TITLE: The Accuracy of Dual Photon Absorptiometry Measurements of Soft Tissue Composition.

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The Accuracy of Dual Photon Absorptiometry Measurements of Soft Tissue Composition

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Abstract

During routine measurements of body composition using a ^{153}Gd based dual photon densitometer, it was observed that negative values were being obtained for the body fat fraction in some adults, in children and in small animals. In these three groups, there appears to be a body size dependent error whereby the measured fat fraction becomes increasingly negative as subject size becomes smaller.

The fat fraction is derived from relating the measured mass attenuation coefficient of soft tissue to an internal calibration based on the use of water and lard as substitutes for muscle and fat. To investigate whether this procedure for instrument calibration is the cause of the fat fraction errors, soft tissue phantoms which contained known amounts of fat, water and protein were prepared. Over the range of fat fractions used, accurate results were obtained.

By using prepared soft tissue and water phantoms it was established that the measured fat fraction incorrectly became progressively smaller as object thickness decreased and incorrectly increased with object thickness. However, accurate measurements were obtained if the equivalent tissue thickness is greater than 9 cm and less than 16 cm of water. Equally reproducible measurements are obtained at all thicknesses investigated.

When dual photon measurements of body composition in 13

adolescent females were compared with measurements obtained from skinfold thicknesses or bioimpedance, there was good agreement between techniques but dual photon results demonstrated a broader range of variation with body size. Comparisons between dual photon absorptiometry derived body composition measurements of 52 male athletes with results obtained from under water weighing allowed for derivation of a simple correction factor for the accuracy errors due to body size.

ACKNOWLEDGEMENT

Like many works of this sort this project represents the combined input of many people.

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CHAPTER 1

WHOLE BODY COMPOSITION MEASUREMENTS

1.0 Introduction

Within the last twenty five years, people have become more conscious of the benefits of regular exercise and a nutritionally balanced diet. This increased awareness of the importance of an active and healthy life-style has highlighted the need for the scientific community to develop and refine various techniques for the measurement of gross body composition. With the emergence of such methods, the fat burning and muscle building ability of fitness regimes can now be studied. Nutritional and dietary programs offered to the general public may be evaluated. Most importantly, the progress of such diseases as anorexia nerovosa and osteoporosis that result in detectable changes in overall body composition may now be studied. Given accurate and precise measurement procedures, the natural history of such diseases can be quantitated and the efficacy of proposed treatment regimes can be evaluated.

A variety of approaches have been used to determine human body composition. One approach is based upon the concept that the body can be divided into two components, one being composed of pure fat and the other consisting of all non fat materials. The various techniques used to measure such a two component body can be grouped into three distinct categories based upon the nature of the

measurement procedure. What follows is a brief description of examples of measurement forms in each of these three groups. Not all measurement techniques currently in use are addressed but those that are considered were chosen because they highlighted the basic principle which was exploited to measure body composition.

1.1 A description of body composition

As a prelude to examining the various approaches used to measure body composition, it is worthwile defining some of the body compartments that will be discussed. The definitions and component masses were gathered from ICRP-23 report on reference man.

Fat is a distinct chemical entity in the body. Depending on its location, it can be grouped into essential and nonessential fat. Nonessential fat is contained in adipose tissue which is principally fat cells closely packed together with connective tissue. Nonessential fat is often labelled storage or excess fat because it is accumulated or utilized by the body in response to caloric intake. Therefore, significant changes in nonessential fat can occur within the body over short time periods (weeks).

The distribution of storage fat is between subcutaneous tissue, marrow fat, and the separable fat around organs. A numerical breakdown is provided in table 1.1. As listed, most of the fat in a reference adult is just below the skin (subcutaneous). Reference man and woman are 70 kg and 58 kg in total body weight respectively. Therefore, nonessential fat comprises approximately

Table 1.1 : The distribution of nonessential fat in males and females.

Site	Weight (kg) Male	Female
Subcutaneous	6.0	10.3
Separable	4.0	3.2
Marrow fat	1.2	1.0
Interstitial	0.8	0.5
Total	12.0	15.0

17% and 26% of the male and female body.

Essential fat is composed of the lipids found in cells. With a large fraction of this lipid component present in membranes for structure, it follows that essential fat levels are not altered by dietary changes. Like its name suggests, essential fat is present even during starvation. Essential fat is approximately 2-5% of the mass of body weight minus nonessential fat. This weight difference is considered as a major body compartment and is commonly labelled as lean body mass(LBM).

Along with essential fat, the lean body mass is made up of the skeleton, muscle and water. The skeleton is defined as an anatomical structure which is comprised of bone, cartilage and certain periarticular tissues. The weight of the skeletons in reference man and woman are 10 kg and 6.8 kg respectively. A breakdown of this skeletal weight is provided in table 1.2. As listed, bone is comprised of bone mineral laid down in a collagen matrix. Consequently, a measurement of bone mineral in the skeleton does not equal the total mass of the skeleton but is approximately 30% of that mass.

The mass of the skeleton changes in response to aging and disease. For persons who experience normal skeletal growth, a peak bone mass is achieved about the age of 20. As earlier highlighted this peak skeletal mass is approximately 10 kg in males and 6.8 kg in females. This level is maintained until the fourth decade of life. At this time skeletal mass starts to decrease. The natural rate of decrease is greater in females than in males. Consequently,

Table 1.2 : The components of the male skeleton. Bone is also divided into its sub components.

Tissue component	Weight (kg)
Bone	5.0
- mineral	2.8
- collagen	2.2
Marrow	3.0
Cartilage	1.1
Periarticular tissue	0.9
Total:	10.0

females experience more skeletal difficulties (such as osteoporosis) later in life due to a weaker skeletal integrity.

The largest contributor to lean body mass is muscle. Of the 70 kg and and 58 kg that comprises a typical male and female, 30 kg(43%) and 20 kg(34%) are due to muscle mass. As a further division, 80% of muscle is water and the remaining fraction is protein. It then follows that water is a major portion (73.2%) of the lean body mass compartment.

Water is not only present in the makeup of muscle tissue but it is also present as extracellular fluid and blood. If essential fat is excluded from the lean body mass, the remaining mass is commonly labelled as fat-free body mass. Further removal of the skeleton results in the remainder being muscle or lean tissue (water). When compared to the body's fat mass, the lean body mass changes slowly over time and is relatively constant over short periods (weeks).

To summarize, each compartment defined earlier is presented in relation to the total body in figure 1.1. The proportions listed are average values adopted from given in ICRP-23. Therefore, for some mass values listed the range of variation between individuals can be quite high. From this figure, it is clear on what grounds the body can be partitioned. This partitioning is independent of sex and so any body composition measurement adopted to measure an adult will not be greatly influenced by gender differences. Having identified and defined the terms commonly used in describing body composition, the compartments being exploited by a given body

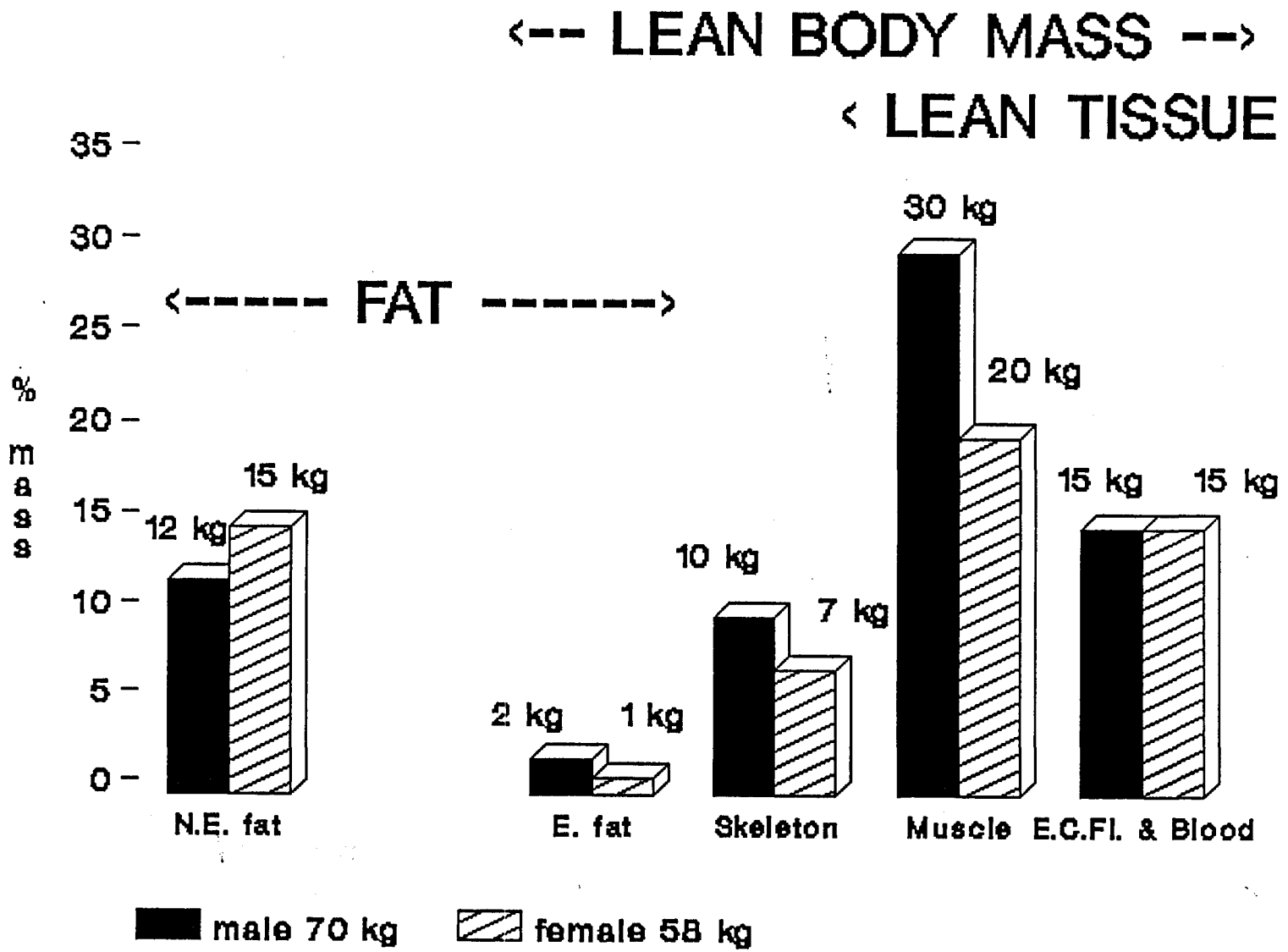


Figure 1.1 : Body composition of reference man and woman.

composition measurement can now be viewed in terms of how it relates to the overall body. Some examples of measurement techniques which best utilize the body's makeup to determine overall composition will now be presented in brief. Again, they will be grouped into three distinct categories based on the nature of the measurement procedure.

1.2 Skinfolds and Bioimpedance

The first set of measurements consist of the use of skinfold calipers and bioimpedance measurements of body resistance. These methods are considered as a single group because they estimate the composition at a local site and predict total body composition based upon this local estimate (Lunar Corporation, 1990). The skinfold method involves the use of calipers to measure subcutaneous adipose tissue thickness at a particular body site. This thickness is related to total body fat mass through established equations. Two fundamental assumptions are made when skin thicknesses measured at a number of sites on the extremities and trunk are translated into an estimation of body fat. The first is that the selected skinfold thicknesses are representative of adipose tissue under the skin. A combination of measurement sites has been found to be a valid indicator of the total amount of subcutaneous fat (Weststrate, et.al. 1989). The second assumption is that subcutaneous adipose tissue mass is directly proportional to total body fat.

The precision of a skinfold measurement can be, at best, 5%.

The accuracy with which a user can determine body fat is dependent upon the type of subject being studied. The relationship between skinfold and total body fat differs with ethnicity, age, and sex (Roche, 1987). For example, fat is distributed differently in women compared to men (see figure 1.1). Therefore, although they are simple, rapid, and easily interpreted, skinfold measurements provide a rather crude estimate of absolute body fat mass.

The technique of bioimpedance uses the measurement of resistance between two subcutaneous tissue sites to predict body fatness. The measurement principle is based on the fact that the body's ability to conduct an electrical current is dependent upon its water and electrolyte distribution. Virtually all body water is confined to the non-fat compartment, contributing 73.2% of the mass. The ease with which an electrical current can be passed through tissue is a direct measure of the water content of the tissue and allows for a calculation of the non-fat mass. Conversely, the resistance that is encountered by the current is a direct measure of the fat content of the tissue.

The bioimpedance measurement is intended to provide an estimate of body make-up. Because an electrical current and resistance can be measured precisely, a typical impedance estimate of body fat and non-fat mass is also precise (2%). However, because the resistance that the applied current encounters is due to subcutaneous fat, it is essential that surface fat mass correlates well with total fat for the impedance measurement to provide an accurate estimates of overall body fat. Although the correlation is

quite good, it varies with age and sex (ICRP, 1975). Consequently, the impedance technique can be quite accurate (5%) for a selected population. Unlike skinfold thickness measurements, highly specialized equipment is required for the bioimpedance technique. It follows that the user must be specifically trained and the sensitivity of the equipment dictates that the apparatus is not mobile. These limitations prevent the use of impedance derived body composition measurements on a widespread clinical scale.

1.3 Densitometry

The second group of methods for the estimation of gross body composition is based on the measurement of density. The density of the whole body is the result of relative contributions of components that differ in density (Mendez, et. al. 1959). The overall density is assumed to be due to the presence of two components: the lean body mass and nonessential fat. Lean body mass is defined as whole body mass minus the nonessential or excess fat. It is worthwile recalling that body fat can be divided into two distinct entities-"essential" and "nonessential" fat (ICRP, 1975). The essential fat is included in the lean body mass because it is composed of the lipid constituents of cells necessary for membrane structure and therefore remains rather distinct from other forms of body fat. Other forms of fat are grouped into the nonessential category. They comprise the storage fat contained in adipose tissue and are located in subcutaneous tissue (approximately half) and in deep seated locations such as yellow bone marrow (ICRP, 1975).

Storage fat changes in response to dietary balance and is therefore quite variable.

The rational for using body density as a means of estimating body composition stems from the concept of a relatively constant lean body mass linked to a variable fat mass. The density of mammalian fat, whether essential or non-essential, has been measured at 0.90 g/cm^3. The density of the lean body varies between individuals ranging from 1.00 g/cm^3 to 1.10 g/cm^3 with an average at 1.05 g/cm^3 (Mendez, et.al. 1959). Therefore, with a relatively constant lean compartment, any change in overall body density results from a change in the body fat mass. As the percentage of body fat increases, the body density decreases.

In order to determine body density a weight and volume must be obtained. Body weight is readily determined from scales. In practice, body volume is obtained by employing Archimedes' principle of water displacement. As given below, volume is calculated by subtracting weight under water from the weight in air.

$$\text{Body Vol.} = [\text{Wt(in air)} - \text{Wt(under water)}]/1.0\ g/cm^3$$

It then follows that

$$\text{Body Density} = \frac{\text{Wt(air)}}{\text{Body Vol.}}$$

This measured density can be related to %fat from tables of density versus body fat established through various animal studies.

The underwater weighing technique provides a more accurate estimate of body fat and lean body mass compared to the bioimpedance and skinfold methods. However, unlike skinfolds and impedance, underwater weighing is much more cumbersome, expensive, and time consuming. These drawbacks exclude its use in a clinical setting.

1.3 Radioisotope based procedures

The third series of measurement techniques available for body composition studies involve the use of radiation or radioactivity in the measurement process. Three measurement forms will be examined.

The first involves the use of neutrons to activate body calcium. This neutron activation system exploit the fact ^{48}Ca which constitutes a small but known fraction of natural elemental Ca can capture thermal neutrons and be converted to radioactive ^{49}Ca, a radioisotope with a half-life of 8.9 minutes. The 3.1 Mev gamma ray emissions from ^{49}Ca can be counted with the use of a whole body counter. The recorded counts can be related back to the calcium mass in the skeleton by appropriate calibration procedures. From the fact that 99% of calcium is contained within bones, a measurement of total body calcium provides an indirect measure of overall skeletal mass. Although unable to provide estimates of fat and lean body mass, the total body calcium measured through activation provides an accurate (5%) and precise (1%) measure of skeletal weight in vivo (Lukaski, 1986). The major disadvantage with the activation procedure is the rather large radiation dose

that is delivered to the subject. Although a single measurement is considered acceptable with respect to dose, repeat measurement over time are regarded as being unethical (Heymsfield, et.al. 1988).

The second use of radioisotope technology involves the calculation of total body potassium. Potassium (K) is present in the lean body mass in a fixed concentration of 68.1 mEq/Kg (ICRP 1975). Therefore a measure of whole body potassium represents a measure of lean body mass. Radioactive ^{40}K comprises 0.012% of naturally occurring potassium in the body. Consequently, total body potassium can be determined from the body ^{40}K content as measured by whole body counting. The 1.46 Mev gamma rays that are emitted from the ^{40}K present in the body are counted and related to lean body mass through the following equation.

$$LBM(Kg) = \frac{\text{Measured total body } ^{40}K(mEq)}{(68.1)mEq/Kg\ (0.00012)}$$

Fat content is then calculated as the difference between total body weight and lean body mass. Given efficient counting, a whole body counter determined measure of ^{40}K can provide a valid estimate of lean body mass and fat mass.

The third use of radiation to probe the body is the technique of Dual Photon Absorptiometry (DPA). In this procedure the body is scanned in a rectilinear pattern using an external ^{153}Gd source. This radionuclide emits photons at two energies. A detector system counts the number of photons at each energy which are transmitted through the body. The differential attenuation of the two photon

energies is analyzed and bone mineral and soft tissue mass is calculated for the scanned subject. The soft tissue mass is then divided into muscle and fat based on attenuation measurements obtained at sites where no bone is present. An in-depth examination of the theory of the DPA technique is reserved for the following chapter.

1.5 The Basis for DPA Assessment

As briefly outlined above, DPA is a recently developed means for measuring skeletal mass, lean body mass, and fat mass simultaneously. Unlike other techniques previously discussed, DPA provides an absolute measure of bone, lean, and fat at each measurement site. This distinct advantage has led to the development of commercial DPA systems. Several examples of these systems are being used on a daily basis in Hospitals and Clinics across Canada. However, being first generation systems, their ability to partition the body has been error prone leading to uncertainties in the results.

In this work, errors observed during clinical measurements performed with the commercially available Norland 2600 Dual Photon Scanner will be examined. The types of errors observed will be considered. An estimation of the uncertainty associated with the absolute measurement of fat and lean masses in a given individual and for the averages of groups of individuals will be assigned. Finally, a means of correcting these errors will be derived.

CHAPTER 2

THE THEORY OF DUAL PHOTON ABSORPTIOMETRY

2.0 Introduction

The Norland 2600 dichromatic densitometer is no different from any other commercially available dual photon scanner. It is based upon the established measurement approach of dividing the human body into two distinct components: bone mineral and soft tissue. In order to analyze a two component system by absorptiometry, attenuation measurements must be recorded at each of two distinct energies for multiple locations throughout the object. To satisfy this criterion, the actual DPA measurement scheme combines a rectilinear scanner with an external ^{153}Gd radionuclide source. The system design is outlined in figure 2.1. As shown, the ^{153}Gd source has an activity of approximately 40 GBq and emits photons at 44 and 100 Kev. In performing a total body measurement the source is moved in a rectilinear raster pattern underneath the body progressing from the subject's head down to their feet. As the source is moved, a sodium iodide detector counts the number of photons at the two energies which are transmitted through the body. A computer stores and manipulates the recorded counts to obtain the absolute mass of bone mineral and soft tissue contained in the total body. What follows is an indepth consideration of the DPA process outlined above. The unique ability of the system to calculate an absolute body mass from raw transmission measurements will be examined in a step by step manner.

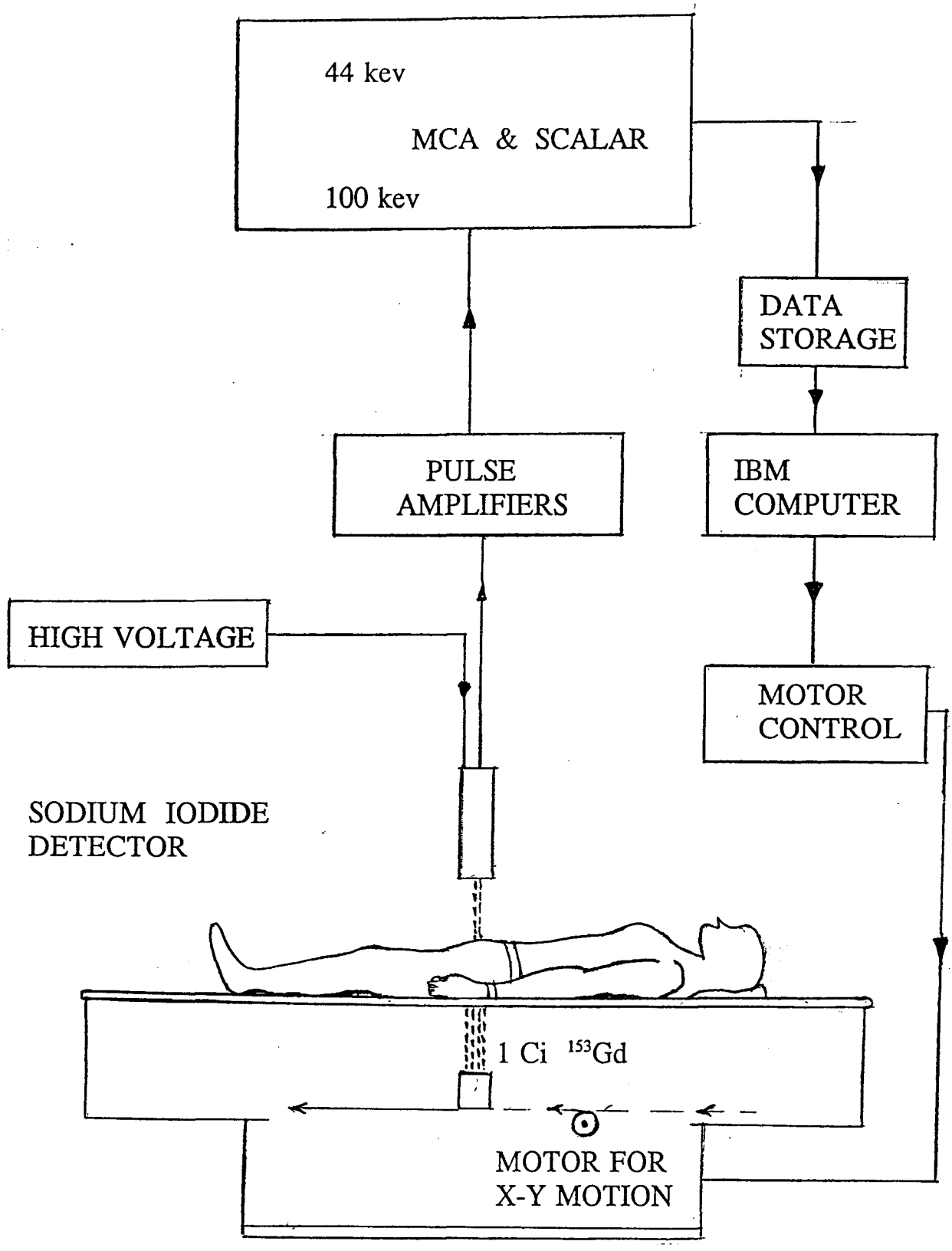


FIGURE 2.1 Basic Electronics and Measurement Geometry for a typical DPA Scanner.

2.1 Defining a measurement pixel.

During a whole body scan performed by the Norland 2600, the ^{153}Gd radioisotope source is scanned throughout the body at a speed of 30 mm/sec and with a line spacing of 15 mm. For a given scan line, the detector continuously records the transmitted photons as they exit the body. The total counts recorded for a scan line is given by integrating the count rate over the length of time it takes to complete a scan line. Integration of the count rate over smaller time intervals will give the counts for sub-lengths or pixels of the scan line. For the Norland scanner each pixel is defined over 0.25 sec intervals. Given a scan speed of 30 mm/sec, a measurement pixel width is 7.5 mm (30 mm/sec X 0.25 sec) across the body. A line spacing of 15 mm dictates that a pixel length is 15 mm along the body.

The maximum scan width that can be accommodated by the scanner is 61.5 cm. The maximum body length that can be scanned is approximately 190 cm. As a means of minimizing scanning time, the scanning motion is adjusted so that measurements are recorded over the body area only and air is not scanned unnecessarily. Consequently, for a typical adult scanned by DPA, approximately 8000 measurement pixels are obtained during a 40 minute scanning period.

2.2 Solving the two component system.

Again, the actual DPA scanning process involves recording transmission measurements through the body. The measurements can be either through bone and soft tissue or through soft tissue only. Assuming that the photons are attenuated exponentially, the intensities of the photon beam at any measuring site after having exited the body can be expressed by:

$$I^{44}=I_o^{44}.EXP(-\mu_{st}^{44}.M_{st}-\mu_{bone}^{44}.M_{bone}) \qquad 2.1a$$

$$I^{100}=I_o^{100}.EXP(-\mu_{st}^{100}.M_{st}-\mu_{bone}^{100}.M_{bone}) \qquad 2.1b$$

I_o^{44}, I_o^{100} and I^{44}, I^{100} are the raw beam and transmitted counts respectively at the two energies. The μ_{st}^{44}, μ_{st}^{100}, and μ_{bone}^{44}, μ_{bone}^{100} terms are mass attenuation for soft tissue and bone at the two energies and therefore have units of cm^2/g. The two unknowns in this equation set are M_{st} and M_{bone}, the soft tissue and mineral masses in units of g/cm^2. The solutions appear consistently in the literature in the following form

$$M_{bone}=\frac{R_{st}.Ln(\frac{I^{100}}{I_o^{100}})-Ln(\frac{I_o^{44}}{I^{44}})}{\mu_{bone}^{44}-\mu_{bone}^{100}.R_{st}} \qquad 2.2$$

$$M_{st}=\frac{Ln(\frac{I^{44}}{I_o^{44}})-R_{bm}.Ln(\frac{I_o^{100}}{I^{100}})}{R_{bm}.\mu_{st}^{100}-\mu_{st}^{44}} \qquad 2.3$$

where the R_{st} and R_{bm} terms have been defined as the following

$$R_{st}=\frac{\mu_{st}^{44}}{\mu_{st}^{100}}=\frac{Ln(\frac{I_o^{44}}{I^{44}})}{Ln(\frac{I_o^{100}}{I^{100}})} \qquad 2.4$$

and similarly

$$R_{bm}=\frac{\mu_{bone}^{44}}{\mu_{bone}^{100}} \qquad 2.5$$

Thus, a determination of M_{st} and M_{bone} at each measurement site along the scanning path requires a knowledge of the mass attenuation coefficients for soft tissue and bone mineral as well as the transmitted fractions at the two energies. The transmitted fractions can be measured directly. The mass attenuation coefficients for bone mineral are well defined because the chemical composition of bone mineral does not vary (ICRP 1975). However, difficulties arise in the determination of the attenuation coefficients for soft tissue and the R_{st} ratio (equation 2.4).

Soft tissues are made up of two distinct components, fat and lean (muscle). Muscle and fat attenuate the 44 and 100 Kev photons differently. The amount of fat and muscle making up the soft tissue mass will vary between measurement sites and between individuals. Therefore the overall mass attenuation coefficient for soft tissue will vary. Consequently, at sites that contain both bone and soft tissue the mass attenuation coefficient of the soft tissue component is unknown and so the values of M_{bone} and M_{st} cannot be calculated.

At sites where no bone is present the R_{st} ratio can be measured directly. This can be seen by setting M_{bone} to zero in equations 2.1a and 2.1b. At sites where bone is present, a direct measurement of M_{bone} and M_{st} cannot be obtained because the original assumption of a two component body is incorrect.

In practice, the problems posed by the unknown attenuation properties of the soft tissue at sites that also contain bone can be circumvented by means of an iterative procedure. This stepwise

process is outlined in fig. 2.2. As shown, an initial R_{st} is guessed and M_{bone} and M_{st} are calculated at each pixel. At this point, the pixels that contain bone mineral can be identified because their calculated bone mass will or will not be non-zero. This identification of the pixels that contain bone mineral allows for a definition of the various bone edges in the subject scanned. For a typical adult body scanned by DPA, approximately half of the 8000 pixels recorded will contain bone mineral. For the non-bone sites, a ratio (R_{st}) of mass attenuation for soft tissue at 44 and 100 Kev is calculated. A weighted average (according to soft tissue mass) of all these R_{st}'s is determined for the whole body. This whole body average value is then compared with the initial guess. If the difference between the new calculated R_{st} and the initial guess is outside a preset tolerance then the new R_{st} is used to recalculate the M_{bone} and M_{st} for each measurement pixel. Again the bone edges are redefined and a new weighted R_{st} calculated. If this new value does not differ significantly from the previous one then the calculation is halted. If there are appreciable differences the iterative procedure is repeated until the difference is within the preset tolerance.

After exiting the iteration loop, the M_{st} and M_{bone} for each pixel in the body is known in units of g/cm^2. The area of each measurement pixel is known. Therefore the product of the masses in each pixel times the area of a pixel results in the mass of bone mineral and soft tissue in units of grams for each pixel. Finally, the M_{st} and

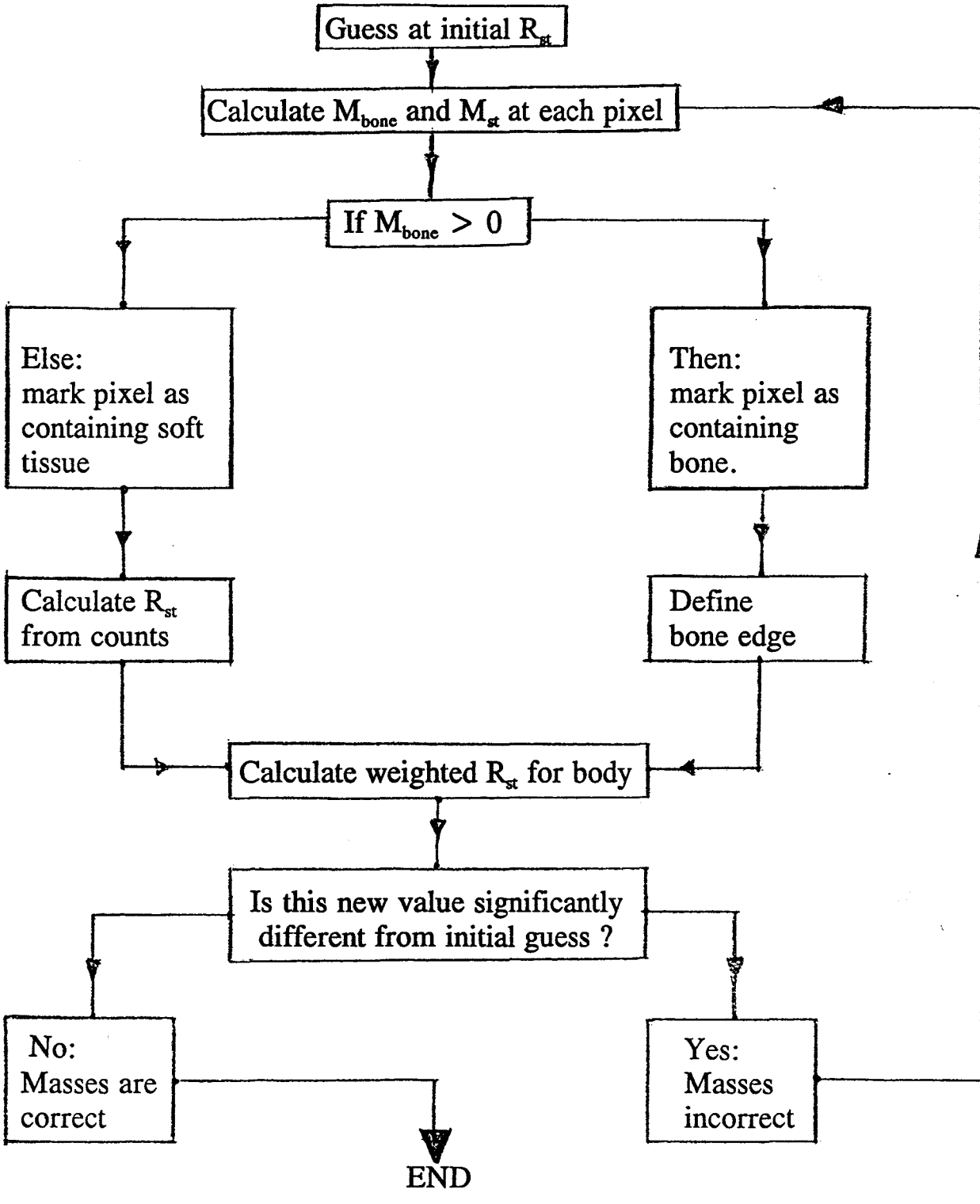


Figure 2.2 The iterative calculation of the mass of bone and soft tissue for each measurement pixel.

M_{bone} for all pixels are summed to obtain the total bone mineral and total soft tissue mass in the body.

2.3 Separating the Soft Tissue mass into muscle and fat

Dual photon absorptiometry has the capability of dividing the total soft tissue mass of the body into its muscle and fat components. The soft tissue mass attenuation coefficients at 44 and 100 Kev will vary depending on the proportion of fat and muscle. That is, the overall mass attenuation coefficient of the tissue will be a linear combination of the attenuation coefficients for fat and muscle weighted by their fractional masses. This can be illustrated as follows.

$$^{44}\mu_{st} = f_{mus}\ ^{44}\mu_{mus} + f_{fat}\ ^{44}\mu_{fat} \qquad 2.6a$$

$$^{100}\mu_{st} = f_{mus}\ ^{100}\mu_{mus} + f_{fat}\ ^{100}\mu_{fat} \qquad 2.6b$$

where f_{mus} and f_{fat} are the fraction of muscle and fat that comprise the total soft tissue mass. The sum of f_{mus} and f_{fat} must be equal to 1. With the soft tissue mass attenuation coefficients varying in response to the fat and muscle content, the R_{st} ratio will reflect the muscle and fat make-up of the tissue. From equations 2.4, 2.6a, and 2.6b, the R_{st} can be rewritten as:

$$R_{st}=\frac{f_{mus}\cdot{}^{44}\mu_{mus}+f_{fat}\cdot{}^{44}\mu_{fat}}{f_{mus}\cdot{}^{100}\mu_{mus}+f_{fat}\cdot{}^{100}\mu_{fat}} \qquad 2.7$$

In this form, it is clear that the R_{st} ratio varies linearly with the lean or fat fraction in the soft tissue. When the fat fraction is zero the ratio will correspond to pure muscle. Conversely, a fat fraction of 1 will result in an R value indicative of pure fat. This relationship is sketched in figure 2.3. The R_{fat} and R_{lean} points shown are the ratios corresponding to pure fat and pure lean tissue. An equation corresponding to this line can be expressed as

$$R_{st} = (\text{slope}) * \%\text{lean} + R_{fat} \qquad 2.8a$$

where slope= $(R_{lean} - R_{fat}) / 100\%$

When combined, a final form of the equation can appear as:

$$\%\text{Lean} = (R_{st} - R_{fat}) / (R_{lean} - R_{fat}) \qquad 2.8b$$

This final form suggests that once the R_{fat} and R_{lean} values are known, then for a given R_{st} measured at a site containing only soft tissue, the fraction of lean tissue at that measurement pixel can be calculated. With the sum of the lean and fat fractions being equal to one, then the fat fraction is easily determined.

In practice, for the body being scanned, the weighted average R_{st} value used in the final step of the iteration to calculate the bone mineral mass is used in the calculation of the lean and fat fractions. This average value can be viewed as an indicator of the body's over-all soft tissue composition. The R_{fat} and R_{lean} points are

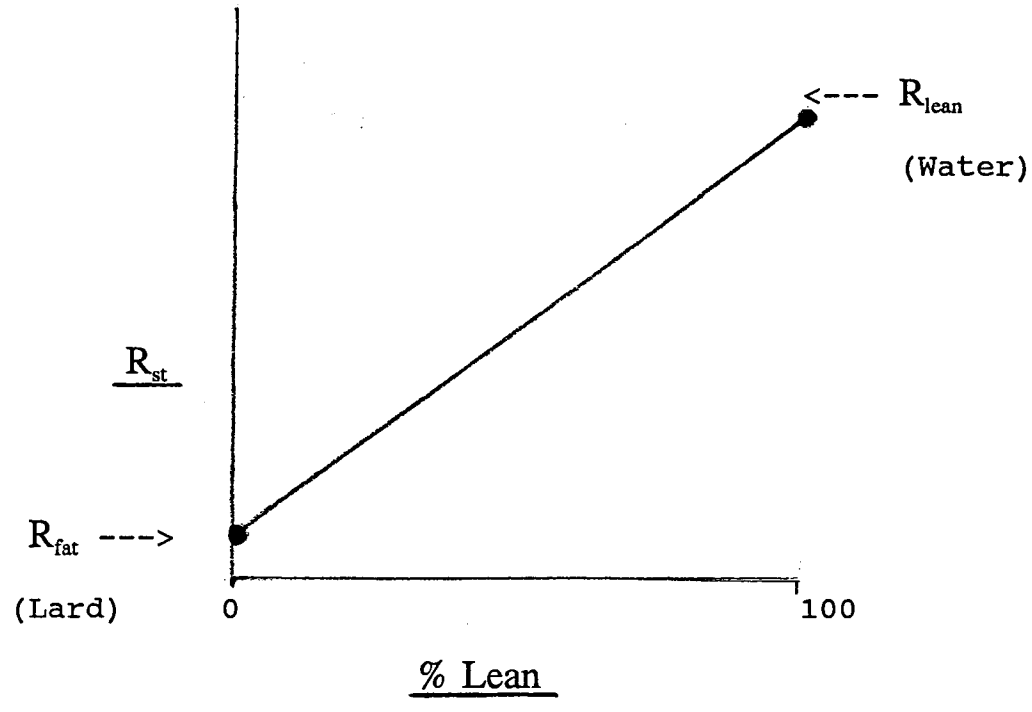


Figure 2.3 The calibration curve used to separate the soft tissue mass into fat and muscle based upon a measured soft tissue mass attenuation coefficient ratio (R_{st}).

established through calibration because there are no pure muscle and pure fat sites in vivo where the R values could be measured directly. For the current Norland 2600 the ratios for pure fat and muscle were determined for lard and water and are incorporated into the analysis software. Water and lard are good substitutes in so far as they attenuate the 44 and 100 Kev photons in a manner similar to that of muscle and fat.

2.4 Summary.

From the recorded transmission measurements made through the body, the Norland 2600 scanner is able to partition the body into bone mineral and soft tissue components. It proceeds to further divide the soft tissue mass into muscle and fat components based upon an internal calibration. The steps involved in the overall partitioning process are summarized in fig 2.4.

The result of a typical whole body scan performed on the Norland machine is illustrated in fig 2.5. The scan parameters are given at the top of the output. The point resolution, line spacing and scan width are fixed. The only variable that can be controlled by the operator is the scan speed. However the maximum speed of 30 mm/sec is recommended. The image represents an outline of the subject's skeleton. It is included in the scan output only for cosmetic purposes. The numbers listed best relay the gross physical make-up of the subject. As shown, the total bone mineral (TBM) and total soft tissue mass (TSM) are calculated for a specific body region and for the total body. Since bone edges are determined

Record raw transmission counts.

- guess at soft tissue mass attenuation coefficient ratio (R_{st})

iterate

- calculate M_{bone} and M_{st} in g/cm^2
- area of a pixel is known in cm^2
- calculate M_{bone} and M_{st} in grams for each pixel
- sum all pixels in body to obtain total bone mineral and total soft tissue
- use average R_{st} to divide soft tissue based upon internal calibration curve.

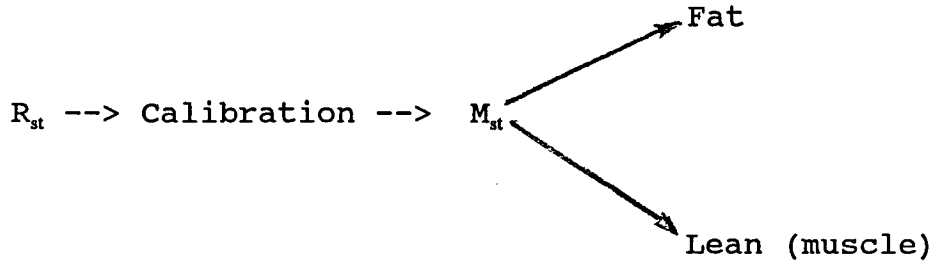
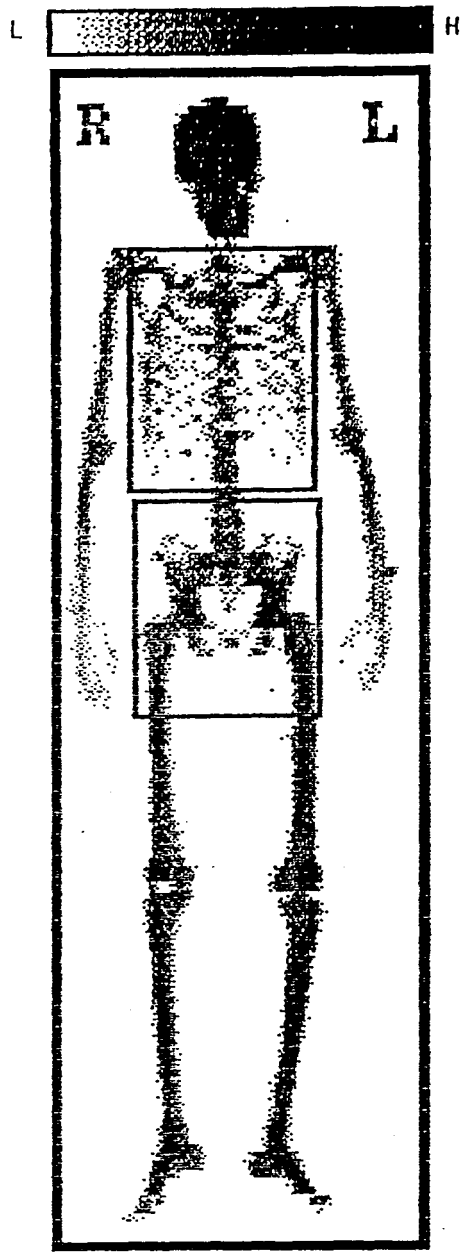


Figure 2.4 Summary of the DPA process from the recorded count rates to the final bone mineral, fat and muscle determination.

HOLE BODY SCAN A ON 11/12/90

Point Res: 7.5 mm Line Spcg: 15.0 mm Scan Width: 61.5 cm
Scan Speed: 30.0 cm/sec Raw Beam: 46211, 31562 Calib Fac: 0.976



egion	TBM (g)	TSM (g)	BMD (g/cm²)
ead	645.6	4882.4	2.360
runk	694.6	19975.4	0.521
elvis	745.6	18886.2	1.954
egs	1265.7	16601.4	1.849
ight arm	252.4	4684.1	1.434
eft arm	235.2	4756.1	1.396
otal	3839.1	69785.5	1.272

egion	Lean Mass (g)	Fat mass (g)	% fat
otal	63837.7	5947.8	8.523

Figure 2.5 A typical whole body scan print out provided by the analysis software.

during analysis, a measure of bone area is also obtained. This allows for a calculation of a bone mineral density (BMD) in g/cm^2 for the total body and at the specific sites outlined. The total soft tissue mass is divided into lean mass and fat mass and a percent body fat is calculated.

CHAPTER 3

THE FACTORS AFFECTING THE ACCURATE SEPARATION OF SOFT TISSUE

3.0 Introduction

The Norland 2600 Dual Photon Scanner has been used to estimate body composition in a variety of subjects. In particular, extensive studies have been performed on humans, dogs, and piglets. In scanning each of these three groups, DPA was used to quantitate the total soft tissue mass, mineral mass, lean mass, and fat mass in the body. Based on the results obtained it appears that the absorptiometry technique inaccurately divides the soft tissue mass into its fat and lean (muscle) components.

The accuracy of separating the soft tissue mass is wholy dependent upon determining the true R_{st} value for the subject and relating it to the internal calibration curve. What follows is an examination of the evidence to support the claim that the present DPA system does not accurately quantitate fat and lean mass. The sources of error that contribute to this apparent limitation will be examined. An estimation of the uncertainty associated with the fat and lean masses as an absolute measure in a given individual will be assigned. Finally, the potential for correcting these errors will be considered.

3.1 Observed errors of soft tissue separation

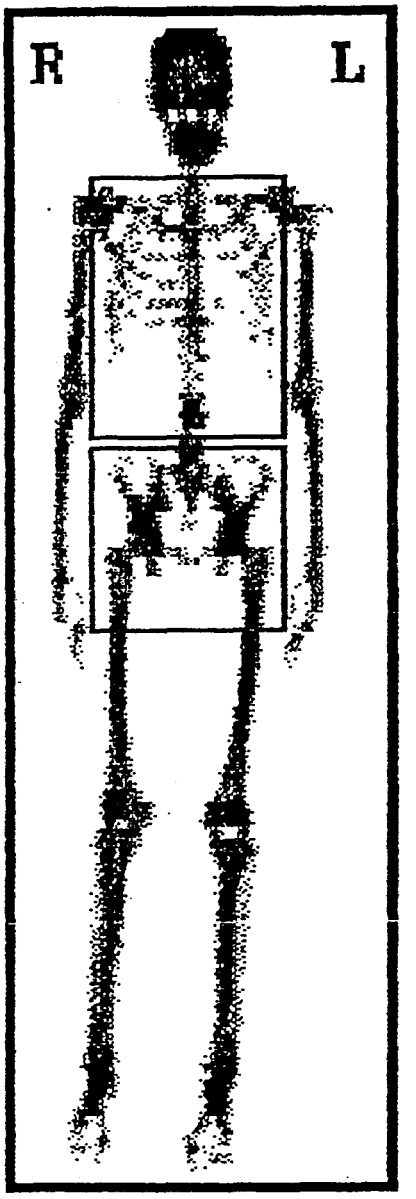
Over the 5 years of using the Norland DPA scanner to perform body composition measurements, there is evidence which suggests that the separation of the soft tissue mass has unacceptably large associated errors. The worst of these errors is the assignment of a negative fat fraction to the body. This is illustrated in the whole body scan of an adult shown in fig 3.1. For this individual DPA determined a total body bone mineral (TBM) of 2862.1 g and a total soft tissue mass (TSM) of 59972.9 g. The soft tissue mass was then divided into its lean mass and fat mass. A lean mass of 62524.7 g and fat mass of -2551.8 g was measured. A negative body fat mass is obviously incorrect. However, the addition of the lean mass plus the fat mass produces the correct total soft tissue mass. The scanner was also accurate in determining total body weight. That is, the sum of TBM and TSM should equal body weight because the body was assumed to be composed of two components. For this particular subject, the DPA determined body weight (62.8 Kg) agrees quite closely with that measured by hospital scales (63.0 Kg).

The Norland company has not placed body size or body outline limitations on the types of subjects upon whom total body composition measurements could be performed. If regional information from a specific body site was desired, the scanner is restricted to human forms only. However, given the basic DPA theory, any reasonably sized mammal could be scanned and its total

OLE BODY SCAN A ON 04/09/89

Point Res: 7.5 mm
Scan Speed: 30.0 mm/sec
Line Spcg: 15.0 mm
Raw Beam: 99189, 69556
Scan Width: 61.5 cm
Calib Fac: 0.969

L H



gion	TBM (g)	TSM (g)	BMD (g/cm²)
ad	471.2	4193.6	1.960
unk	532.1	19640.7	0.398
lvis	517.6	13356.4	1.981
gs	881.6	15003.4	1.409
ght arm	250.9	4154.6	1.352
ft arm	208.6	3624.2	1.307
tal	2862.1	59972.9	1.018

gion	Lean Mass (g)	Fat mass (g)	% fat
tal	62524.7	-2551.8	-4.255

Figure 3.1 A whole body scan of an adult for which the scanner determined a negative percent body fat and fat mass.

body bone mineral and soft tissue mass can be determined. It was in light of this basic fact that DPA derived body composition was extended to other mammals. For example, along with numerous adults and children, several scans were performed on dogs and piglets. In each of these groups negative percent body fat errors, similar to that outlined in figure 3.1, were observed. The occurrences were more frequent in non-human subjects. To illustrate the magnitude of these errors, a summary of some negative percent fats observed in humans, dogs, and piglets is provided in table 3.1. As tabulated, there appears to be an inverse relationship between the total soft tissue mass and the range of negative percent body fat determined for each group. It appears that lower soft tissue masses tend to produce larger negative percent fats. This suggests that in leaner and smaller subjects DPA is underestimating fat and so in the process overestimates the lean fraction. Again, from figure 3.1, the lean fraction is overestimated to such a point that it exceeds 1 (104.3%).

A second type of error observed in the process of soft tissue separation is an overestimation of body fat. Body fat percentages exceeding 50 have been observed in a number of people scanned by DPA. These determinations are thought to be incorrect because these persons were not obese. Quite simply, they did not look that fat and so the DPA result was questioned. It is important to note that this trend of overestimating body fat while at the same time underestimating lean tissue in larger subjects was not immediately apparent. It evolved as a result of numerous clinical observations

Table 3.1 : Range of negative percent body fat recorded for the various subjects scanned.

Subject	Average Soft Tissue Mass(kg)	Range of (-)%fat observed
Piglets	4	-8 to -17
Dogs	15	0 to -4
Humans		
- Rygiel pat. 1	16	-5
- Rygiel pat. 2	24	-9
- Jogger 1	60	-4
- Jogger 2	61	-2
- Cystic Fib.	48	-1

and comparisons to other body composition measurement techniques.

The errors observed in soft tissue separation occur at the extremes, in the very lean and in the very fat. Therefore, the reliability of a DPA measurements performed on subjects between these body extremes is questionable. For DPA to be used as an accurate and precise tool in body composition measurements, the limitations and magnitude of these soft tissue measurement errors must be established for clinically relevant body types.

3.1.1 The origin of the negative %fat errors

Separation of the total soft tissue mass into muscle and fat is linked to the internal calibration. The inaccuracies observed during clinical trials suggests that the calibration curve relating the average R_{st} ratio to the lean or fat fraction present in the soft tissue mass is not an accurate representation of the true relationship. This calibration line was defined in equation 2.8b as being:

$$\%Lean = (R_{st} - R_{fat}) / (R_{lean} - R_{fat})$$

With the (Lean+Fat) fractions equaling 1 (100%), then a negative percent fat can only be obtained if the lean fraction exceeds 1. That is

$$(R_{st} - R_{fat}) / (R_{lean} - R_{fat}) > 1$$

or

$$(R_{st} - R_{fat}) > (R_{lean} - R_{fat})$$

which reduces down to

$$R_{st} > R_{lean}$$

It is due to this condition that the negative percent fat(lean fractions exceeding 100%) errors occur. There are two potential sources of error in the measurement process that can lead to the above condition. The first is that the measured R_{st} values are representative of the true tissue state but the R_{lean} endpoint is not an accurate substitute for pure lean tissue. The second possibility is that the calibration points are representative of lean and fat but the measured R_{st} is not reflective of the tissue's true muscle/fat composition. To understand the first possibility, the differences (if any) between the true line and that incorporated into the Norland analysis software must be examined. This comparison is made in the following section.

3.2 Accuracy of the calibration line.

For the current Norland 2600 the ratios for pure fat and muscle were determined for lard and water. Water and lard are good substitutes in so far as they attenuate the 44 and 100 Kev photons in a manner similar to that of muscle and fat. However, it is of interest to compare how well a lard/water calibration represents the true curve for pure mammalian fat and lean tissue.

The theoretical R values for pure fat and lean can be derived from a knowledge of their elemental compositions and atomic cross-

Table 3.2 : Composition of mammalian fat.

Fatty Acid.	g/(100g fat)	μ_{44}	μ_{100}
Myristic	2.7	0.221	0.170
Palmitic	26.5	0.221	0.170
Stearic	8.4	0.220	0.170
Oleic	49.4	0.218	0.172
Linoleic	10.5	0.219	0.169
Erucis	2.5	0.218	0.171

sections (Hubbell, ICRP 1975). Mammalian fat is a mixture of a number of fatty acids. The proportions of the mixture may vary somewhat from one individual to the next but these variations do not alter its attenuation properties significantly. The constituent fatty acids are shown in table 3.2 along with their respective mass attenuation coefficients at 44 and 100 Kev. The overall mass attenuation coefficients of fat can be derived from a linear combination of the attenuation coefficients of the individual fatty acids weighted by their fractional masses. This mixing to determine an effective mass attenuation coefficient is commonly expressed as

$$\mu_{eff} = \Sigma(n_i \ \mu_i)$$

where n_i = fractional mass of i'th component

μ_i = mass attenuation coefficient of i'th component

When applied to mammalian fat the results are:

FAT $\mu_{44}=0.220$ cm^2/g , $\mu_{100}=0.170$ cm^2/g

$$R_{fat} = 1.294$$

Lean tissue can be considered as the muscle which comprises the skeletal muscle system. The composition of wet skeletal muscle is given in table 3.3 along with an elemental breakdown of its

Table 3.3 : Composition of wet skeletal muscle

	μ_{44} (cm^2/g)	μ_{100} (cm^2/g)
78% - Water	0.248	0.171
19% - Protein - 52% Carbon - 24% Oxygen - 16% Nitrogen - 8% Oxygen	0.220	0.163
3% - Fat	0.220	0.170

protein content. If the percent masses of water and protein are normalized to eliminate the 3% fat, the calculated mass attenuation coefficients for pure lean tissue are:

$$\text{LEAN} \quad \mu_{44}=0.248 \text{ cm}^2/\text{g} \ , \ \mu_{100}=0.168 \text{ cm}^2/\text{g}$$

$$R_{lean} = 1.476$$

Again, Norland's substitute of pure fat and pure lean in humans are lard and water. The mass attenuation coefficients for water at 44 and 100 kev are included in table 3.3. When expressed as the R_{st} ratio, the theoretical value for water is 1.451.
That is:

$$R_{water}=1.451$$

The chemical composition of commercially available lard is similar to that listed in table 3.2 for mammalian fat. However there are slight differences in the weighting of the various fatty acids (CRC). When the differences are accounted for, the calculated mass attenuation coefficients for lard are

$$\mu_{44}=0.216 \text{ cm}^2/\text{g} \quad \mu_{100}=0.166 \text{ cm}^2/\text{g}$$

$$R_{lard}=1.301$$

Clearly then, based on theoretical derivations of the various mass attenuation coefficients, the water/lard substitution for pure

lean/fat is not accurate on the basis of matching up the respective R_{st} ratios. This numerical difference between the predicted true lean/fat R values and those for water/lard are sketched in figure 3.2. With these differences between the two curves, it appears that the current calibration system will tend to slightly overestimate the fat in obese subjects while underestimating it in those who are very lean.

To test the validity of this "calibration to true" relationship illustrated by figure 3.2, a series of scans were performed on a set of well characterized pork phantoms. The true fat content of each phantom was determined chemically by using an established procedure (Floch et.al. 1957). The water fraction in the lean component was derived from freeze drying of the wet tissue. The protein content was then derived from a knowledge of the water and fat contents. A full description of the steps used to quantitate the components of a phantom is reserved for appendix 1.

The water fraction of each phantom was constant at 0.78 but the fat content was varied. The total mass of each phantom was known and so the ability of the DPA to divide this mass into its muscle and fat components can be summarized in table 3.4. It is quite clear that, on the average, the Norland 2600 is able to divide the phantom masses into their correct proportions. It was also quite accurate (2.5%) in obtaining the total soft mass (TSM) for each phantom. The precision in the percent fat was obtained from repeated measurements and ranged from 1-2%. To determine the

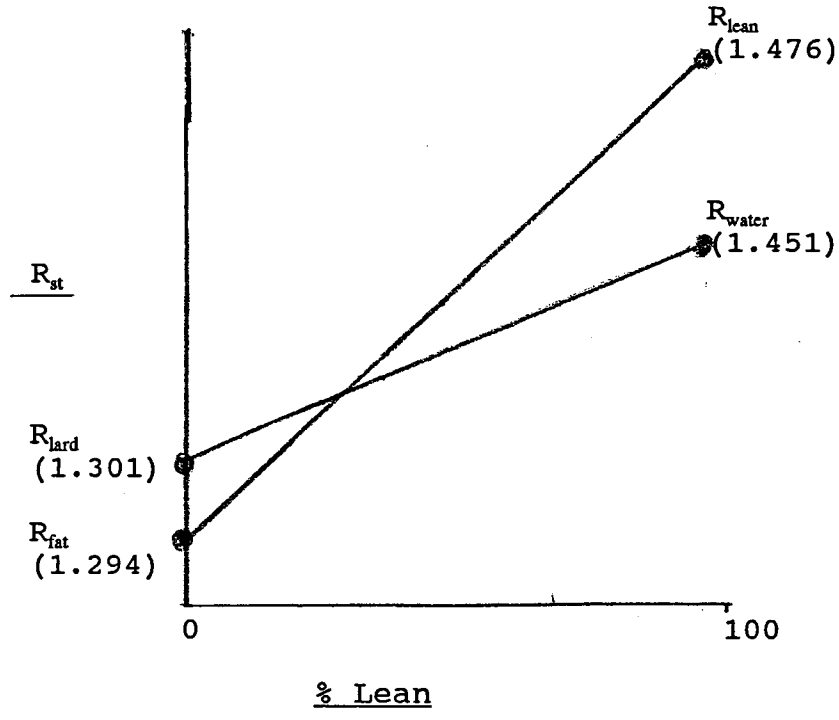


Figure 3.2 Relationship between the Norland water/lard calibration and that expected for pure muscle and fat tissue. The R_{st} values were calculated from the mass attenuation coefficients predicted by Hubbell.

Table 3.4 The correlation between DPA determined fat, lean and total soft mass versus the expected values in each phantom.

<------- True Values ------->			<----------DPA ---------->		
Total Soft mass (g)	Lean tissue mass (g)	% Fat	TSM(g)	Lean(g)	%Fat
4000	3800	5.0	3932+16	3701+100	5.9+1.1
4402	3800	13.7	4285+25	3421+91	17.8+1.6
3846	1909	52.8	3856+54	1731+30	55.1+1.2
4361	1909	56.5	4205+54	1654+65	60.5+2.0
3805	0	100.0	3657+37	-98+28	102.7 +1.8

(Mean of 4 measurements)

accuracy with which the phantoms were divided into their correct proportions, the true percent fat in each is plotted against the DPA determined fraction in figure 3.3. As plotted, the measured points correspond well to the line of identity. Least squares analysis revealed that the slope of a best fit line was not significantly different from 1 (1.014 +/- 0.020). However, an intercept of (1.81 +/- 0.97)% suggests that there is an absolute error in the percent fat and lean determinations of approximately 2-3%.

Given the proposed relationship between the internal R_{st} calibration and the true one outlined in fig. 3.2, the accurate determination of the 95% lean : %5 fat phantom by the scanner was rather surprising. A negative fat result was expected and figure 3.3 should have had a configuration similar to that sketched in figure 3.4. because of the $R_{st} > R_{lean}$ condition derived earlier. Therefore, given the results obtained it is clear that for (at least) this set of well characterized tissue phantoms, the present soft mass calibration allowed for accurate and precise tissue separation into muscle and fat and the differences between the two calibration lines in figure 3.2 are of no significance.

The level of accuracy and precision achieved in this phantom trial is in keeping with those reported by others. Gotfredsen et al. reported a 2% accuracy and precision error in the percent lean and fat determination in ox muscle/lard phantoms by DPA (Gotfredsen et al. 1988). A high degree of precision in separating soft tissue in vitro was also reported by Heymsfield et al. A coefficient of variation for DPA measured percent fat of < 1% allowed them to

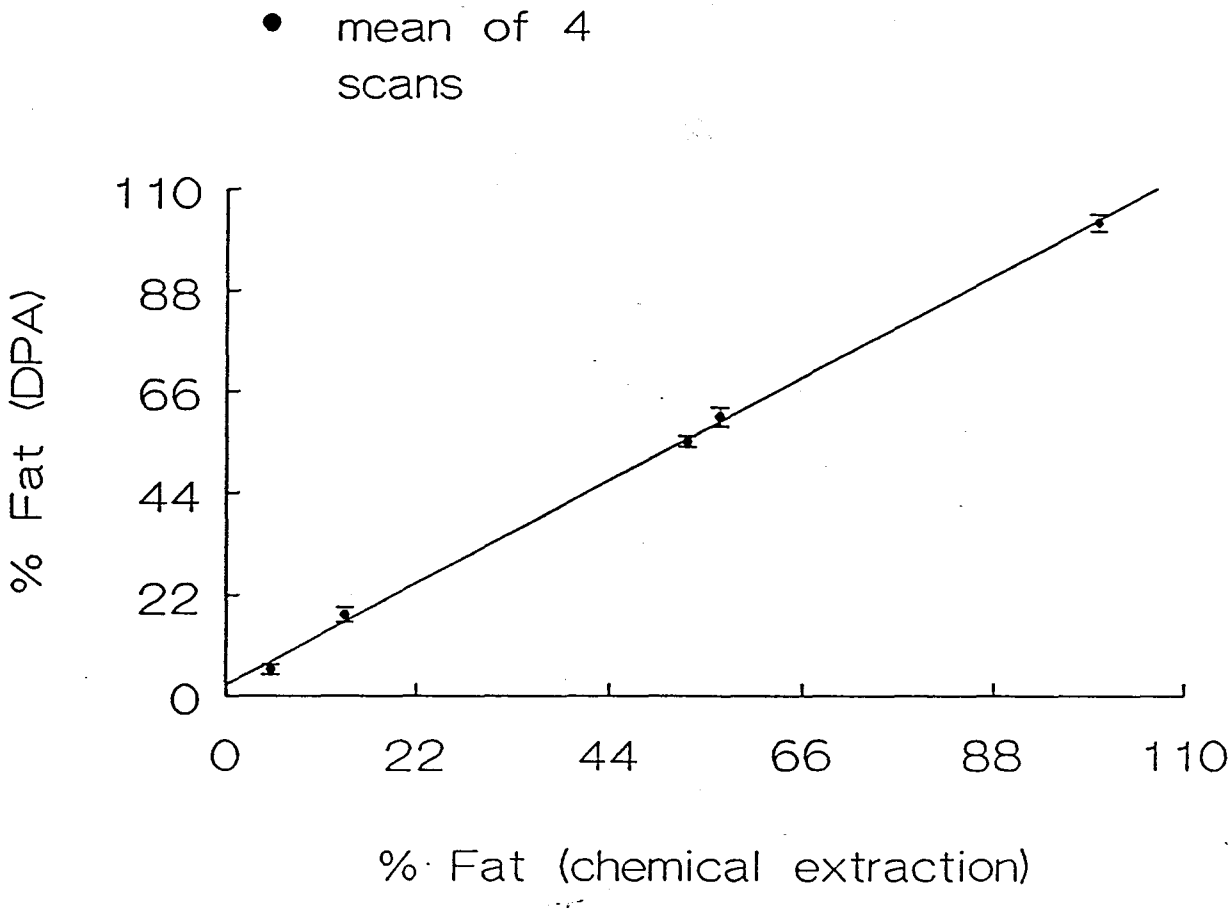


Figure 3.3 The relationship between DPA derived fat fractions and those expected from chemical analysis.

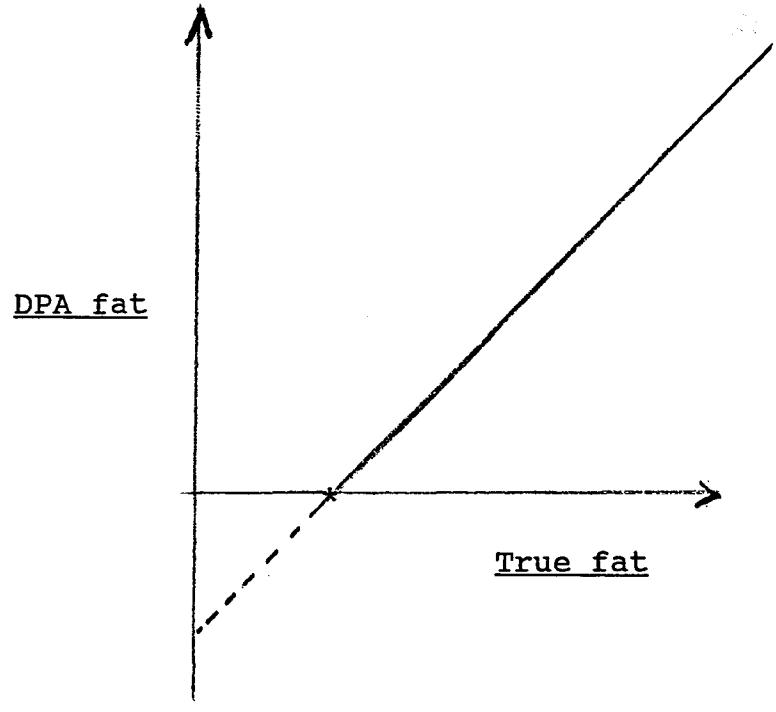


Figure 3.4 The relationship expected between the true fat fraction and that determined by DPA if the measured R_{st} exceeds the R_{water} endpoint. Below (*), DPA will give (-) fat.

conclude that DPA would be very sensitive to small changes in fat and lean tissues (Heymsfield et al. 1989).

Although in clinical trials it has been established that the soft tissue separation in people and animals can be incorrect, for this phantom series DPA divided the soft tissue into its correct muscle and fat proportions. The high degree of accuracy achieved in the separation establishes confidence in the calibration. Consequently, further discussion focuses on the differences between soft tissue as it is packaged in a phantom compared to how it is distributed in the body and how these differences can affect the accuracy of the R_{st} determination.

3.3 The effect of body size on soft tissue separation

The photon counts detected by the sodium iodide detector will be influenced by scattering in the body, beam hardening, and dead time effects due to the limitations of the electronics. Therefore, to achieve purely exponential attenuation, it is necessary to adjust the recorded counts to correct for dead time losses, cross-over from the 100 kev to the 44 kev channel and beam hardening. Several correction algorithms have been outlined in depth in the literature (Gotfredson, et al. 1984; Peppler, et al. 1981). Each algorithm is designed to maintain a constant R_{st} ratio independent of tissue thickness for a fixed tissue composition. This count rate correction is required because the thickness and composition of the soft tissue mass varies from site to site within an individual and between individuals.

To demonstrate the effectiveness of the count rate corrections adopted by Norland, transmission counts were recorded through various thicknesses of water to determine the stability of the R_{st} value. Water is an homogeneous lean tissue substitute and is therefore expected to have a constant ratio of 1.48. The results of R_{st} measurements through thicknesses ranging from 1 to 23 cm are displayed in figure 3.5. Ten measurements were recorded at each thickness and so the spread in the raw data points highlights the deviation associated with a single measurement. Clearly, below 8 cm of water the ratio is too high but approaches the desired level between 8 and 16 cm. It then decreases below the required level for thicknesses exceeding 16 cm. When the natural logarthims of the transmitted fractions at 44 and 100 kev are plotted against thickness the slope of the resultant lines are equivalent to the linear attenuation coefficients at the respective energies. The results of this analysis for water are given in figure 3.6. The linear attenuation coefficients listed were determined through least squares analysis. As water has a density of 1 g/cm^3, the mass attenuation coefficients will also be 0.239 cm^2/g and 0.162 cm^2/g at 44 and 100 kev respectively. It is interesting to note that these measured values are not in good agreement with theoretical predictions from Hubbell (table 3.3). These measured versus theoretical differences in mass attenuation coefficient suggests that the effects due to scatter, deadtime, and beam hardening are present. However, when the measured mass attenuation coefficients

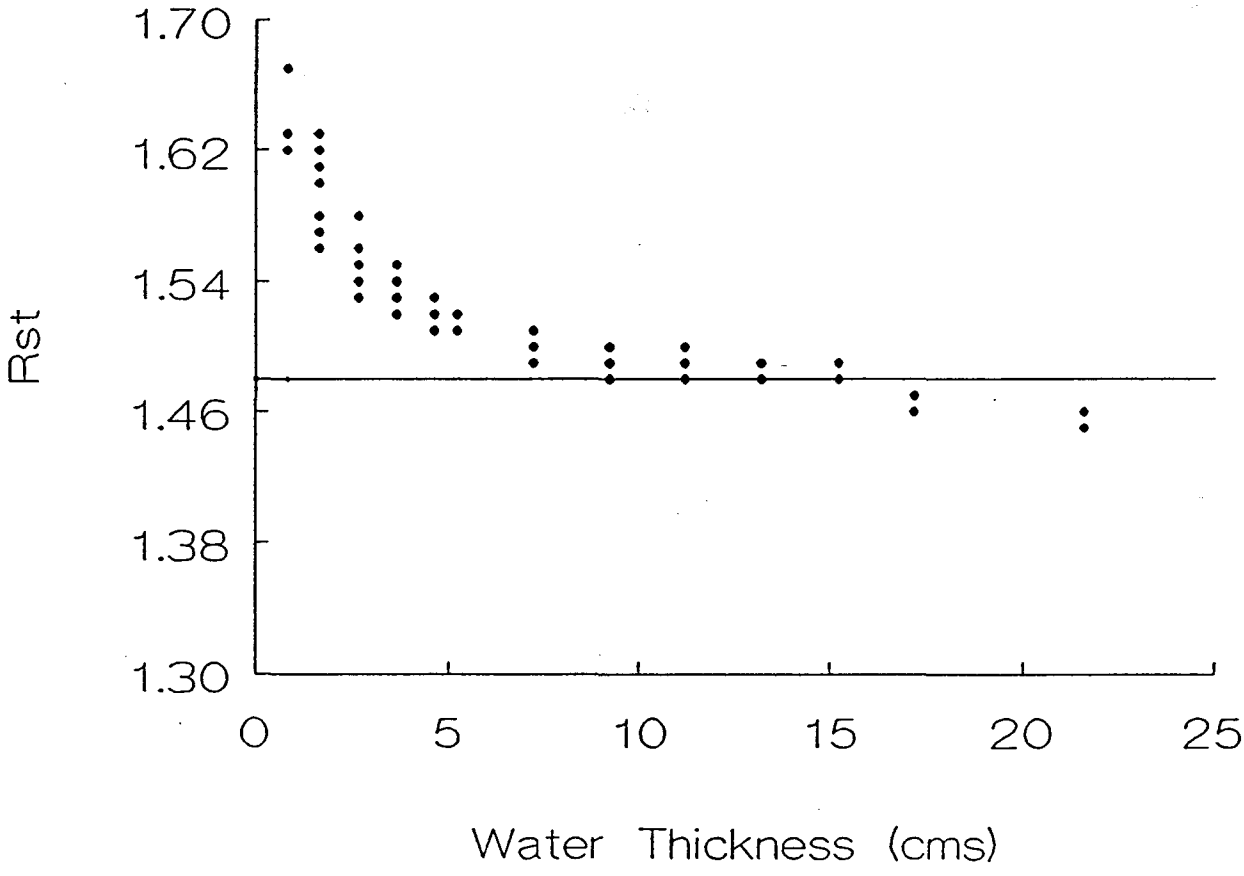


Figure 3.5 The stability of the R_{st} ratio through varying thicknesses of water. The expected value of 1.48 is indicated by (——).

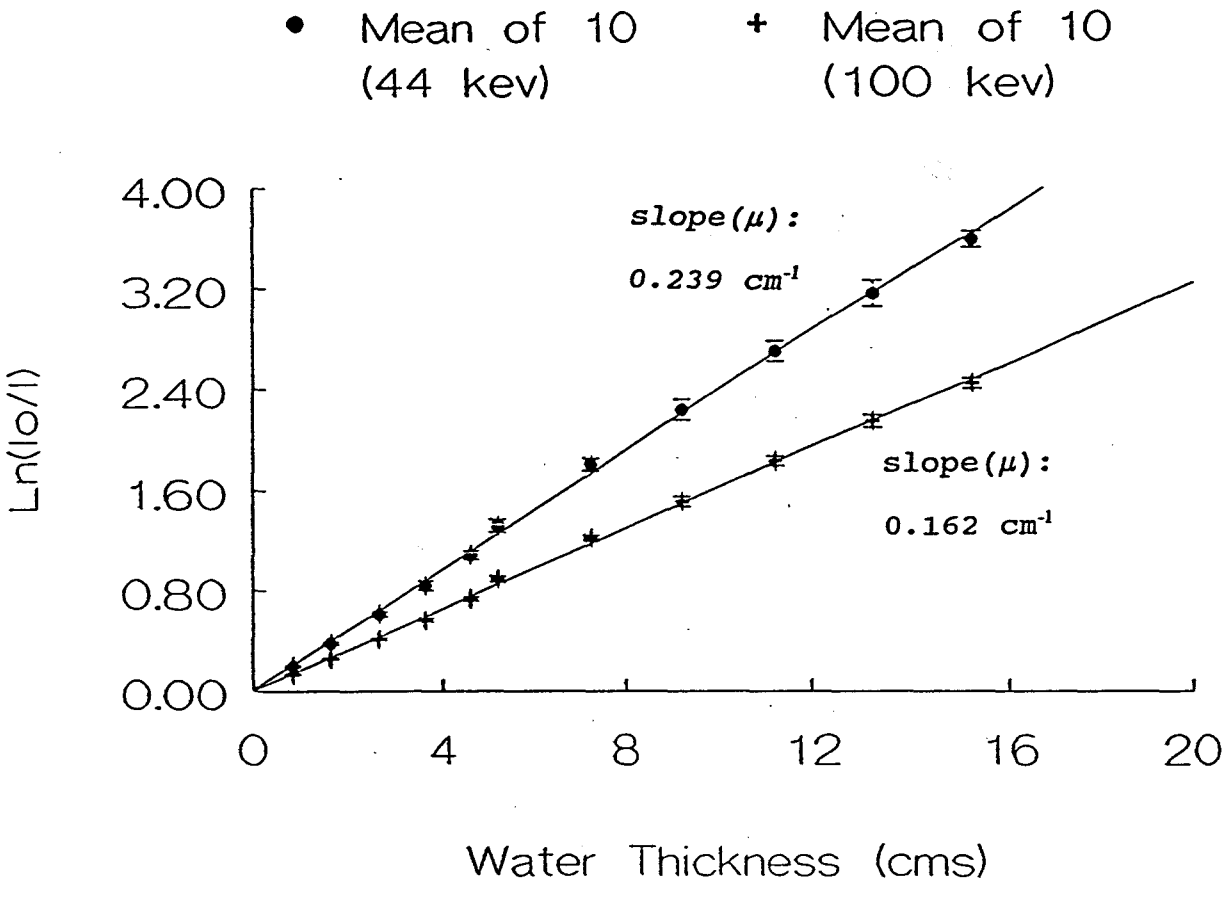


Figure 3.6 Determination of the mass attenuation coefficients for water at 44 and 100 Kev by recording transmitted fractions at different thicknesses.

are expressed as the R_{st} ratio 1.475 is obtained. This value is in excellent agreement with the 1.476 corresponding to pure lean tissue. Therefore, when measured in the source - detector - collimator geometry of the Norland 2600 and even when the recorded count rates are not corrected completely, water becomes an effective lean tissue substitute.

3.3.1 DPA scans of pure water phantoms

Whole body scans were performed on the pure water phantoms which contained a bone mineral sample. The bone mineral sample had to be included in the scanning field because the analysis software expects to see a two component system, one of which must be bone mineral equivalent material. The water was contained in a box of fixed dimensions. Only the thickness through which the photon beam traverses was varied. That is, the total soft tissue mass was changed by varying its thickness and not its area (the number of pixels in the scan). Fixing the area ensures that the scan results indicate the effect of thickness and not the statistical fluctuations due to changing the number of pixels comprising a scan. A lean fraction of 100 % was expected (0% fat). The effect of thickness on fat determination is plotted in figure 3.7. Clearly, there is a limited range over which DPA separates water into fat and lean equivalent fractions. Below 8 cm the fat fraction is underestimated to a point where it becomes negative. Between 8 to 16 cm it accurately determines the fat and lean fractions to within 2%. Beyond 16 cm the fat content is grossly overestimated and

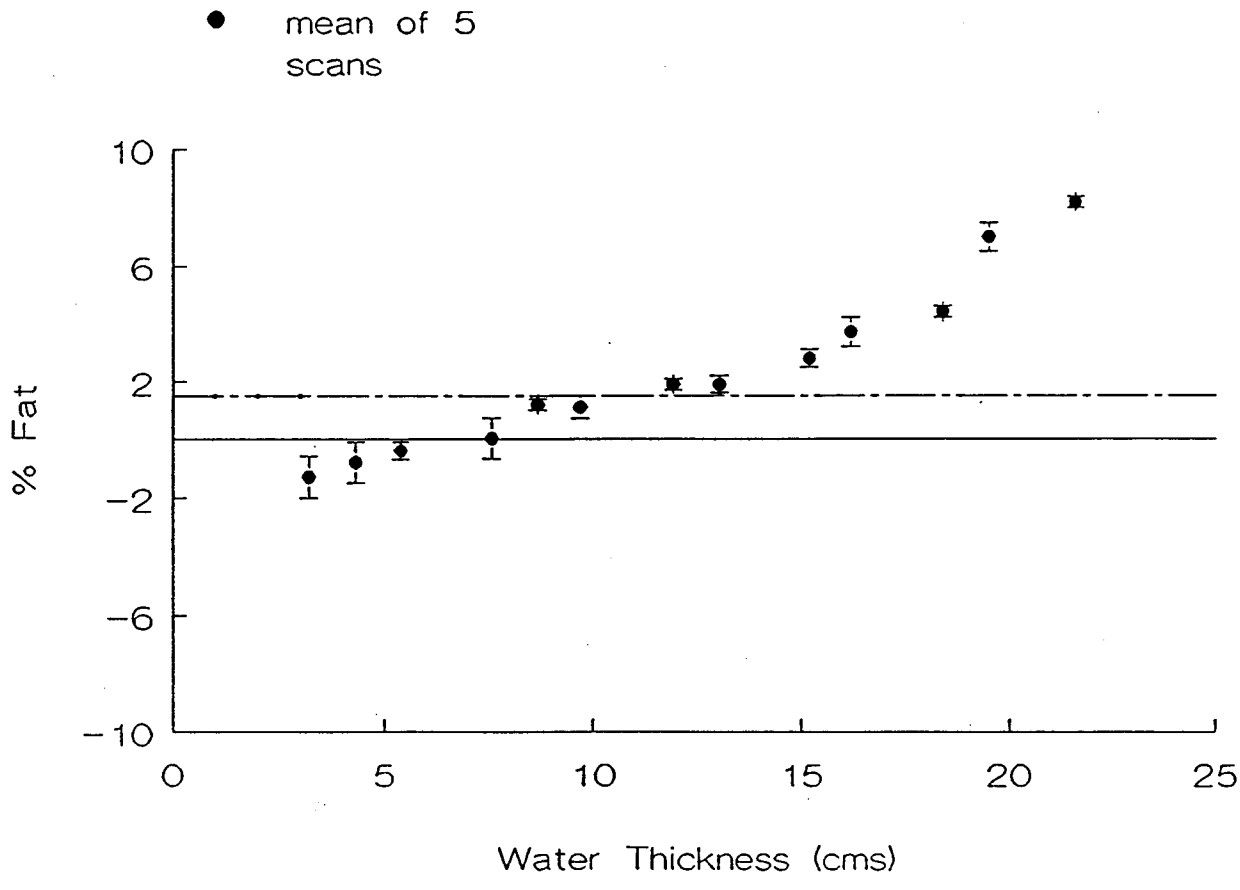


Figure 3.7 The effect of thickness on the accuracy of fat determination for water. The measured mean value is indicated by (——).

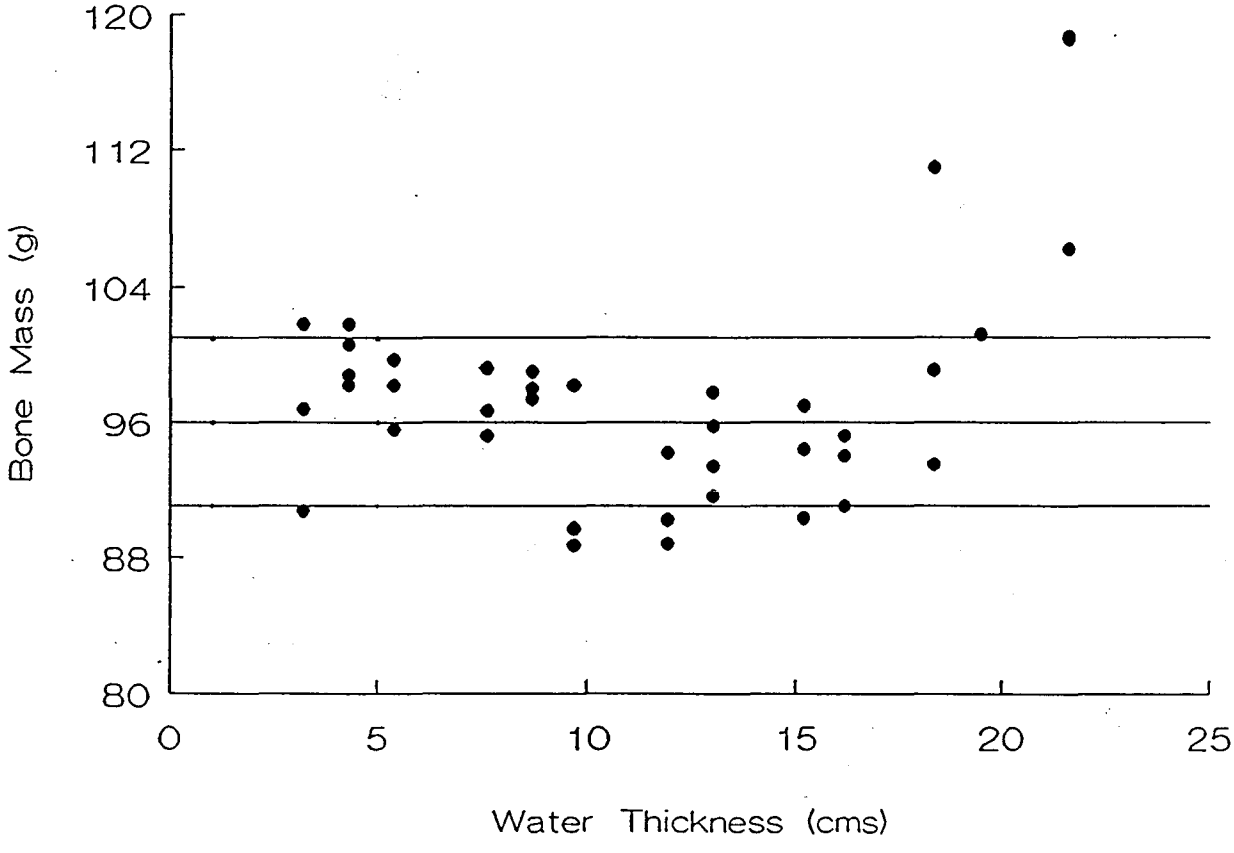


Figure 3.8 The stability of bone mineral content with changing overlying water (tissue) thickness. The average value along with a +/- 2% uncertainty range is indicated by (——).

consequently the lean tissue mass is underestimated by the same margin of error (5-10%). Interestingly, the profile shown in figure 3.7 appears to be the mirror image of that found in figure 3.5. This correspondence between the two curves lends support to the fact that soft tissue separation is wholly dependent upon an accurate determination of the average R_{st}.

Although the soft tissue separation was only accurate over a limited thickness range, the bone mineral content did not vary significantly over the full range of thicknesses. As shown in figure 3.8, there is less than a 3% precision error in bone mineral determinations for an overlying tissue thickness of up to 16 cm. It is important to note that clinically relevant thicknesses of soft tissue overlying bone are between 1 to 10 cm and so the 3 outlying points that occur at thicknesses of approximately 20 cm represent unrealistic body situations.

In concluding, the results obtained from scanning water and shown in figure 3.9 verify that the total soft tissue mass is accurately determined. There is excellent correlation between DPA determined water mass and true water mass as determined volumetrically. Least squares analysis revealed that the slope of this line was not statistically different from 1 and its intercept not different from 0.

To verify that the thickness effects observed for a homogenous medium such as water are applicable to actual mammalian soft tissue mass, the results of figure 3.7 were repeated on a 4 Kg pork phantom that was 3% fat and 97 % muscle by mass. Quite simply, the

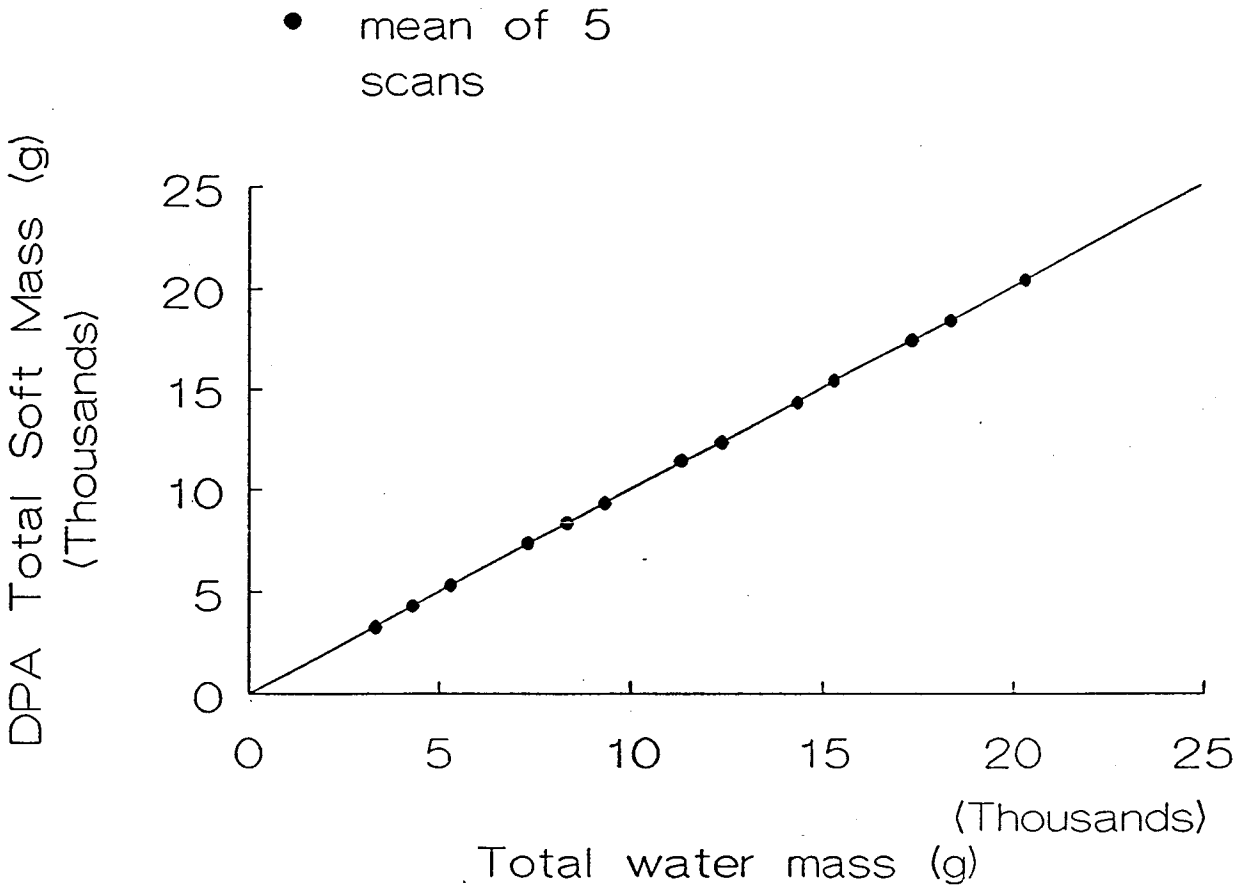


Figure 3.9 The correlation between DPA derived total mass and the true value.

the pork tissue was squeezed to different dimensions to produce various thicknesses through which the radiation beam traversed. The results of this trial are plotted in figure 3.10. Again, the same error profile is obtained as compared with water. When the 4 Kg mass is spread out over an area to produce a mean thickness of 3 cm, a fat mass of -250 g is obtained. For thicknesses between 10 to 16 cm, an accurate fat and lean mass determination is obtained. Beyond 16 cm the fat content is overestimated. At all thicknesses, however, the total soft tissue mass was accurately measured as (4.0 +/- 0.15) Kg.

3.4 The effect of hydration changes in the lean mass

It has been estimated that there is up to a 10% variation in the mass attenuation coefficients between subjects due to normal biological variations (Webber, 1986). A large fraction of this can be attributed to the normal variation in the water content of skeletal muscle. For normally hydrated adults the average fluid fraction is 0.78 of the lean mass with a range of variation from 0.72 to 0.82 (ICRP-23 1975). For Dual Photon Absorptiometry measurements to be applicable to all persons in a population, its ability to partition the body into bone mineral, muscle, and fat must not be influenced by this variation in water content between individuals. That is, if two individuals were composed of the same fat and bone mineral fractions but had equal muscle masses that contained different fractions of water, then a DPA measurement should reveal equal amounts of fat, lean tissue and bone mineral.

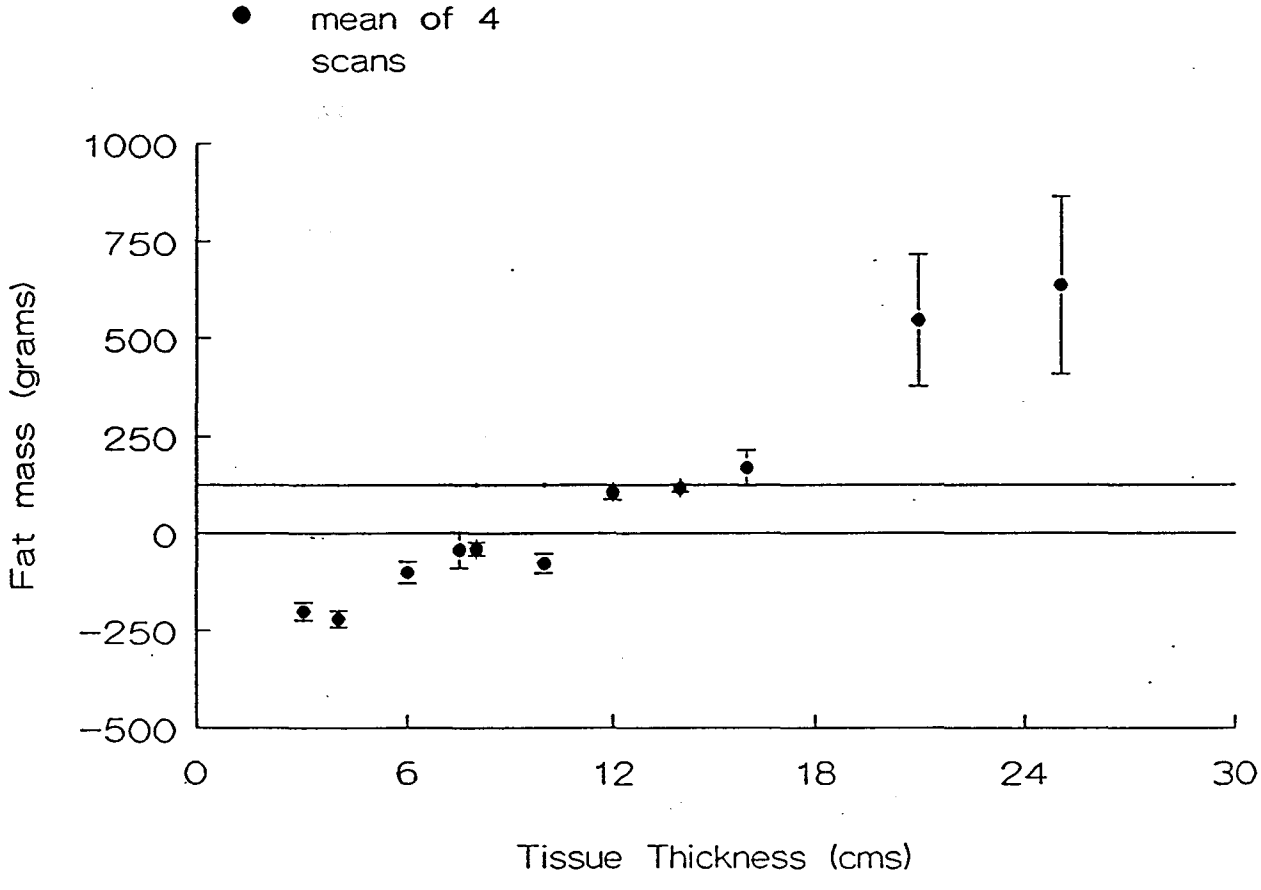


Figure 3.10 The range of errors in fat determination that occur by changing tissue thickness. The true fat content as determined chemically is indicated at 125 g.

To examine whether soft tissue separation is influenced by water change, 4 tissue phantoms were constructed. These phantoms contained the same percent fat by mass but the percent water in the muscle mass was varied from 69% to 86%. Whole body scans were performed on each phantom plus a sample of bone mineral. The results of soft tissue division into muscle and fat are provided in figure 3.11. What is plotted is the change of muscle water from the average versus the DPA determined fat fractions. The true percent fat in each phantom was 52.8, but as revealed by the plot an accurate fat and lean determination is only achieved at the average water fraction of 78% (0% change from the average). Clearly, there is a reciprocal relationship between fat determination and water content. For a 10% change in water from the average there is an incorrect but opposite absolute change of 5% in percent fat determination.

For the phantoms that contained a water fraction of 0.78 in the lean compartment the scanner was quite accurate in dividing the total mass into its respective compartments. This suggests that the present internal calibration with lard and water works well for persons who's lean mass contains 78% water by weight. Any increase or decrease from this water level leads to an incorrect soft mass separation. If the water fraction exceeds 0.78 then the DPA technique overestimates the lean fraction thereby underestimating fat. The source of this error is the slope of the calibration line. One must be able to adjust the R_{lean} end point in response to the different mass attenuation coefficients of muscle tissue with

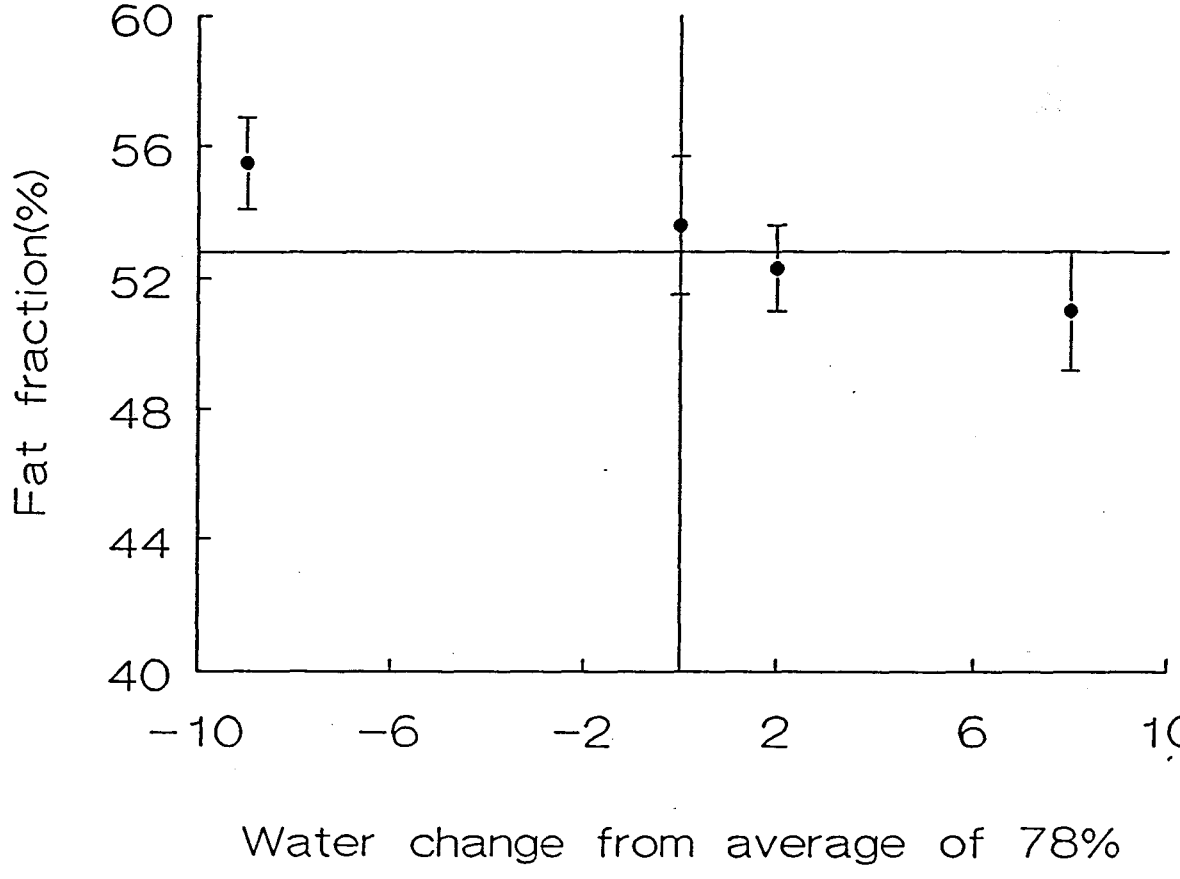


Figure 3.11 The effect of varying water content in muscle on fat fraction determination in a given soft tissue mass. The expected fat fraction is indicated by (——).

differing water and protein contents. In practice, a range of calibration curves similar to those sketched in figure 3.12 should be included in the analysis software to enable accurate soft tissue separation in the presence of hydration changes from the average.

It is of interest to note that there exists a reciprocal relationship between water and fat in the body. Leanness predisposes one to a higher water fraction in the lean compartment. Consequently, in light of these phantom results, the trends observed in the daily use of the Norland scanner are not surprising. Leaner subjects will be estimated too lean while those who are obese will produce slightly higher fat readings from their true value.

The ability of DPA to follow water changes in the lean body has not been widely reported in the literature. However, the results from the phantom trials outlined above are consistent with those results that were reported. For example, by performing DPA scans on a fixed region of the forearm, Whitt and Mazess showed that a +/- 10% change in fluid mass (soft tissue) could be detected with an accuracy of 4%. However, they also revealed that beam hardening effects caused the R_{st} value to decrease as the mass of water increased. This R_{st} trend translated into an apparent increase in fat fraction (from 3% to 10%) and a decrease in lean tissue mass at the forearm site (Whitt and Mazess, 1977). Most recently, Lands and co-workers found that DPA was excellent for the evaluation of changes in total body soft tissue mass. However, by changing body water through a saline transfusion or through sweating, they showed

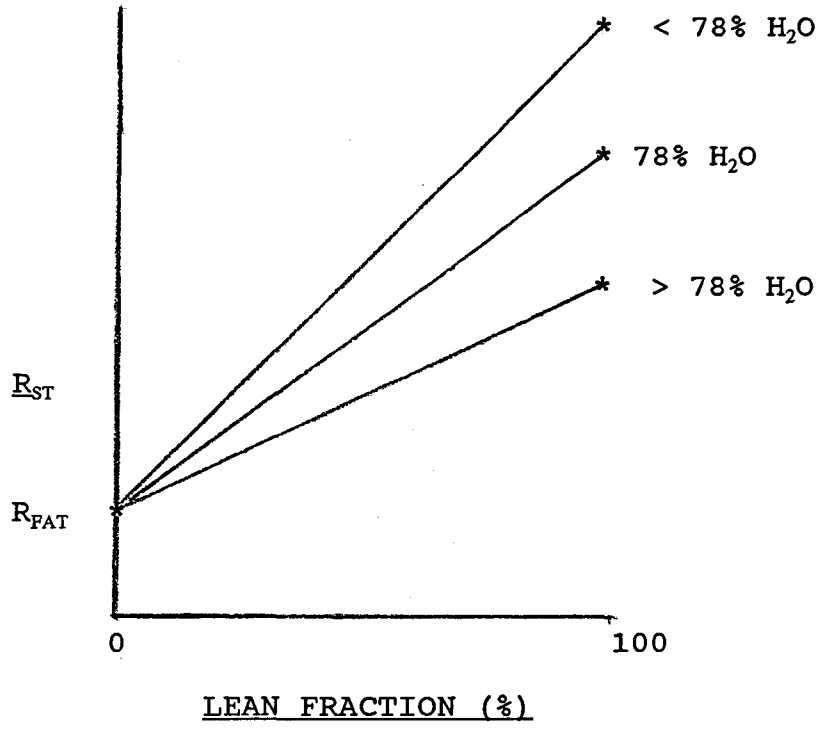


Figure 3.12 The effect of changing the water fraction at the lean endpoint on the slope of the calibration line.

that the DPA technique was unable to assign the change to its lean and fat components with any degree of accuracy (Lands et al.,1991).

3.5 Summary of errors

Much work has been done on the validity of bone mineral measurements by DPA. Although there are non-exponential counting contributions (Gluer, 1988; Ross et al., 1988), in general, the conclusions drawn emphasized that counting irregularities affected bone mineral measurement accuracy by as much as 10-15%. However, precision errors were considerably lower (< 3%). Such a large accuracy error does not eliminate Dual Photon Absoptiometry as the measurement tool of choice for bone mineral studies. Because skeletal changes are so small, a high degree of precision is more desirable than a high degree of accuracy and so DPA works well. To date, the effects of these counting factors on soft tissue separation have not been reported as rigorously as has been done for bone mineral determinations. However, these phantom trials are indicative of the types of approach that can be adopted in order to quantify the errors in soft tissue separation due to count rate effects.

The results obtained from scans performed on the water and soft tissue pork phantoms are most useful if the observed trends are extrapolated to clinical subjects. Such an extension is provided in table 3.5 and was based on the following considerations. From earlier findings, it is clear that the R_{st} ratio is reflective of the true soft tissue state only over a limited soft tissue thickness

range. This thickness range, however is comparable to that found in the average adult male and female. Subjects can be placed into three general categories based on body size; small, average and large. Although the errors listed in table 3.5 are from phantom trials, they should also represent resonable estimates of those present in clinical trials

Those subjects classified as small have soft tissue thicknesses of less than approximately 10 cm through which the R_{st} values are measured and averaged. As tabulated, the Norland DPA scanner provides accurate measurements of body weight (BM+TSM), bone mineral masses(BM),total soft mass(TSM) but underestimated fat and overestimated lean tissue masses by the same percent errors. For the average adult who's mean body soft tissue thickness is between 10 to 16 cm, body weight, BM, TSM, fat, and lean are determined with a high degree of accuracy and precision. In larger subjects (>16 cm) again body weight,BM, and TSM are accurately measured but fat mass is overestimated and so the lean tissue mass is underestimated. As an example, for larger subjects, this percent error in fat mass translates into an absolute mass error of approximately 1-2 Kg in males and 2-3 Kg in females. Because lean tissue masses are approximately 2.5 times that of fat in males and 1.5 in females (see figure 1.1), the errors for lean tissue determination can be as much as 2.5-5 Kg in males and 3-4.5 Kg in females.

The consideration of these sources of error has reemphasized the fact that the DPA technique can be unreliable in quantitating the components of the soft mass in absolute terms for any given

Table 3.5: The predicted % accuracy and [% precision] with which DPA can determine body weight, bone mineral mass(BM), total soft tissue mass(TSM), lean tissue mass and fat mass in people.

Body Size	Body Weight	BM	TSM	Lean	Fat
Small	< 1% [1%]	** [2%]	2-3% [2%]	2-5% [1-3%]	-(2-5%) [1-3%]
Average	< 1% [1%]	** [2%]	2-3% [1-2%]	3% [2%]	3% [2%]
Large	< 1% [1%]	** [2%]	2-3% [2%]	-(5-10%) [3-4%]	+(5-10%) [3-4%]

** = not determined

-( )= underestimation error

+( )= overestimation error

individual within a study population. Improving the reliability of the division of the soft mass, in all subject types, will entail minimizing the effects of subject thickness and perhaps quantitating the magnitude of the biological differences (due to body water) between individuals. However, given the predictable behaviour in the accuracy errors of fat and lean tissue determinations, the potential for correction exists.

3.6 Correcting for Body Size

Under Water Weighing (UWW) is considered as the body composition technique which most accurately (within 2%) determines body fat. If the body size dependency of DPA determined fat and lean tissue is real then a comparison of the two methods can disclose any fundamental variables which can be used to generate correction equations for the DPA inaccuracies.

UWW and DPA were used to measure body fat in 52 males enrolled in a study designed to examine the effects of jogging on bone mineral density and mass. There was a control group and 5 other groups that were determined according to the number of kilometers that each subject ran per week. The body characteristics of the study population is summarized in table 3.6. Three points are worth highlighting from this table. The first is that the range of %fat determined by DPA is much wider than that for by UWW and DPA includes some negative percent fat determinations. Secondly, there is no difference in the population mean %fat whether it is determined by DPA or by UWW. This correspondence between the two

Table 3.6 : The body size characteristics of the 52 males enrolled in study.

	Mean	SD	Range
Height(cm)	175.42	7.29	147 - 190.5
Weight(Kg)	74.11	7.57	60.4 - 89.9
Body Mass Index (Kg/m^2)	24.13	2.54	19.6 - 30.92
%Fat(DPA)	11.26	8.97	-3.96 - 35.43
%Fat(UWW)	12.60	4.82	5 - 25.50

means implies that although DPA may provide unreliable body fat and lean estimates in a given individual, it provides an accurate estimate of the population mean fat and lean tissue masses. Thirdly, the standard deviation (SD) in the population mean fat is twice as large for the DPA measurements. This suggests that although DPA is precise for an individual, the differences between individuals greatly affects the measurement process.

A direct comparison of the %fat determined by the two techniques is shown in figure 3.13. Least squares analysis of this data set produced a slope of 0.2696, an intercept of 9.59%, and a correlation coefficient of 0.5049. When two quantities are compared in this manner perfect agreement between the two measurements exists when the regression line has a slope of 1 and an intercept of 0. It then follows that, there is only a moderate agreement between %Fat(UWW) and %Fat(DPA). To account for this difference in fat as measured by the two techniques, [%Fat(UWW) - %Fat(DPA)] was regressed against the various parameters listed in table 3.6. The resulting slopes, intercepts, and correlation coefficients are provided in table 3.7. It is clear that the difference in fat is not due to UWW (r^2=0.0336) but is strongly dependent upon a DPA measurement (r^2=0.8276). The hypothesis that a DPA result is size dependent is supported further by the good correlation between fat difference and the Body Mass Index (BMI) which is an indicator of body thickness. That is by multiplying the BMI by density, the units appear in dimensions of thickness (m).

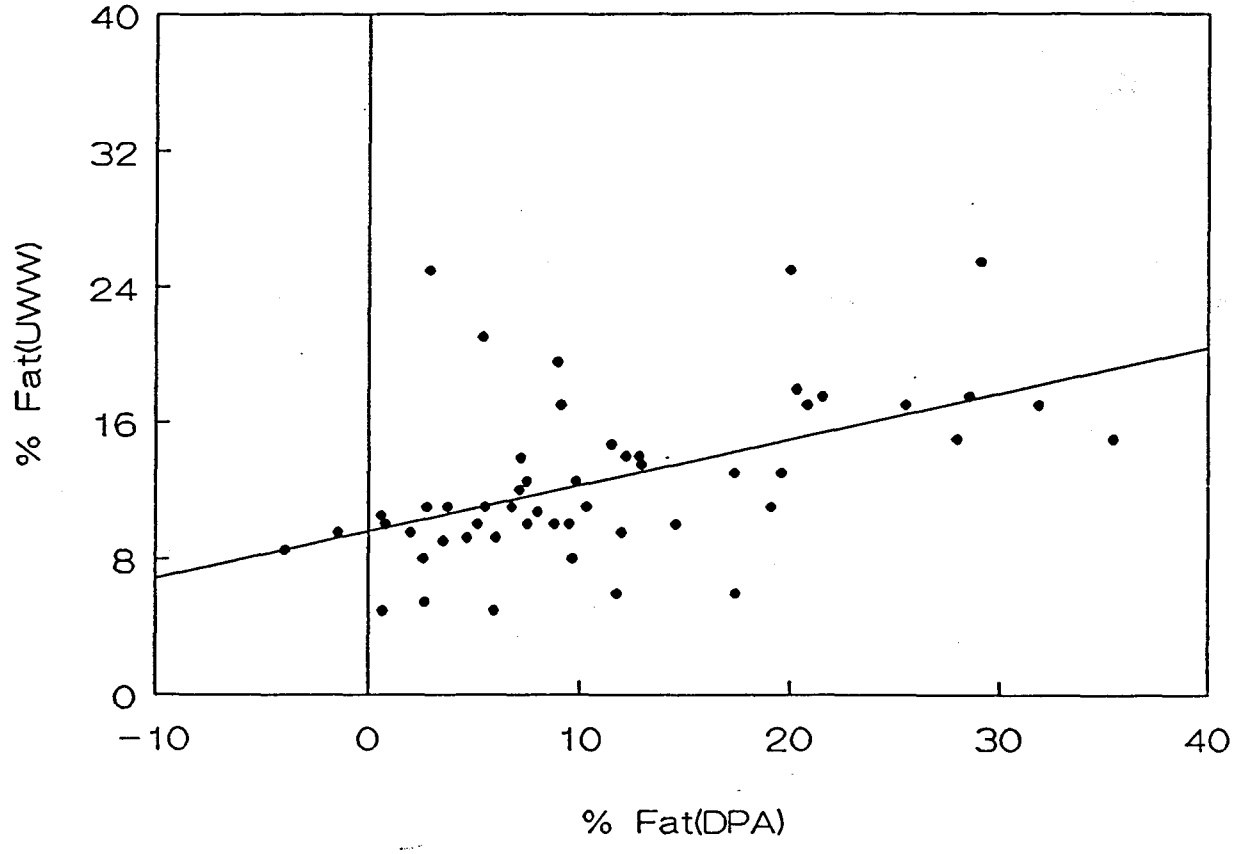


Figure 3.13 The correlation between fat measured by under water weighing (UWW) and DPA.

Table 3.7 : Variables that can be considered as indicies of difference in fat [%Fat(UWW) - %Fat(DPA)]

Variable	Slope	Intercept	Correlation(R^2)
Height	0.1235	-20.56	0.1116
Weight	-0.4306	32.79	0.3935
BMI	-1.4520	36.16	0.4579
%Fat(DPA)	-0.7447	9.49	0.8276
%Fat(UWW)	0.0543	0.74	0.0336

It would be most useful if the errors in a %Fat(DPA) could be corrected by a height and weight measurement of the individual subject. With these body indicies being combined to form the Body Mass Index, corrections based upon the BMI will be explored. The regression line of [%Fat(UWW) - %Fat(DPA)] versus %Fat(DPA) which yielded a high correlation (r^2=0.8276) is displayed in figure 3.14. Upon close examination of this plot it appears that below a %Fat of 13%, DPA underestimated fat relative to UWW (positive difference in fat). Beyond 13%, the difference in fat is negative and so indicates that a DPA measured body fat is an overestimation relative to that determined by UWW. Although not as dramatic, a similar profile is obtained when the difference in fat is displayed against BMI (figure 3.15). However to reveal the presence of any underlying trends a 5 point smooth was applied to the data to minimize the influence of any statistical fluctuations. That is, with respect to incrementing BMI's, every 5 points were averaged and displayed as one. The results are given in figure 3.16. It is again clear from this plot that the underestimation and overestimation tendencies of the Norland DPA scanner are a function of body size. Linear regression of this smoothed data revealed a high degree of correlation (r^2=0.8596). The corresponding slope and intercept were -1.533 and 38.11 respectively. If this slope and intercept of the smoothed data is compared to that obtained from a regression performed on the raw data set (table 3.7, BMI) there is little difference in the two slopes (-1.45) and intercepts (36.16).

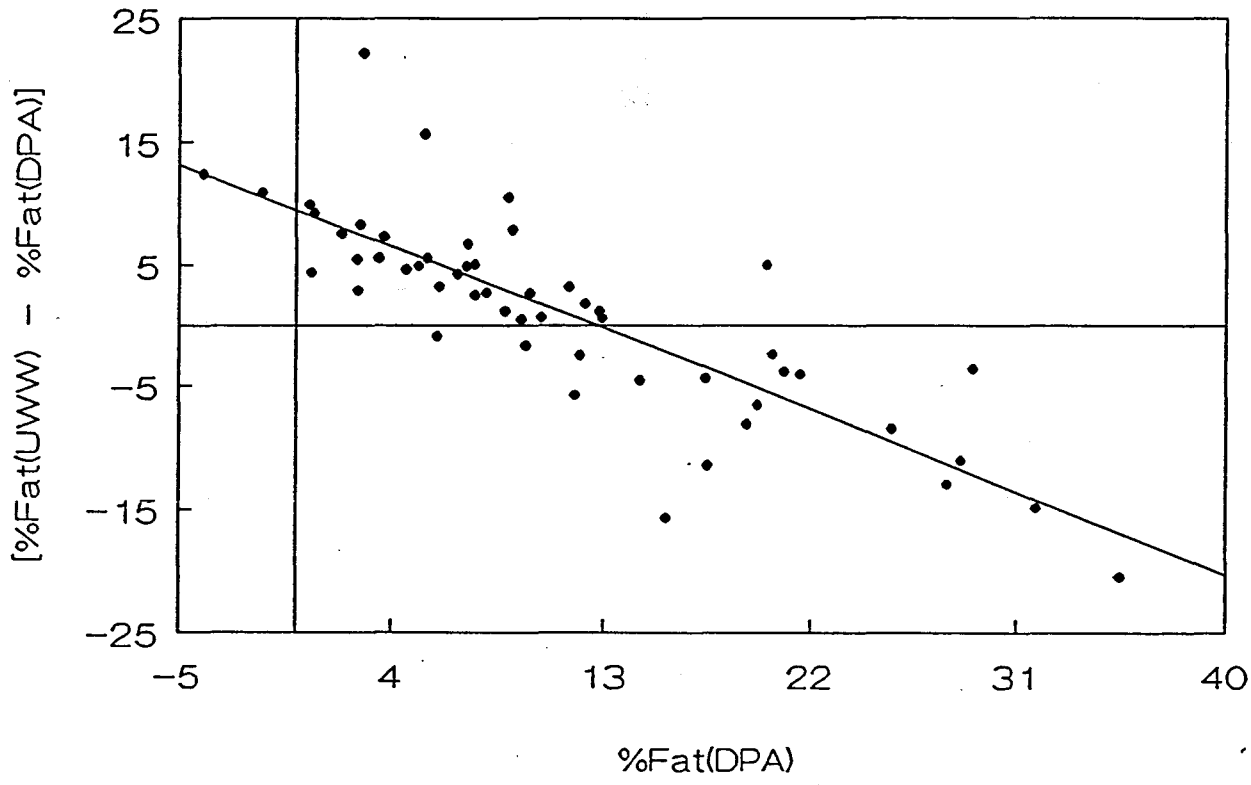


Figure 3.14 Comparison of difference in fat measured by the two techniques as a function of fatness (%Fat(DPA)).

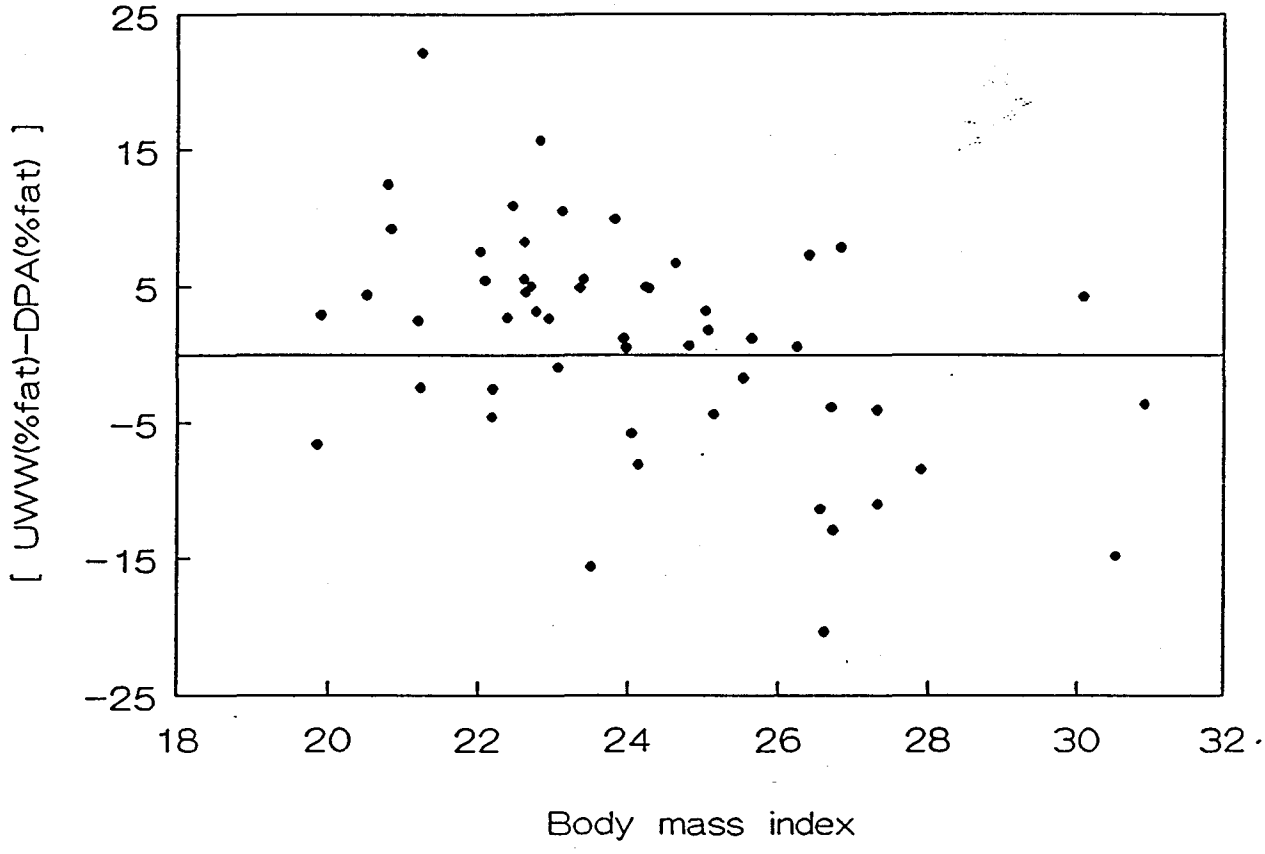


Figure 3.15 The correlation between fat difference and body size as indicated by the Body Mass Index.

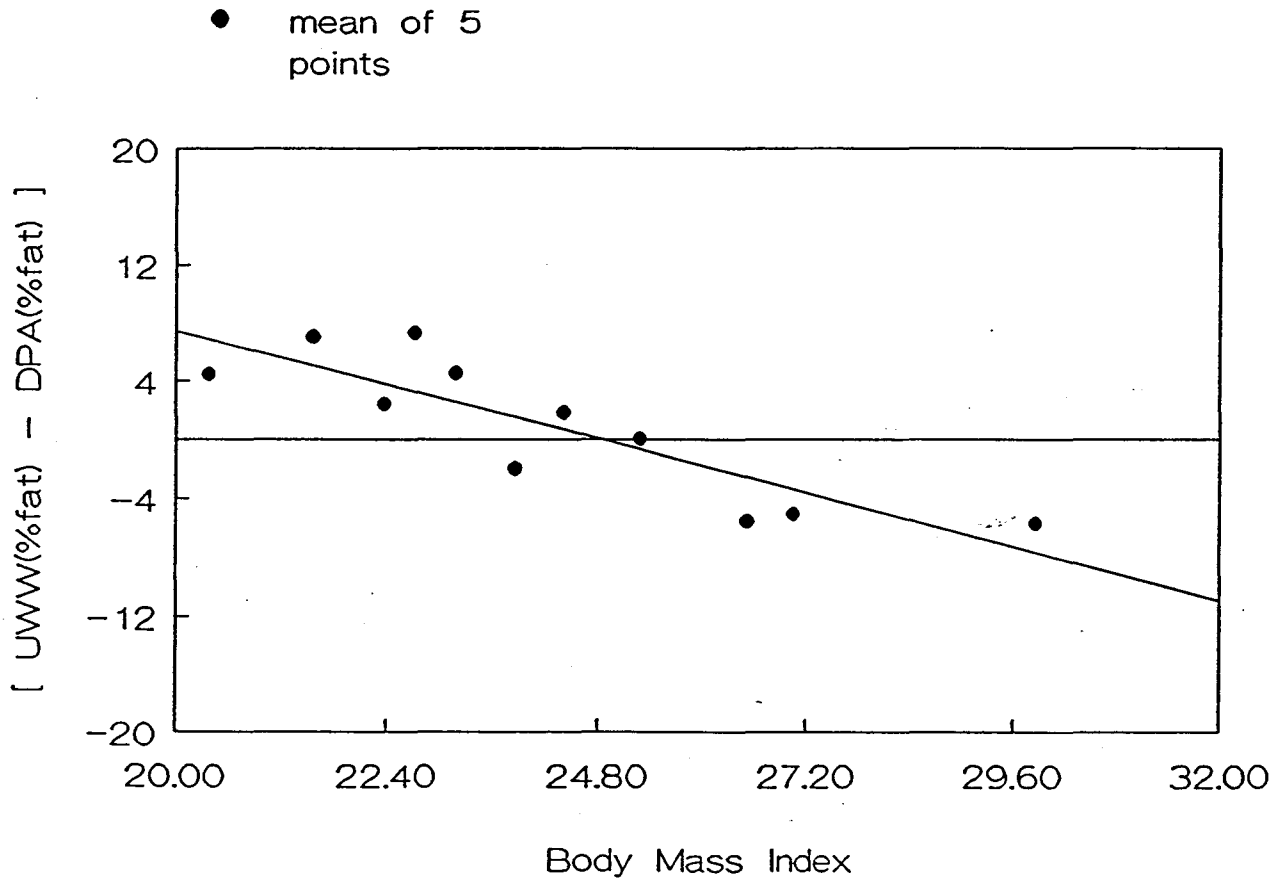


Figure 3.16 The correlation between fat difference and Body Mass Index after a 5-point smooth is applied to the 52 raw data points.

Therefore, through the technique of smoothing, the expected error profile is enhanced while conserving the integrity of the data set. Interestingly, at approximately 24 (BMI corresponding to the average male) the difference in %fat is 0. Again, as with the phantom data, around this average there is a small region over which the DPA measurement is correct. Although there exists this plateau in the data profile, the high degree of correlation suggests that the relationship between the fat differences and body mass index is linear. To the extent that an UWW determined %Fat is an accurate measure of the true one, then the differences in a DPA measured fat in adult males from the truth can be defined as follows:

$$\%Fat(True) - \%Fat(DPA) = 38.11\% - 1.5333\ (Weight/Height^2) \qquad 3.1$$

This correction equation is only applicable to adult males but a similar profile is expected for females. With (%Lean+% Fat = 100) it follows that the lean tissue mass is easily obtained with a knowledge of the corrected fat mass.

Wang et al.(1989) performed a similar type of regression analysis on a population of 99 males and 187 females between the ages of 19-94 yr to disclose any fundamental differences between fat estimated by dual-photon absoptiometry and under water weighing. For their population, the range of the differences in measured fat was greater in females than males. In their analysis process they defined the parameter D_{lbm} (lean body mass density) as

being a function of total body density (determined by UWW) and a function of fat fraction (determined by DPA). This variable was shown to be the best predictor of the measured differences [%Fat(UWW) - %Fat(DPA)], more so in females (r^2=0.98) than males (r^2=0.90). With D_{lbm} being based upon a DPA measured %Fat, the confounding influence of body size was not addressed. It follows that not only was the lean body mass density a good predictor of the measured differences but the effects of body size on the D_{lbm} may have been of greater importance.

3.7 Usefulness of DPA for determining group averages.

The direct comparison of body fat measured by under water weighing and by dual-photon absorptiometry of the previous section revealed that there was no significant differences between the group average %fat when determined by either technique. To pursue this notion further, body fat measurements were made in 13 subjects using the techniques of skinfold thickness (SKF), Bioimpedance (BIA), and photon absorptiometry (DPA). The body characteristics of the subject are provided in table 3.8 along with the group fat averages determined by each technique. As tabulated, although the mean %fats are within one standard deviation (SD) of each other, the DPA determined mean fat is lower than that determined from skinfolds and impedance. What is more significant is the SD associated with each mean. The SD in the DPA determined mean is approximately twice as large as the deviation associated with the others. Again, this increased SD for photon absorptiometry is a

Table 3.8: Comparison of the average body fat in a group of 13 males and females measured by skinfolds(SKF), bioimpedance(BIA), and dual-photon absorptiometry(DPA).

	Mean	SD	Range
Height(cm)	142.71	16.13	119.0 - 172.0
Weight(Kg)	34.54	9.78	20.3 - 53.1
Body Mass Index (Kg/m^2)	16.57	1.65	13.6 - 20.2
%Fat(BIA)	16.96	2.99	10.1 - 24.1
%Fat(SKF)	15.30	4.14	8.2 - 24.0
%Fat(DPA)	11.08	6.23	1.2 - 27.02
[%Fat(BIA)-%Fat(DPA)]	5.88	3.64	-2.9 - 9.67
[%Fat(SKF)-%Fat(DPA)]	4.22	4.44	-5.4 - 8.32
[%Fat(BIA)-%Fat(SKF)]	1.66	2.71	-3.9 - 5.93

reflection of its dependency on body size. For this study population the range of variations in height and weight are quite wide and this is paralled by the range of %Fat(DPA) (1.2 - 27.0)% being much wider that determined by skinfolds (8.2 - 24.0)% and impedance (10.1 - 24.1)%. It has been established that in smaller subjects, DPA consistently underestimates body fat. This trend is supported by the mean fat differences presented in table 3.8. That is, given that DPA is underestimating fat, then the mean difference [%Fat(BIA)-%Fat(DPA)] or [%Fat(SKF)-%Fat(DPA)] will exceed 0. This is indeed true for this group of subjects. For example, in the case of DPA and BIA, the mean difference is just under 2 standard deviations away from 0. The excellent agreement between skinfolds and bioimpedance (despite the wide range of body sizes being measured) further reinforces the hypothesis of a DPA body size dependency.

Although the analysis of these 13 subjects only focused on a body fat measurement it is expected that DPA will also be able to correctly determine the averages for lean tissue mass. In the case of the measured differences between techniques, the tendency of DPA to overestimate lean tissue masses will be bourne out in the mean differences being less that 0.

CHAPTER 4

CONCLUSION

4.1 Introduction

Dual-photon absorptiometry is a recently developed method of measuring skeletal mass, lean tissue mass and fat mass. It measures these three body components simultaneously and therefore has an advantage over other body composition measurement techniques which generally only provide an estimate of one body compartment per measurement. This distinct advantage has led to the development of several commercially available photon absorptiometry systems. One such system is the Norland 2600 which was advertised as being able to provide accurate and precise measures of bone mineral mass and soft tissue mass and reliable estimates of fat and lean tissue mass in people. During clinical trials, however, the unreliability of the lean and fat tissue became apparent. It was clear that in persons who were very lean, the Norland system far underestimates their body fat and overestimates their lean tissue. At the other end of the scale, in persons who were very fat the Norland system assigned them too much body fat and underestimated their lean tissue mass. Consequently, the reliability of the system to correctly measure fat and lean tissues between these two extremes had to be examined in order to assess the value of photon absorptiometry as a tool of choice to perform body composition measurements on a study population containing various body types.

4.2 Body Size and Muscle water effects.

With the use of soft tissue and water phantoms it was shown that the scattering of the 100 Kev photons into the 44 Kev window influenced the accuracy with which a soft tissue mass could be divided into its muscle and fat proportions. By changing the thickness of the soft tissue mass it became evident that as the amount of scatter increased, the accuracy of the measurement was also being influenced. It was found that for fat and lean measurements performed on tissues below 10 cm thick, the absoptiometry technique consisently incurred an accuracy error of -(2-5)%. That is, it consistently underestimated the fat mass by 2-5% and overestimated the lean tissue mass by 2-5%. In fact, the occurrence of negative percent fat determinations were not uncommon. For tissue thicknesses between 10 to 16 cm the dual-photon absorptiometer was able to determine fat and lean tissue masses to within +3%. Beyond 16 cm, the amount of photon scatter resulted in accuracy errors of +(5-10%). That is, fat was consistently overestimated by 5-10% and lean tissue was underestimated by the same margin of error. At all thicknesses, however, the total tissue mass was accurately measured to within 2% The precision errors for fat and muscle determinations at all tissue thicknesses ranged from 1% to 3%.

Lean tissue (muscle) is composed of approximately 78% water and 22% protein by mass. However the water content can vary from 72% to 82% for individuals in a normal population. This variation in water content translates into a slight but measurable change in

the mass attenuation coefficient of muscle tissue. When this measured mass attenuation coefficient is related to the current muscle/fat calibration curve of the Norland system, inaccuracies were apparent. For a +10% change of the water content in muscle tissue mixed with fat, the fat mass was underestimated by 10% and the muscle mass overestimated by 10%. A decrease of 10% in water brought about an apparent increase in fat of 10% and a corresponding decrease in muscle. Consequently, there exists a reciprocal relationship between the accuracy error in fat determination and the water content in the lean tissue that is being measured in combination with the fat mass. Again, however, the overall tissue mass was determined to within +/-2% for all water fractions tried.

4.3 DPA Errors for individual and population studies

From the soft tissue phantom trials and the comparison with under water weighing the accuracy errors were assessed for a given individual. In general the results support the notion that for males who's body thickness was below the average (body mass index of 23-25), dual-photon absorptiometry may inaccurately measure their body fat by approximately 1.55*(BMI-24.5)%; where BMI is the index for the individual in question. The lean tissue mass for this individual is overestimated by the same margin of error. This equation defining the error was derived from a population of 52 males with body indicies ranging from 18 to 30. Therefore, it should only be applied to male subjects in this body mass index

range. Further work is needed to establish the nature and magnitude of the errors in females and children.

Although the accuracy errors associated with a lean and fat measurement are at times large (10-15%), the precision error is quite small (< 3%). This precision error appears to be independent of body size for subjects who fall within a clinically relevant range. That is, for very small subjects such as babies and the very obese this precision error may become more variable.

The usefulness of DPA to follow total body soft tissue changes for a group of subjects was shown by comparing the %fat measured by DPA to that determined by bioimpedance, skinfolds, and under water weighing. There were no significant differences in the mean %fat as determined by these body composition techniques. However, the standard deviation associated with the DPA determined mean was consistently larger than those of the other techniques. This greater variance for DPA is again reflective of the influences of body size in an individual measurement.

4.4 The Future of Dual Photon Absorptiometry

The next generation of photon absorptiometers are advertised as being superior to the first generation systems. Their superiority stems from the fact that an x-ray tube is used to supply the dual energy photons needed to perform the measurement. Five times as many photons are now being used to make the transmission measurements throughout the body and so a corresponding increase in scanning speed reduces the scanning time

for a whole body scan to approximately 10 minutes. These new dual photon x-ray systems (DPX) are also expected to reduce the radiation dose to the patient by half while still providing reliable total body and regional fat mass, lean tissue mass, and bone mineral information.

Given that improved correction algorithms for non exponential counting effects are included in the analysis software, then the accuracy and precision of DPX will exceed those achieved by DPA. It should also not be influenced by body size. Consequently, with the proposed levels of accuracy and precision associated with a DPX determined body composition measurement, there is no reason why this x-ray based photon absorptiometry system cannot become the gold standard by which all other body composition measurement techniques are judged.

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Appendix 1

1.0 Chemical Fat Extraction

Wet skeletal muscle is composed of approximately 78% water, 19% protein, 3% fat and trace amounts of carbohydrates and DNA (<1%). Once freeze dried the water fraction is removed form the tissue. Therefore a fat extraction procedure applied to dried tissue will involve the separation of fat from protein and trace amounts of carbohydrates and DNA. The following sections outline a simple protocol for the extraction.

Reagents:

Chloroform.
Methanol
4 mM Magnesium Chloride ($MgCl_2$) or 0.9% saline (NaCl)
Chloroform : Methanol mixture (2:1 by vol.)
Crisco Oil (5 drops - 0.080 g)

Equipment:

Hamilton Syringe - 2 ml
4 Gram vial (15 ml) with teflon caps
15 ml Graduated centrifuge tube
25 ml Graduated cylinder
Funnel
Filter paper (Whatman #1 or #41; 11 cm diameter)
Fumehood & centrifuge

Procedure:

(1) Weigh 100 mg of dry tissue into 4 gram vials or centrifuge tubes. If centrifuge tubes are used, add tissue to solvent to prevent packing of tissue to bottom of tube.

(2) Re-hydrate tissue by adding 0.6 ml H_2O per 100 mg of dry tissue.

(3) Add 10 ml Chloroform:Methanol mixture using a dispenser. Cap and allow to sit overnight (~16 hrs). Because of the sensitivity of chloroform, cover with foil to protect from light.

(4) Bring the contents to room temperature in a fumehood. Add 2 ml of $MgCl_2$ or 0.9% saline.If dry tissue was not re-hydrated (step #2) then add 2.6 ml of either salt solution. Mix gently and return to 4 C for 30 minutes.

(5) Balance tubes and centrifuge at 1000 rpm for 60 minutes or allow to stand at 4 C until a clear separation into 2 phases is obtained. After separation, allow contents to equilibrate to room temperature.

(6) Estimate volume of the organic phase by reading directly off centrifuge tube. The organic phase is the lowest of the two distinct phases.

(7) Using a Hamilton syringe remove 4 ml from the organic phase and deliver into a preweighed culture tube. The organic phase is composed of fat dissolved in chloroform.

(8) Dry under a stream of air under fumehood. This allows for the evaporation of the chloroform and subsequent precipitation of fat.

(9) Reweigh the culture tube containing the fat.

2.0 Determination of water and protein content

Water fraction was determined through freeze drying by vacuum. The ratio of the dry to wet tissue weight is equal to the fraction of water removed.

Once tissue has been dried it is composed of fat and protein. Therefore when the fat fraction in the dried tissue is determined the protein content is equal to 1 - fat fraction.

3.0 Phantom construction

Appropriate amounts of fat and dried muscle tissue were weighed out. The desired amount of water was then added to rehydrate the tissue. This total soft tissue mass was then stored

in a polyethlene container (14 cm x 14 cm x 14 cm) and sealed. A radiation dose of 2-3 MRads was delivered to each phantom for sterilization to allow reuse for up to one year. A small pin-hole was made in the tops of each phantom to allow the escape of any gaseous products.