factors relating to the plasticity of tissues or fluid movement within the body, or differential loading of the spine in different orientations.

*** Figure 2.3 *** near here.

Although both CT and MRI generate accurate total and regional body volumes and dimensions, these procedures are expensive and neither is risk free for the subject. Neither method is widely used for body composition purposes due to their high cost and in the case of CT, high radiation exposure. They remain expensive for routine, but can validate other more affordable methods.

Diagnostic ultrasound scanning has been routinely used for safe pre-natal scans for the delineation of structures in a growing foetus. More recently, technological advances have enabled its use for the assessment of body composition, but due to its portable nature, it is described in Chapter 3.

Both CT and MRI are considered to be sophisticated laboratory body composition methods. However, neither of these methods has been validated for the assessment of total body fat against a robust method such as the 4-compartment method approach. More commonly, MRI and CT total body composition is determined by acquiring slices at specific anatomical locations and interpolating between these slices to determine volumes using a geometric model based on either a parallel trapezium or truncated cone. Tissue volumes are then related to mass via assumed densities.

Some studies have attempted to validate CT and MRI by comparison with cadavers (Rossner *et al.*, 1990; Mitsiopoulos, Baumgartner, Heymsfield, Lyons, Gallagher & Ross, 1998) but only do so for certain sections of the body (e.g. subcutaneous and visceral adipose tissue in the abdomen, and subcutaneous adipose tissue in arms and legs). Total percentage body fat determined by MRI was compared to % body fat by underwater weighing (Sohlstrom, Wahlund & Forsum, 1993), however, as discussed above, the limitations of UWW preclude its use as a valid reference for quantifying accuracy. This underscores the need to assess the true accuracy of CT and MRI for the determination of total % body fat compared to the 4-compartment model, if it is to be used for total % fat determination. More commonly, both techniques are used for diagnostics, and composition of specified regions, and not the whole body.

2.6 Dual-energy X-ray absorptiometry (DXA))

Dual-energy X-ray absorptiometry (DXA) was developed from single photon absorptiometry using Iodine-125 on the proximal and distal ulna. This was replaced with dual photon absorptiometry using

Gadolinium-153. This produces gamma emissions at 44 and 100 KeV which enabled estimates of bone mineral density. DXA emerged when radioactive isotopes were replaced with an X-ray source. The filtered beam resulted in high and low energies which are absorbed to differing extents according to the tissue characteristics. Absorption/attenuation characteristics can be measured using chemicals and tissue equivalents to simulate the human body.

When a photon source directed at one side of an object, the intensity of the beam at the other side is determined by the object's thickness, density and chemical composition. This phenomenon is referred to as attenuation - and this enables a DXA scan to produce a composition map of the scanned area of the supine body. A measurement is made of the attenuation of the X-ray beam at both energies, at every point in the scanned area using the ratio of attenuation of the beam at the two energies – the R value. With this, different components of the body are grouped into three different categories by their X-ray attenuation properties, calibrated against tissue-equivalent standards. Attenuation properties of different tissues vary according to the differing atomic masses of the elements they contain. Bone mineral contains calcium and phosphorous in large quantities while lean soft tissue contains small quantities of potassium, chlorine, sulphur, calcium and iron, mostly as electrolytes. Fat, however, contains hydrogen, carbon and oxygen.

When measuring bone mineral density, bone mass is referred to as bone mineral content (BMC). Bone mineral density (BMD), however, is the quantity of bone mass per unit area, and is therefore an areal density in $g \cdot cc^{-2}$. Bone area is calculated as the area of pixels containing bone.

For soft tissue composition assessment (i.e. determining fat and fat-free soft tissue mass), at established energies of 40 and 70 KeV, fat produces an attenuation coefficient of 1.18 (R_f) and 1.399 for fat free soft tissue (R_l). Given the consistency between subjects of these, the ratio of attenuation at the lower energy relative to the higher energy soft tissue (R_{st}) is a function of the proportion of fat (R_f) and fat free soft tissue (R_l) in each pixel:

$$\text{Lean fraction } L_f = [R_{st} - R_f] / [R_l - R_f]$$

Where soft tissue and bone are layered, if the relative intensity of the photon beam can be measured, and the mass attenuation coefficients are known, estimates of the bone mass and overlying soft tissue mass can be calculated.

Although DXA does not provide three independent measurements, values for three different body composition tissues are calculated (BMC, fat-free soft tissue mass and fat mass). To obtain these values, it is assumed that the composition of the soft tissue layer overlying the bone has the same fat-to-lean ratio as that for non-bone pixels in the same scan region (Ellis, 2000). Note that the use of

'lean' tissue can be misleading, because elsewhere it is defined as including essential lipids. Fat-free mass and fat-free soft tissue mass (which excludes BMC) are less ambiguous.

DXA can also be used to estimate muscle mass. The mass of appendicular fat-free soft tissue (AFFST) can be estimated from DXA but muscle mass predictions rely on the assumption that all non-fat and non-bone tissue is muscle mass, and that the appendicular and torso muscle occur in a known ratio. In reality, AFFST's largest proportion comprises skeletal muscle, but it also comprises skin, connective tissue and the lean portion of adipose tissue. This assumption of AFFST equating to muscle should be reasonably accurate in the arms, legs, and regions in between joints where the amount of tendons and cartilage are small (Visser, Fuerst, Lang, Salamone, & Harris, 1999). A number of models have been developed to determine skeletal muscle mass based on measurements of AFFST, but often require adjustments for age and gender which will have an effect on AFFST. The models available require to be validated across different populations, but still provide an important alternative to CT and MRI for the assessment of muscle mass.

The use of DXA for determining composition requires us to make a number of assumptions. It is assumed that body tissues are represented by attenuation coefficients; soft tissue composition in pixels containing bone can be estimated from neighbouring pixels according to the fat distribution model; and that anterior-posterior thickness does not affect measurements. It has been reported that DXA significantly underestimates percent body fat in lean individuals and overestimates fat in the obese (LaForgia, Dollman, Dale, Withers & Hill, 2009). Although there is a trend for DXA to progressively underestimate percent body fat of leaner individuals, which could be accounted for using correction factors, in the obese there appears to be no systematic bias. Previous work using DXA found that large errors in percent body fat were associated with increasing tissue thickness, where the thicker the tissue under analysis, the greater the degree of beam hardening which results in greater attenuation of the lower energy X-rays. The results from LaForgia *et al.*, (2009) suggested that the precision of estimating percent body fat using DXA remains questionable. Due to this, they proposed that DXA may be suitable for providing acceptable descriptive cross-sectional body composition data for obese cohorts but is less suitable for providing accurate measures at the individual level.

As highlighted previously, DXA is unable to measure soft tissue overlying the bone and assumes that the soft tissue adjacent to the bone has the same tissue composition as the soft tissue overlying the bone. This assumption may cause a greater error associated with all soft tissue measurements in muscular athletes due to larger variations of soft tissue over bone associated with various distributions of muscle and fat.

There are also a number of practical issues that need to be considered with DXA. These include X-ray dosage, obtaining ethics permission and consent for the measurement; ensuring that the individual to be measured is not pregnant, difficulties for fitting on the equipment in relation to height, weight and overall body mass (for obese individuals and larger athletes); clothing; jewellery; calcified tissue; metal implants and movement.

DXA scanning is a very useful tool for the assessment of body composition, as the resultant radiation exposure from a whole body scan is relatively minor (depending on the machine model) and is comparable to the unavoidable daily background radiation. This has made it a more acceptable method of determining body composition in at-risk groups including children. DXA is also useful when investigating the effects of disability on body composition where changes in bone mineral density and lean-tissue hydration are evident, and where the presence of artefacts such as surgical implants can be adjusted for. But DXA scanning is not without its limitations. DXA scans are stable for normal ranges of adiposity and size (up to 25cm thick), but not as accurate for larger individuals where the error is magnified. Bone data are altered via measurement artefacts in a person with changing adiposity, while clothing and gut content are included in the calculations for fat free soft tissue/fat. Significant variation in measurement for the same individual using different manufacturers' instruments (Ellis, 2000). Disparities in potential error exist among different DXA machines and models, making comparisons between investigations difficult and underscoring the need for standardisation of the procedures. Protein mass is not specifically measured and therefore, the estimate for the lean mass using DXA can vary from the true protein mass (Ellis, 2000).

Errors in estimating percentage body fat in women due to fluctuations in total body water through various stages of menstrual cycle were also shown and there is evidence to suggest that DXA may not be appropriate for estimating percentage body fat in Caucasian female athletes (Moon *et al.*, 2009). Finally, DXA scanning can be limited for very obese individuals where body size "allowance" is limited by the scan area for the DXA machine. More recently, the GE Healthcare Lunar iDXA has become available which has a scanning bed which can withstand greater weights and provides a larger scanning width as well as producing higher resolution images. In addition, this equipment allows one side of the scanning table to be extended to allow for half-body scans of extremely large individuals. This new equipment appears to provide good precision for total body measurements of body composition and fat distribution (Hind, Oldroyd & Truscott, 2010) and accurate measurement of nonbone lean and fat masses, and percent body fat in obese adults (Rothney, Brychta, Schaefer, Chen & Skarulis, 2009) using half-body scans.

Despite the limitations and assumptions required, DXA scans can be performed within minutes and provide immediate results and is able to provide region specific data via computer software. Future

work for the development of DXA scanning would require precision studies for a variety of populations (e.g. children, obese, athletes or disabled). The unique ability for DXA to estimate regional composition means that it has considerable potential for future body composition research. Pixel size and regional boundary placement during analysis, invariably mean that regional precision, especially in the arms, is poorer than that of the whole body. It is widely recognised that different manufacturers scanners yield different results, and despite limited success of previous standardisation attempts, more work needs to be undertaken to standardise DXA results between different manufacturers.

2.7 3D photonic scanning

Three dimensional photonic imaging was developed in the late 1950s from stereo photogrammetry and was subsequently developed as a technology for whole-body surface measurements in humans. Several different models of scanners have been developed and improved on over the years, enhancing the data density and the speed of acquisition for measurement to ~ 10s, generating values for regional body volumes and dimensions as well as for total body volume. Measurements and ratios of different body girths such as waist-hip or waist-chest ratio can provide important information about weight distribution. This can be done by measuring skin folds and anthropometry, but multiple manual measurements can be time-consuming, supporting the potential usefulness of three dimensional photonic scanning (3DPS). Also, manual anthropometric measurements and 3DPS may not be directly equivalent as hand-held tape measures on the skin surface, even although they aim not to indent the skin, may do so to a limited extent in order to locate the tape itself and hence compresses the circumference being measured, which is not an issue for 3DPS.

Three dimensional laser imaging is a non-invasive optical method that uses high speed digital cameras and trigonometric principles to detect the actual position of laser-light points projected onto the surface of an object and reflected to cameras. A 3D laser scanner consists of a number of category 1 laser columns positioned inside a scanning cabin delivering light at a wavelength of ~690nm (depending on the manufacturer and model). The participant is asked to wear close-fitting clothing to avoid obscuring the details of skin topography, and is required to stand still in a standard pose to minimise data shadow effect. The system calibration ensures lasers detect a stationary object in focus, but postural sway and breathing artefacts affect human scans. System software approaches involve automatic landmark recognition resulting in the generation of a 3D-body shell as well as values for standard dimensional analyses for girths, surface areas and volumes. Further technical detail on 3D scanning is provided in chapter 4, whereas specific body composition studies are discussed here.

In a study by Wang et al (2006), a C9036-02 laser scanner (Hamamatsu Photonics, Hamamatsu, Japan) was compared to under water weighing and anthropometry for validating the accuracy and reproducibility of total and regional body volumes and dimensions measurements in human subjects with a wide range of age, weight and body fatness and a commercially available mannequin. The protocol involved exhaling completely and maintaining a stationary posture for the scan duration. The body volumes and circumferences were significantly larger when measured using 3DPS when compared to underwater weighing, but there was no significant difference in percentage body fat when comparing the two methods. Despite these differences in total body volume of 62 participants were significant, these were considered to be small and not necessarily clinically significant. However, scientifically, the finding bears closer scrutiny. Interestingly, age, sex and obesity did not affect the relationship between 3DPS and underwater weighing or tape measurements. In scientific (as opposed to clinical) terms, the lack of significant differences in the calculated % fat using the 2C method and the Siri equation, may indicate the inadequacy of this densitometric approach, as small volume differences have considerable influence over calculated density. In addition, the large age range (6-83 y) means the variability in the constituents of the FFM was likely to be considerable in this sample.

Measurements for the mannequin demonstrated the effect of clothing on 3DPS where the total body volume was significantly greater than that measured without clothing. This is simply due to the fact that the data points used to generate body volume when using 3DPS were reflected from the surface of the body or clothing. Overall, the authors concluded that 3DPS is an accurate and repeatable technique for measuring body volume and dimensions, provided participants wear suitable clothing.

An alternative light-based scanner was used for the UK National Sizing survey of adults (Size UK; Wells, Treleaven & Cole, 2007). The study used a cross section of these data to examine the relation between shape and BMI as well as to identify the interactive effects of age and sex on shape. The results suggested that the two main factors associated with weight in men after adjustment for height are chest and waist, while women hip and bust, hence, the genders differ in the body regions that appear to be most influential in terms of shape. The authors further suggest that chest in men but hips in women reflect physique, while waist in men and bust in women reflect fatness. In addition, BMI has limitations in accounting for age-associated changes in the distribution of body weight, which also differ noticeably between genders. Associations of shape with age were significantly stronger in women than in men where shape implied a shift in fat toward the upper body with age for women. The development of a more android pattern of fat distribution with age in women and a lack of such an association in men, would suggest these changes are related to hormonal changes of female reproductive biology changing the strategy of energy deposition with age. As central obesity has been associated with increased risk of cardiovascular disease, this association of age with shape suggests a delay in the detrimental effect of central fat in women which may contribute to the

average greater life expectancy of women in industrialised countries. The study also found that differences in shape between genders were more pronounced in young adults and lessened with age.

Body scanning can acquire useful anthropometric measures for health research, without recourse to quantifying body fat. For instance, sagittal abdominal diameter has been found to be more strongly associated than waist circumference to visceral fat, which in turn is closely associated to cardiovascular risk while thigh girth has been found to have a protective effect for both heart disease and type 2 diabetes. Thus, 3DPS has potential for application in the prevention, classification, and monitoring of diseases that are related to body shape, size or fatness such as growth and development, age, weight management, fitness management and pre- and postsurgical procedures. The association of different variables of shape with different diseases demonstrates the potential that monitoring changes in body shape has for informing clinicians about the risk of outcomes or even in respond to diet, exercise or drug treatment in individual patients. Shape data have the potential to be developed into proxies for physiological markers of health and disease such as blood pressure, blood biochemistry and body fat distribution.

The technique of 3DPS has certain advantages over other body composition measures. The digital nature of data facilitates archiving and retrieval of scans, software allows superimposition of repeat scans to highlight changes. It is a safe and easy method for working with children, providing the child can stay still for the duration of the measurement. The technique also produces a 3-dimensional topographical image which is more complete than a one dimensional measurement like waist circumference and it remains cheaper than other imaging techniques such as CT or MRI and does not require specialised conditions or intensive technological support.

The main limitation of this technique is the requirement for the individual being measured to remain perfectly still for approximately 10 - 15 seconds (depending on the selected resolution and the model of 3D scanner) which would limit its use in the very young or the very ill. Similarly, body hair of sufficient density has been shown to increase the apparent size of body segments which may explain the differences between 3DLS and densitometrically-determined volume

Future work would require further validation to assess the repeatability and accuracy of the diverse measurements that can be obtained from photonic scans. Special attention should be given to posture, where a standard protocol for adopting and maintaining the stance during the measurement should be developed. Investigation of the relation between body shape and risk of morbidity and mortality and surrogate biomarkers for disease for different ethnicities is an important future research priority. Advances in computer graphics and scanning technology mean that 3DLS is likely to become more affordable and widespread for health and sports applications for body composition measurement in the future.

Many of the methods mentioned in this chapter they require considerable research funding or clinical imperative before the costs can be justified. As a consequence, such laboratory techniques are beyond the means of many end users of body composition. This includes competitive athletes, and those following conditioning programmes, for whom less invasive and more affordable methods are warranted. For a review of current methods of body composition methods applied in sport, the reader is directed to Ackland et al. (2011) which highlights some of the issues involved with measuring athletes, and threats to the validity of the assumptions which this involves.

*** Table 2.2 near here ***

Multicompartment models

As discussed above, each laboratory-based method is available for the estimation of body composition. Their attributes are summarised in Table 2.2. However, it is clear that each of these approaches is limited due to the assumptions associated with them. One way to overcome these limitations is through the use of multicompartment models. As discussed in chapter 1, Wang Gallagher, D., Thornton, J.C., Yu, W., Horlick, M. & Pi-Sunyer (1992) noted that body composition could be measured at the atomic, molecular, cellular, tissue, and whole body level. The laboratory methods, which measure at these different levels of analysis, can be combined to form multi-compartment models which are now considered to be the gold standard for body composition estimation. Multi-compartment models have often been used for validating single methods of body composition estimates. The theory is that by combining data from several laboratory methods into a multi-component model, that the error associated with the assumptions for each individual method is reduced. Each measurement, however, needs to be compositionally independent of the other measurements. There are several published multi-compartment models available based on a number of different methodologies (e.g. body volume can be estimated either by air displacement plethysmography or underwater weighing). However, there is some discussion about the best multi-compartment model to use. The four-compartment model is currently very popular and is considered to be the criterion method for body composition is depicted in figure 2.4. Nevertheless, it remains costly and time consuming which unfortunately limits its use at a clinical and research level.

*** Figure 2.4 near here ***

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