The distribution of body fat in man

THE DISTRIBUTION OF BODY FAT IN MAN

by

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A Master's Thesis

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ABSTRACT

This thesis is concerned with the calculation of the proportion of fat situated subcutaneously (PFSS) in man.

Chapter I describes some of the work that has been carried out in which the PFSS has been determined, and attempts to show that the variation in values of the PFSS found could be due not only to biological variation, but to the techniques and methods used in its calculation.The calculation of the PFSS for an individual requires knowledge of total body fat mass, and subcutaneous fat mass (SFM) or internal fat mass (IFM). In this thesis the SFM is calculated using a dimensional equation formulated by Skerlj, Brozek and Hunt (1953). This equation requires that the mean subcutaneous adipose tissue thickness of the body be known, as well as body surface area. A number of suggestions are made which might improve the accuracy of the SFM calculation using this equation. These include the use of ultrasound to measure the thickness of subcutaneous adipose tissue at body sites which are representative of, or can be related to, the mean thickness of subcutaneous adipose tissue over the entire body's surface. Also the accuracy of the equation of Dubois and Dubois (1916) for the estimation of body surface area is questioned, and a new equation for the estimation of body surface area is introduced.

Chapter II deals with the theoretical considerations of measuring subcutaneous adipose tissue thickness with ultrasound, and describes the ultrasonic equipment to be used in this study.

Chapter III contains a review of the literature relating to the measurement of subcutaneous adipose tissue thickness with ultrasound, and also presents the results of a validation of the ultrasonic technique using cadaverous material and human subjects. It is suggested that with appropriate care and expertise acceptable measures of subcutaneous adipose tissue thickness can be obtained at most body sites using ultrasound.

In Chapter IV are presented the results of a study where sixty ultrasound measurements of subcutaneous adipose tissue thickness had been taken on thirty male and female subjects aged eighteen to twenty-four years. It is suggested that the mean of the sixty measurements will be close to the true mean of subcutaneous adipose tissue thickness

over the entire body's surface. The mean of the sixty measurements was related to measurements of subcutaneous adipose tissue at a subset of four body sites, by multiple regression equations. For the males the four sites used were the chin, medial calf, abdomen and triceps, whilst in the females they were at the anterior thigh, posterior thigh, abdomen and triceps. The selection of these particular sites is discussed.

The PFSS was calculated in a group of forty-six male and female subjects aged eighteen to twenty-five years. The results are shown in Chapter V. The SFM value used in the calculation of the PFSS was derived using ultrasound, skinfold calipers and the multiple regression equations produced in Chapter IV to estimate mean subcutaneous adipose tissue thickness, whilst body surface area was assessed by two different equations. The PFSS values so produced varied, and many proved to be significantly different from each other. The implications of the large range of PFSS values found for different individuals are discussed in relation to the prediction of body density from measurements of subcutaneous adipose tissue. The poor relationship between subcutaneous fat mass and internal fat mass found in this work is also highlighted.

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CHAPTER I

Man has been concerned with the composition of the human body for many centuries. Since c. 440 B.C., when Hippocrates postulated his ideas of the four constituents of man, we have come a long way in our understanding of the composition of the body. Describing the body as two 'compartments' began in earnest when a number of workers produced equations relating the specific gravity or density of the body to the percentage of fat within it (Rathbun and Pace, 1945; Siri, 1956). The principle on which these equations are based was derived hundreds of years ago by Archimedes, namely, that if a body has two components, of different densities, the proportion of the components can be calculated from the density of the body. Using these and other equations a great deal of work has been done to assess the percentage of the body which is fat, and hence calculate body fat mass. Much of the early work involved U.S Navy personnel (Behnke, Feen and Welham, 1942; Morales, Rathbun, Smith and Pace, 1945). Subcutaneous 'fatness' has also been assessed for many years using skinfold calipers, and often the skinfold measurements have been related to total body fat measurements.

The first attempt to combine knowledge of total body fat mass and subcutaneous fatmass in order to investigate the distribution of fat between internal and external stores was carried out by Skerlj, Brozek and Hunt in 1953. These workers calculated the proportion of fat situated subcutaneously (PFSS) in a group of women subjects and found the mean value to be 0.264 for women in the age range eighteen to thirty years, 0.260 for the age range thirty-one to forty-five years, and 0.218 for the age range forty-six to sixty-seven years.

Since this pioneering work a number of studies have produced differing mean values for the PFSS ranging from 0.218 (Skerlj et al, 1953) to 0.670 (Jones, Bharadwaj, Bhatia and Malhotra, 1976).

The mechanisms, if they exist, by which internal and external fat stores are regulated are as yet unknown, and it is still to be established whether, even within a homogenous group, a constant relationship exists between the two fat stores. The distribution of body fat between the two stores, within a homogenous group, can be expected to be subject to biological variation, however, some of the large variations in the values of the PFSS may be attributable to the differences in experimental techniques adopted by different workers.

In order that the PFSS can be calculated for an individual, it is necessary to determine quantitative values for the total body fat mass and the subcutaneous fat mass or internal fat mass. All workers have measured total body fat mass from values of body density, obtained by either hydrostatic weighing or by volumetric displacement techniques. However, it is the varying methods of measuring the quantity of fat situated subcutaneously, and the conversion of body density values to body fat mass, that may have led to the varying estimates of the PFSS.

In the initial work by Skerlj et al (1953), the total fat mass was found from values of body density obtained using the equation of Rathbun and Pace (1945), and a dimensional equation for the calculation of the subcutaneous fat mass (SFM) was produced, which has been the basis of later calculations of SFM by other workers. The equation is shown in figure 1.1.

In the work of Skerlj et al (1953), the proportion of fat in adipose tissue was taken as 0.42 after the work of Mitchell, Hamilton, Steggerda and Bean (1945). The density of adipose tissue was used as opposed to the density of fat, and a value of 0.94×10^3 kg m^{-3} assigned to it. The body surface area was estimated from height and weight using the equation of Dubois (1936), and thus the problem remaining was to calculate mean subcutaneous adipose tissue thickness. This was achieved by finding a mean skinfold thickness based on a sample of ten body sites using skinfold calipers. This mean skinfold thickness was taken to be equivalent to a double layer of subcutaneous adipose tissue and two thicknesses of skin. This value was therefore divided by two and 1 mm subtracted from this; 1 mm being an estimation of skin thickness. Thus the remaining value was taken to represent the mean thickness of adipose tissue over the entire body.

In retrospect, the values obtained by Skerlj et al for the PFSS using this method of SFM calculation, seem low in comparison with later works (Allen, Peng, Chen, Huang, Chang and Fang, 1956; Jones et al, 1976; Brown and Jones. 1977; Marfell-Jones, 1977). There are, however, possible reasons for this low value of the PFSS to be found within the experimental techniques used by Skerlj et al (1953).

$$\text{Subcutaneous Fat Mass} = \text{Mean Subcutaneous Adipose Tissue Thickness} \times \text{Body Surface Area} \times \text{Proportion of Fat in Adipose Tissue} \times \text{Density of Fat}$$

Figure 1.1. The equation used by Skerlj et al (1953) to calculate subcutaneous fat mass.

Firstly, the compressibility of subcutaneous adipose tissue is well documented (Brozek and Kinsey, 1960; Jones,1970) and therefore the assumption by Skerlj et al that half the mean skinfold thickness represented a single layer of subcutaneous adipose tissue was inaccurate. This error would lead to an underestimation of mean subcutaneous adipose tissue thickness and hence SFM and PFSS.

Secondly, the figure chosen to represent the proportion of fat in adipose tissue, 0.42, was the result of a single cadaver analysis, and is considerably less than later estimates of about 0.8 obtained by Baker (1969) and Garrow (1974).

One must also consider whether the ten skinfold sites used truly represent the thickness of subcutaneous adipose tissue over the entire body's surface. Without taking many skinfold caliper measurements over the entire body it is difficult to assess the accuracy or validity of the mean thickness obtained using Skerlj's sites. However, it is probable that the choice of sites will produce differences in the mean thickness of subcutaneous adipose tissue calculated and hence differences in the PFSS.

Allen et al (1956) carried out experiments in Taiwan on eighty-seven men and women which led to the calculation of the proportion of adipose tissue situated subcutaneously (PASS), as distinct from the PFSS. Total body adipose mass was derived from body density measurements using an equation based on that of Rathbun and Pace (1945), but using different values for the densities of 'lean tissue' and 'fatty tissue'. The calculation of the mass of subcutaneous adipose tissue followed similar lines to that of Skerlj et al, being the product of mean subcutaneous adipose tissue thickness, body surface area, and the density of adipose tissue. Using these techniques Allen et al calculated that the PASS was between 0.2 and 0.6 depending on the total adiposity of the body. For the women the mean PASS can be calculated to be 0.47. If we accept the hypothesis that the composition of adipose tissue is a constant, in terms of the ratio of fat to water, this means that the PFSS for these data is also 0.47. The difference between this value and the value of 0.264 found by Skerlj et al for a similar age group, must be due to either biological variation or differing techniques. As the two sets of subjects have different ethnic backgrounds the probability of the difference being due to biological variation is enhanced. However, if the body density measurements of the Taiwan women are placed in the same equation as used by Skerlj et al, and the same values used to represent the proportion of fat in adipose tissue, and density of fat, are also applied to Allen's data the mean PFSS for the Taiwan group drops to 0.25, which is much closer to the value found by Skerlj et al (1953).

Nevertheless, the same limitations apply to the technique of Allen et al (1956) as to the work of Skerlj et al (1953). No account is taken of the compressibility of skinfolds, therefore, an underestimation of the PFSS will occur. The sites of measurement used by both sets of workers are identical, except for one site, and therefore the validity of the choice os sites is still questionable. Therefore, when the same techniques are applied to both sets of data comparable results are produced. However, it may be possible at this point to conclude that both the values derived for the PFSS are consistent but inaccurate.

Brown and Jones (1977) studied the PFSS in forty-two women, aged seventeen to twenty-four years, who had been selected to form three activity groups; very active, active and sedentary. There was no statistically significant difference in the PFSS found between the three activity groups, but the mean PFSS of all the subjects, at 0.653, was higher than the values found by both Skerlj et al (1953) and Allen et al (1956). The higher values reported in this study can be explained by the fact that Brown and Jones took into account the compression of skinfolds which occurs when measured with skinfold calipers. In order to arrive at a value for a single layer of uncompressed subcutaneous adipose tissue, the skinfold caliper measurements were adjusted using correction factors derived from a comparison between soft-tissue radiographic and skinfold caliper measurements of subcutaneous adipose tissue (Lee and Ng, 1965; Jones, 1970). Following the dimensional approach as previous workers, the mean subcutaneous adipose tissue thickness was found from eleven body sites and this multiplied by body surface area as assessed from the equation of Dubois and Dubois (1916) and by values to represent the density of fat and the proportion of fat in adipose tissue. The latter value was taken as 0.8 after the work of Baker (1969) and Garrow (1974), and the former as 0.9×10^3 kg m^{-3} after the work of Fidanza, Keys and Anderson (1953). The mean PFSS values for all the female subjects so far discussed are shown in table 1.1.

If the skinfold values obtained by Brown and Jones (1977) had been simply halved, as was done by Allen et al (1956) and Skerlj et al (1953), the mean PFSS value decreases from 0.653 to 0.460. This value is then very similar to the figure of 0.470 derived from the data of Allen et al (1956). If the value for the proportion of fat in adipose tissue, as used by Skerlj et al (1953) is applied to the data of Brown and Jones (1977) the mean PFSS decreases further to 0.240. This value is then

WORKERS AND TOTAL NUMBER IN GROUP (N)	SUBGROUPS AND NUMBER IN GROUP (N)	PFSS	
		MEAN	STANDARD DEVIATION
SKERLJ ET AL (1953) N=62	AGE RANGE 18-30 YEARS N=23	0.264	-
	AGE RANGE 32-45 YEARS N=19	0.260	-
	AGE RANGE 46-67 YEARS N=20	0.218	-
	TOTAL GROUP	0.248	-
ALLEN ET AL (1956) N=26	-	0.470	-
BROWN AND JONES (1977) N=42	VERY PHYSICALLY ACTIVE N=14	0.674	0.106
	PHYSICALLY ACTIVE N=14	0.648	0.105
	SEDENTARY N=14	0.636	0.129
	TOTAL GROUP	0.653	0.113

TABLE 1.1. THE MEAN AND STANDARD DEVIATIONS FOR SOME PFSS VALUES FOR FEMALES.

very similar to the mean PFSS found by Skerlj et al (1953).

Jones et al (1976) calculated the PFSS in a group of one hundred and twenty Indian subjects. The subjects consisted of four groups: Gurkhas, Rajputs and South Indians - all being servicemen, and a group of civilians from the Utter Pradesh region. Within these groups the mean PFSS varied from 0.57 in the Gurkhas to 0.67 in the civilian group. These values are much higher than the mean value for the male subjects in Allen's study, which can be calculated as being 0.259.

The work of Brown and Jones (1977) was repeated in 1977 on men by Marfell-Jones. The only difference in technique between the two studies were that Marfell-Jones included a triceps skinfold measurement in the calculation of mean subcutaneous adipose tissue thickness, and that different correction factors were used to adjust the skinfold caliper measurements to a single layer of adipose tissue. This work produced a mean value for the PFSS of 0.52 for the entire group of fifty-one subjects, with varying but not significantly different values for the very active, active and sedentary groups of 0.590, 0.500 and 0.480 respectively. These values, however, are significantly lower than the values found for the equivalent groups in the study of females of Brown and Jones (1977). This finding contradicts the statements of Durnin and Womersley (1974) and Forbes and Amirhakimi (1970) that males carry a higher proportion of their body fat subcutaneously than do females.

The mean values for all the male subjects so far discussed are shown in table 1.2.

It can be seen from tables 1.1 and 1.2 that the values for the PFSS have varied between workers, and that, from a number of examples cited in the text, that the differences can be partially reduced by applying consistent techniques to the data. However, even when techniques have been relatively similar, such as with Brown and Jones (1977) and Marfell-Jones (1977), there are improvements which could be made to the calculation of the SFM, and hence PFSS.

A number of workers have pointed to the errors that could be produced in the PFSS calculation due to the compressibility of skinfolds, and the choice of sites on the body at which to measure subcutaneous adipose tissue thickness in order to obtain a representative value for the mean

WORKERS AND TOTAL NUMBER IN GROUP (N)	SUBGROUPS AND NUMBER IN GROUP (N)	PFSS	
		MEAN	STANDARD DEVIATION
ALLEN ET AL (1956) N=57	-	0.259	-
JONES ET AL (1976) N=120	GURKHAS N=30	0.571	-
	RAJPUTS N=30	0.592	-
	S.INDIANS N=30	0.607	-
	UTTAR PRADESH N=30	0.670	-
	TOTAL GROUP	0.610	-
MARFELL-JONES (1977) N=51	VERY PHYSICALLY ACTIVE N=14	0.598	0.172
	PHYSICALLY ACTIVE N=19	0.500	0.148
	SEDENTARY N=18	0.483	0.137
	TOTAL GROUP	0.521	0.151

TABLE 1.2. THE MEAN AND STANDARD DEVIATIONS FOR SOME PFSS VALUES FOR MALES

subcutaneous adipose tissue thickness over the entire body's surface. The correction factors employed by Brown and Jones (1977) to convert skinfold caliper measurements to a single layer of adipose tissue are obviously better than the assumption that half a skinfold caliper measurement is a single layer of adipose tissue as used by many workers, as the later method does not take account of skinfold compressibility. However, the need for population specific correction factors, as suggested by Jones et al (1976), limits the use of these correction factors to groups on whom a comparison between skinfold calipers measurements and direct measurements of adipose tissue thickness has been completed. As skinfold compressibility is believed to vary with age, sex, physical activity, ethnic background and body site, the number of different correction factors becomes large and restrictive. There are other ways in which the thickness of subcutaneous adipose tissue can be measured directly, however many of these, for example, soft-tissue radiography, needle puncture and electrical conductivity, are unacceptable for widespread use because of ethical and practical considerations. An alternative, which is non-hazardous, socially and ethically acceptable, and which could offer a practical and viable method of assessing subcutaneous adipose tissue thickness, is ultrasound.

If it were known at which body sites to measure subcutaneous adipose tissue thickness to obtain a true representation of the mean thickness over the entire body's surface, using these sites would be preferable to using sites chosen for alternative reasons, such as ' ease of location ' (Skerlj et al , 1953). Many of the sites used in previous studies were chosen on the basis that they were in areas of known adipose tissue deposition, as opposed to representing the true mean, and it is therefore probable that an overestimation of mean adipose tissue thickness occurs on this basis.

It has been suggested (Brown, 1976) that the equation of Dubois (1916) to estimate body surface area consistently underestimates the true body surface area, and recent work (Wilkinson, Jones and Davies, 1982) confirmed this belief. These workers produced a new equation based on body weight and the circumference of the calf immediately below the knee. Underestimation of the body surface area will reduce SFM and PFSS values.

This thesis describes attempts to improve on the calculation of the SFM and hence PFSS by :

1.) Using ultrasound to measure the thickness of subcutaneous adipose tissue directly, and hence remove the necessity for population specific correction factors in conjunction with skinfold caliper measurements.

2) Determining which body sites best represent, or can be related to, the mean thickness of adipose tissue over the entire body surface.

Once this has been done, the PFSS can be calculated using the ultrasonic technique at the appropriate sites, and the values so obtained compared with values in the literature, and also with values obtained using a different assessment of body surface area, and values found using skinfold caliper measurements of subcutaneous adipose tissue.

CHAPTER II

The use of ultrasound in the measurement of subcutaneous adipose thickness.

Theoretical Considerations.

Ultrasound consists of sound waves, whose frequency are above the upper limit of human hearing. This parameter is obviously variable but generally speaking ultrasound has a frequency greater than 20,000 Hz.

Although ultrasonic waves can be produced in a number of ways, the method of production used in this study, and most other studies measuring subcutaneous adipose tissue thickness, involves the production of ultrasound by the piezo-electric effect.

The basic physical principle of the piezo-electric effect was described over a hundred years ago by Jacques and Pierre Curie. They described the phenomenon that certain materials possess the property that when they are exposed to an electric field a change occurs in the physical dimensions of the material. If a piezo-electric material is placed in an alternating electric field, in the correct orientation, the material will alternatively expand and contract, producing longitudinal ultrasonic waves in the surrounding medium. These ultrasonic waves can then be directed into a coherent beam, which may, if required, be focused. The piezo-electric material can be conveniently housed in a small probe which is called a transducer.

Piezo-electric materials also have the reciprocal property that if high frequency sound waves strike the transducer, a voltage is produced by the material. This can be amplified and the voltage displayed on a cathode ray oscilloscope (CRO). This is termed A-mode ultrasound, the 'A' being an abbreviation for amplitude, as the detection of ultrasound by the transducer is shown as either an increase or decrease in amplitude of an electronic trace on a CRO, the direction of amplitude displacement is simply dependent on the electronics of the ultrasound equipment. A typical display produced on a CRO from a reflecting surface is shown in figure 2.1. This display can be explained as follows - at the same instant as the pulse of ultrasound is produced by the transducer, a spot on the screen of the cathode ray tube begins to move from left to right at a constant velocity. This spot will trace out a horizontal line on the screen. After a very short period of time the ultrasonic wave strikes the reflecting surface, and a proportion of the ultrasonic energy is reflected back towards the transducer. When this reflected energy strikes the transducer a voltage is induced which is then amplified and displayed as the peak, 'B', as shown in figure 2.1. The initial peak, 'A', is produced by the piezo-electric material itself and is often called the 'main bang'. If the process is repeated with enough rapidity, that is, more than about twenty times a second, a steady trace is observed on the CRO. The distance between the peaks 'A' and 'B' on the CRO is related to the speed of sound in the surrounding medium, in this case water, the sweep speed of the CRO, and the distance between the transducer and the reflecting surface. As the speed of sound in water is well documented, and the sweep speed of the CRO is known, it is therefore possible to calculate the distance between the transducer and the target from the trace on the CRO.

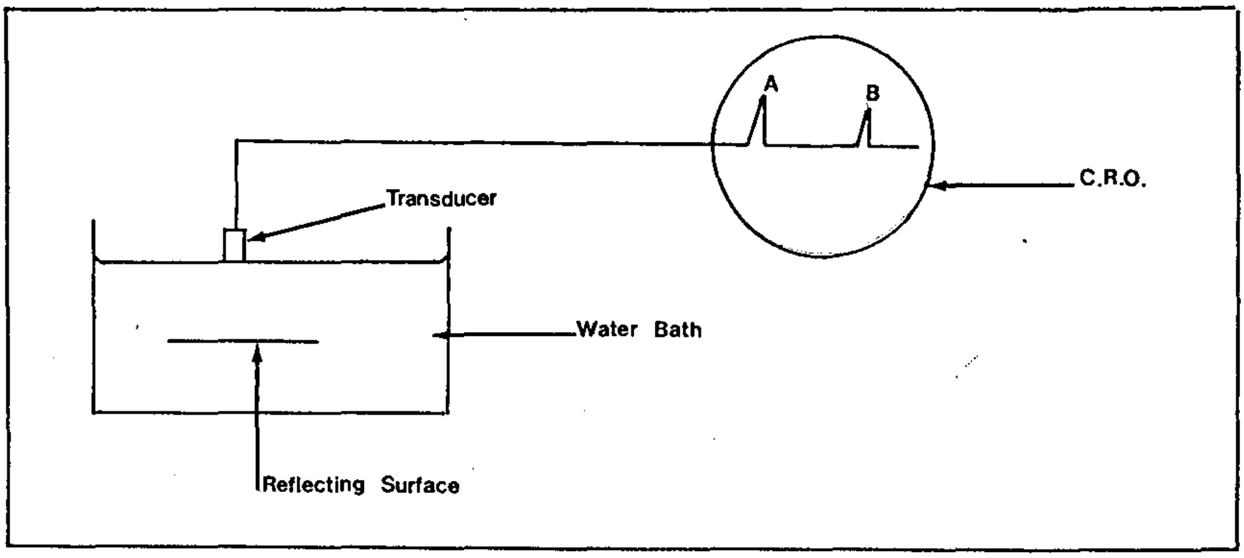


Figure 2.1. A typical A-mode display of a single reflecting surface.

It is now possible to consider the way in which A-mode ultrasound can be used in the assessment of subcutaneous adipose tissue thickness. Figure 2.2 shows a diagramatic section of subcutaneous adipose tissue and its surrounding tissues.

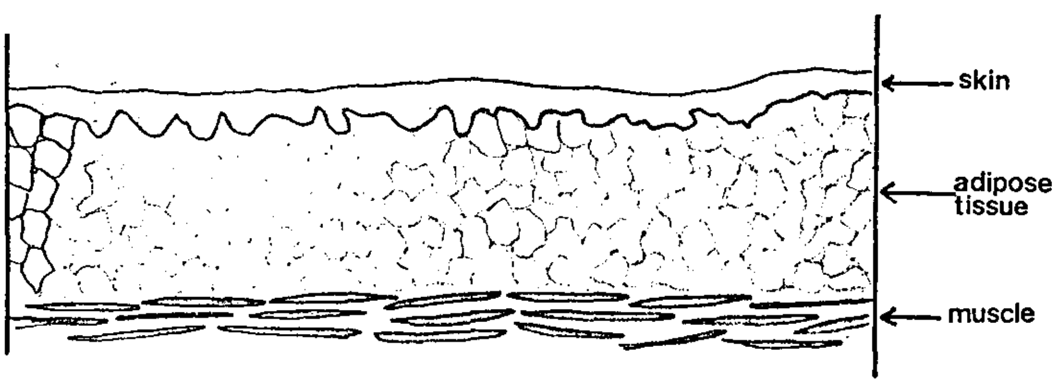


Figure 2.2. A diagramatic section of subcutaneous adipose tissue.

If a transducer is placed on the skin surface one would imagine that peaks would be displayed on the CRO corresponding to the reflective skin-adipose tissue and adipose tissue-muscle interfaces. In practice, as the mean thickness of human skin is about 1.7 mm, (Brown and Jones, 1977) the skin-adipose tissue peak is often lost within the main bang. Visualisation of an ultrasonic echo from the adipose tissue-muscle interface is initially dependent on the reflectiveness of the interface. The reflectiveness of this particular biological boundary is only nineteen decibels below that of a perfect reflector, thus no immediate problems should be encountered. In the theoretical considerations of the use of ultrasound, it is assumed that the reflective boundary is flat, and that the ultrasonic beam strikes the interface at an angle of incidence of 0 degrees. If the angle of incidence is not 0 degrees the amplitude of the returning ultrasonic echo decreases, and this reduction of the amplitude may make the visualisation of the adipose tissue-muscle interface echo more difficult. Figure 2.3 shows the relationship between echo amplitude and the angle of incidence for a flat target, in water, at two different transducer-target distances.

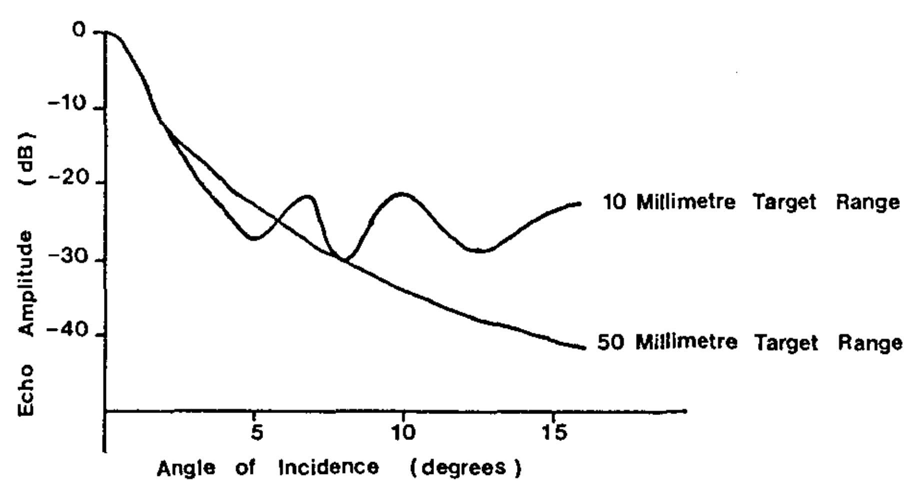


Figure 2.3. The relationship between echo amplitude and angle of incidence for a flat target in water at two ranges. Each curve is relative to 0 dB at 0° incidence. Frequency 1.7 MHz, transducer diameter 2 cm. Reproduced from 'Physical Principles of Ultrasonic Diagnosis' by kind permission of P.N.T. Wells.

Also, it is necessary to consider the fact that the amplitude of the returning ultrasonic echo changes with the range of the relecting surface. Figure 2.4 shows the relationship between echo amplitude and target range for a flat target in water at normal incidence.

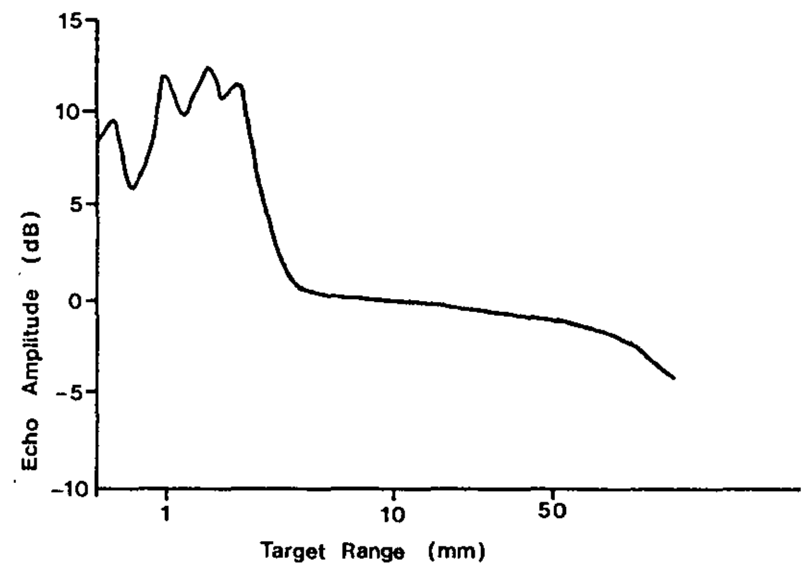


Figure 2.4. The relationship between echo amplitude and range, for a flat target in water at 0° incidence. Levels relative to 0 dB at 1 cm range. Frequency 1.7 MHz, transducer diameter 2 cm. Reproduced from 'Physical Principles of Ultrasound Diagnosis' by kind permission of P.N.T. Wells.

The production of multiple echoes could also cause difficulty in the identification of the adipose tissue-muscle interface. These extra echoes are referred to as multiple reflection artifacts and their existence can be explained as follows - consider again figure 2.2 in which a transducer has been placed on the skin surface and ultrasound is directed through the tissues. The basis of the system is that differences in tissue impedence cause reflections which can be detected by the transducer. As the pulse of ultrasound that has been reflected by the adipose tissue-muscle interface travels back towards the transducer it will strike the adipose tissue-skin interface. The majority of the sound energy passes through the skin, and is detected by the transducer, but a portion is reflected away from the adipose tissue-skin interface and so acts as a second pulse of ultrasound which will be reflected again at the adipose tissue-muscle interface. Thus in due time a second echo returns to the transducer and although its amplitude is reduced it can cause a peak to be displayed on the CRO. By the same token, more artifacts may appear until the multiple reflection echoes fall below the threshold level of the ultrasonic equipment. Similar artifacts may be produced by reflection at the skin-transducer interface, hence it is possible that a whole range of ' false ' echoes may be visualised on the CRO.

Thus, when attempting to use ultrasound to measure subcutaneous adipose tissue thickness care should be taken in the manipulation of the transducer, so as to minimise the angle of incidence the ultrasonic wave subtends to the adipose tissue-muscle interface and so reduce the returning signal amplitude loss. A certain degree of knowledge of the anatomy of the tissue structure underlying the site of measurement is therefore desirable. The loss of returning signal amplitude due to target range will be minimal in measuring subcutaneous adipose tissue thickness. Signal amplitude loss occurs significantly when target range is greater than 5 cm, as can be seen in figure 2.4. As it is likely that the majority of adipose tissue thicknesses encountered in a normal population will be less than this value, no real problem should arise due to returning signal amplitude loss unless very obese individuals are to be measured.

Multiple reflection artifacts could feasibly cause a significant problem in the identification of the adipose tissue-muscle interface. However, electronic damping of the returning ultrasonic echo should aid in the reduction of multiple reflection artifact amplitudes below the threshold of detection of the ultrasonic equipment, and so eliminate many of the ' false ' echoes that could be visualised as the adipose tissue-muscle interface

Ultrasonic equipment used in this study.

The ultrasonic equipment used in this study was an Ekoline 20A diagnostic ultrasonoscope, manufactured by Smith Kline Instruments Inc. This piece of apparatus is specifically designed for medical use. It can operate transducers at frequencies ranging from 1.75 MHz to 11 MHz. The transducer used in this study operated at 5 MHz and was unfocused. The Ekoline 20A has an effective tissue depth range of 35cm, and according to in vitro tests, can detect objects as small as 0.01 mm. The ultrasonic echoes are displayed on a CRO which has electronic markers at 2 mm intervals. The equipment is capable of receiving and displaying signals from structures only a few millimetres from the face of the transducer which would normally be lost in the main bang due to special damping circuits. The Ekoline 20A is electronically coupled to an Ekoline 21 video strip chart recorder. This apparatus uses a fibre optic cathode ray tube (FO CRT) to record ultrasonic signals onto Kodak 2295 direct print paper. The paper is marked by the FO CRT at 1 cm intervals, so enabling the distance between the main bang and tissue interface echo to be calculated from the paper trace. The basis of the system is therefore to identify the adipose tissue-muscle interface on the CRO and then record a hard copy of the trace onto the direct print paper, which can then be read at a later time. The ultrasonic equipment used in this study is shown in figures 2.5 and 2.6.

Throughout this work when the thickness of subcutaneous adipose tissue thickness is measured ultrasonically in effect the measurement is one of subcutaneous adipose tissue and skin thickness. To obtain a true measure of the thickness of subcutaneous adipose tissue the thickness of the skin must be measured or estimated. This is discussed in more detail later in the text.

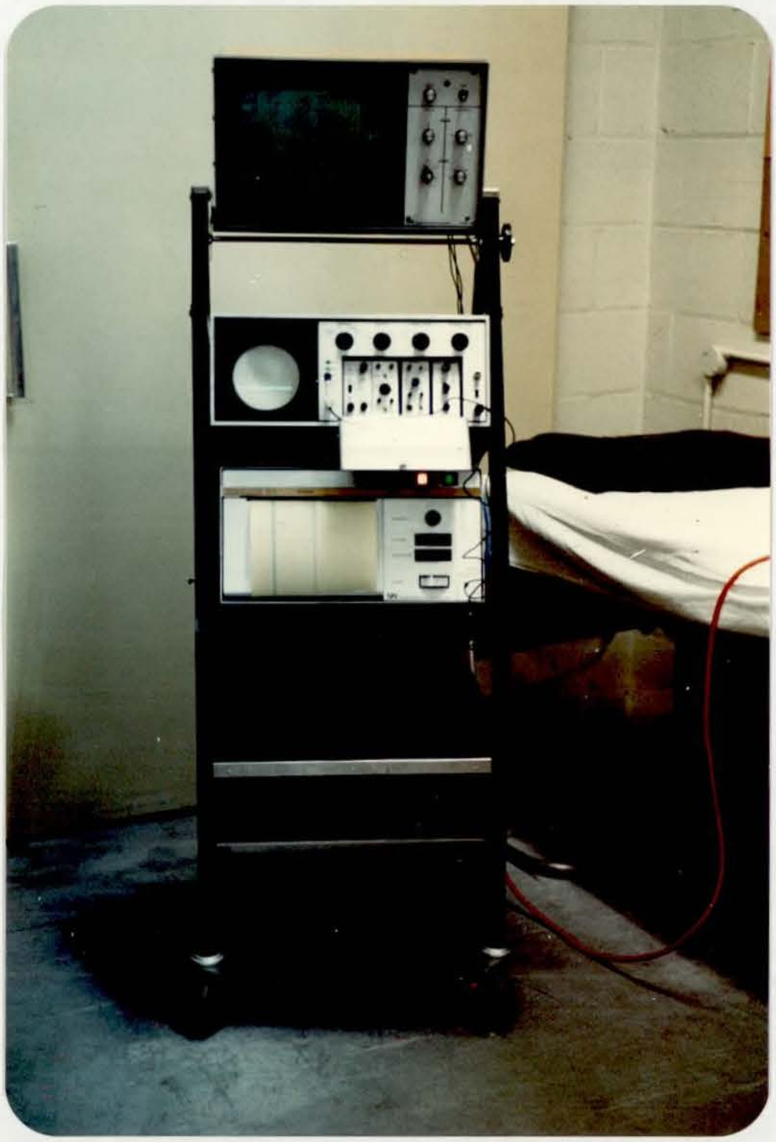


Figure 2.5. The Ekoline 20A ultrasonoscope used in this study, with the Ekoline 20A video strip chart recorder shown beneath.

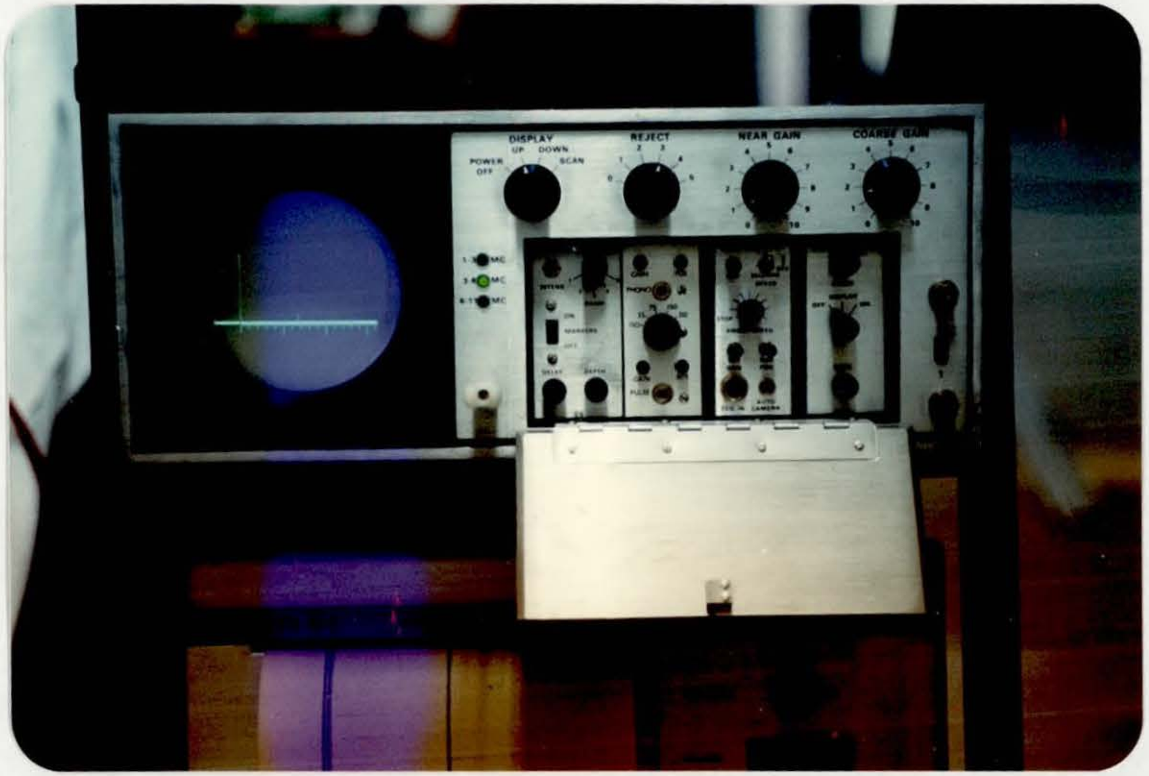


Figure 2.6. The Ekoline 20A ultrasonoscope.

CHAPTER III

INTRODUCTION

Ultrasound has been used in the meat and livestock industry for carcass evaluation for over twenty-five years (Temple, Stonaker, Howry, Posakony and Hazaleus, 1956), and although the ultrasonic technique had been used for diagnostic investigation in medicine in the 1950s (Wild and Neal, 1951; Donald, MacVicar and Brown, 1958; Brown, 1959; Jefferson, 1959), it was Whittingham in 1962 who first considered in detail the use of ultrasound for measuring subcutaneous adipose tissue thickness in humans. Whittingham investigated the technique in order to assess body composition in RAF aircrew by a method which was simple and did not "interfere unduly with a man's activity or composure."

Many of the theoretical and technical aspects were reviewed by this worker, and indeed, Whittingham drew attention to the inaccuracies that could be induced by compression of the subcutaneous adipose layer by the transducer probe, this being an extremely important consideration in the technique. A number of ways of overcoming this problem were suggested and a method involving coupling the probe with olive oil to a block of perspex, which was in turn placed on the site of measurement, was recommended for its simplicity and convenience. Whittingham also refers to preliminary work carried out in his investigation where ultrasonic measurements of subcutaneous adipose tissue were compared with direct measurements, using a ruler, on patients before surgery. Six subjects were used in this part of his study, and the results were referred to as "encouraging", although further details were not reported. However, Whittingham points to the work of Lauprecht, Scheper and Schroder (1957) who compared ultrasonic with direct measurements in slaughtered pigs. These workers concluded that the accuracy of the ultrasonic measurement was entirely adequate for pig breeding and economic purposes.

Bullen, Quaade, Olesen and Lund (1965) indicated the possible advantages that the ultrasonic technique could have compared to subcutaneous adipose tissue thicknesses as assessed by skinfold calipers. They indicated the problems of variation in skinfold compressibility (Brozek and Kinsey, 1960), and the difficulty in measuring skinfolds on obese individuals. These workers compared skinfold thicknesses, as measured with Lange skinfold calipers, with ultrasonic measurements in one hundred subjects at two bodily sites. The correlation coefficients between the two techniques at both sites were high, for the triceps site $r = 0.8$ ($p < .001$) for both males and females, whilst for the abdomen site the male sample yielded a correlation coefficient of 0.9 ($p < .001$), and for females $r = 0.85$ ($p < .001$).

Moreover, Bullen et al, carried out a more direct assessment of the ultrasonic technique's ability to measure subcutaneous adipose tissue thickness. Ultrasonic measures of adipose tissue thickness were compared with measurements obtained using the method of direct needle puncture on thirteen subjects. The correlation coefficient between these two techniques was again high ($r = 0.98$ $p < .001$). The exact nature of the relationship is not expressed, but from an illustration cited in the publication, the relationship seems close to the 1:1 ratio which would be expected.

After comparing the ability of Harpenden skinfold calipers, electrical conductivity and ultrasound to measure subcutaneous adipose tissue thickness, Booth, Goddard and Paton (1966), concluded that the ultrasonic technique was the more accurate. However, they were also the first to stress the importance of operator experience in the manipulation of the transducer and in the interpretation of the echoes obtained. Thus those workers stated that in the hands of uninitiated observers extremely inaccurate results could be produced.

Correlation coefficients between the three techniques tested by Booth et al were all high, ranging from 0.81 to 0.98, however, again, more detailed statistical descriptions of the relationships between the techniques were not reported.

As part of the procedure used for the in vivo estimation of plutonium-239 and americum-241 in the lungs of workers using these radioactive isotopes, Ramsden, Peabody and Spreight (1967) found it necessary to evaluate the thickness of soft-tissue on the chest. To achieve this, an ultrasonic technique based on that of Whittingham (1962) was used. Once again it is noted in the report by these workers that "a certain degree of expertise" was required before the measurement of soft tissue thickness could be achieved. The occurrence of extraneous ultrasonic reflections were reported, which made the tissue thickness more difficult to assess accurately, especially when there was an appreciable thickness of soft-tissue.

Extraneous ultrasonic echoes could possibly explain the large differences in mean values of fat thickness as measured by ultrasound and soft-tissue radiography over the greater trochanter, as reported by Hawes, Albert, Healey and Garrow (1972). These workers concluded that the difference in means of 7.3mm between the two techniques in thirty-two subjects is probably due to the inclusion of a muscle layer in the ultrasonically assessed tissue thickness. Such a large discrepancy did not occur, however, over the iliac crest, with a difference of only 1mm in mean values. Even taking into account these discrepancies Hawes et al concluded that ultrasound provided a simple and acceptable method for measuring fat thickness over bony points, with an accuracy comparable to that of a soft-tissue radiograph but without the associated radiation hazard.

From data given by Wells (1969) the reflectivity of a fat-bone interface can be calculated to be 3.2 decibels below that of a perfect reflector. Whereas, as previously mentioned, the same value for a fat-muscle interface is 20 decibels (Wells 1969). These differences in reflectivity at the different boundaries could explain the difference in the mean values of the two techniques obtained at the sites studied, because these workers indicated that a small muscle layer overlies the greater trochanter and at this site the dominant ultrasonic echo would be that of the muscle-bone interface. Indeed, the lateral surface of the iliac crest is free of muscle attachment or overlying muscle tissue, while the greater trochanter would be covered by the distal portion of the gluteus medius (Warwick and Williams 1973). However, as the fat boundary encountered over most of the body's surface would be one with underlying muscular tissue, this work casts doubt on the ability of ultrasound to measure accurately the depth of the fat-muscle interface, and hence the subcutaneous adipose tissue thickness.

The work of Haymes, Lundergren, Loomis and Buskirk (1976) also showed large discrepancies in subcutaneous adipose tissue measurements using ultrasound and soft-tissue radiography. These workers compared measurements of two bodily sites, at the midtriceps and suprailiac, on twelve male and six female subjects. Consistent with the work of Hawes et al (1972) the correlation coefficients between the two techniques were high with $r = 0.88$ ($p<.001$) at the midtriceps site and $r = 0.78$ ($p<.001$) at the suprailiac site. However, these workers also expressed the intercept and gradient of the least squares line for the ultrasonic and radiographic measurements. If we accept that the two techniques are measuring the same parameter, i.e., the thickness of subcutaneous adipose tissue, the ideal relationship between them is one described by the regression equation:

$$Y = 0 + X$$

where Y is the ultrasonic estimation of adipose tissue thickness, and X is the radiographic measure. In fact the regression equation differed from this ideal relationship, being

$Y = 1.44 + 0.7X$ at the midtriceps site, and

$Y = 5.67 + 0.4X$ at the suprailiac site.

For example, radiographically determined thickness of 20mm would produce an error in the measurement of subcutaneous adipose tissue of 4.6mm at the midtriceps site and 6.3mm at the suprailiac site using these relationships.

The differences in the measurements obtained by the two techniques might be partly explained if the application of the transducer on the skin's surface caused local indentation, and hence compression of the subcutaneous adipose tissue. The use of a perspex block to avoid such local indentation as described by Whittingham (1962) was rejected by those workers as it was found that multiple extraneous echoes were produced by the passage of the ultrasonic waves through the perspex. Extraneous ultrasonic echoes produced by the adipose layer itself, however, are more likely to explain the poor relationship between the two techniques found by Haymes et al. Such echoes were reported, and indeed, a discrete interface in the adipose tissue layer at the waist site could be visualized in 72% of the subjects on the soft-tissue radiographs. These workers concluded by stating that "further work is needed in discriminating accurately the fat-muscle interface from the other discontinuities within the adipose deposits."

Sanchez and Jacobson (1978) described the use of ultrasound as a possible new anthropometric tool. These workers were evaluating a portable ultrasonic device, manufactured by Ithaco Inc., Ithaca, New York, to assess adipose tissue thickness. Their evaluation consisted of comparing the ultrasonic measurement obtained with this piece of equipment with direct measurements on cadaverous material. These workers endorsed the observations of Ramsden et al (1967) and Haymes et al (1976), that extraneous ultrasonic echoes could be visualized, and also the effect of transducer pressure in compressing of the adipose tissue layer as previously reported by Whittingham (1962) and Haymes et al (1976). Sanchez and Jacobson found that when subcutaneous adipose tissue thickness was greater than 10mm the direct measurements were within 2mm of the ultrasonic reading, however, below 10mm of adipose tissue thickness they state that greater differences occur, indicating an error of measurement in excess of 20%, in this range of tissue thickness. The same piece of ultrasonic apparatus as used by Sanchez and Jacobson was used by Balta, Ward and Tomkins (1981). Thirty-six measurements of adipose tissue thickness were made on thirteen patients (seven male and six female), who had undergone surgery, using ultrasound and a sterile steel ruler. An excellent correlation coefficient between the two techniques is reported, ($r = 0.99$, $p<.001$) with a difference of 1.4mm between the mean value of each technique. However, the inclusion of both males and females in the statistical calculation gives a bivariate distribution which will lead to a false high correlation coefficient. If males and females had been considered separately, the correlation coefficient would be probably much less impressive.

A number of workers (Booth et al 1966; Ramsden et al 1967), have stated that expertise and experience is required in using the ultrasonic technique to measure the thickness of subcutaneous adipose tissue. The difficulty in identifying the adipose tissue boundary due to extraneous ultrasonic echoes has also been highlighted (Booth et al 1966, Ramsden et al 1967, Haymes et al 1976). Considering these statements, in conjunction with the poor relationships between ultrasonic and radiographic measures found by Haymes et al and Hawes et al 1972, it was decided that an attempt to validate the ultrasonic technique against more direct methods of tissue thickness should be a prerequisite to any work involving the ultrasonic measurement of adipose tissue thickness.

If the production of extraneous ultrasonic echoes from within the adipose tissue layer is to be a major problem in the measurement of tissue thickness using this technique, it could be valuable to be able to identify the structure or structures within the adipose tissue responsible for these echoes. For this reason the first part of this investigation, involved the measurement of adipose tissue thickness in cadaverous material, from which the adipose tissue could be dissected and ultrasonic echoes produced by the tissue, matched to interfaces within it.

METHOD

Twenty-four sites of measurement were marked on a female cadaver. The cadaver weighed 83 kg after embalming with 12 - 15 litres of fluid. The cause of death had been left ventricular failure at the age of seventy-eight years. The thickness of subcutaneous adipose tissue at the sites were then measured ultrasonically to the nearest 0.1 millimetre. Once a permanent copy of the ultrasonic echoes obtained at a particular site had been recorded, the site was dissected down to the muscle interface, and the thickness of adipose tissue measured using a depth gauge, accurate to 0.1 millimetre. At ten sites two ultrasonic and depth gauge measurements were taken, a number of hours apart, so that the standard error of measurement for the two techniques could be expressed.

RESULTS

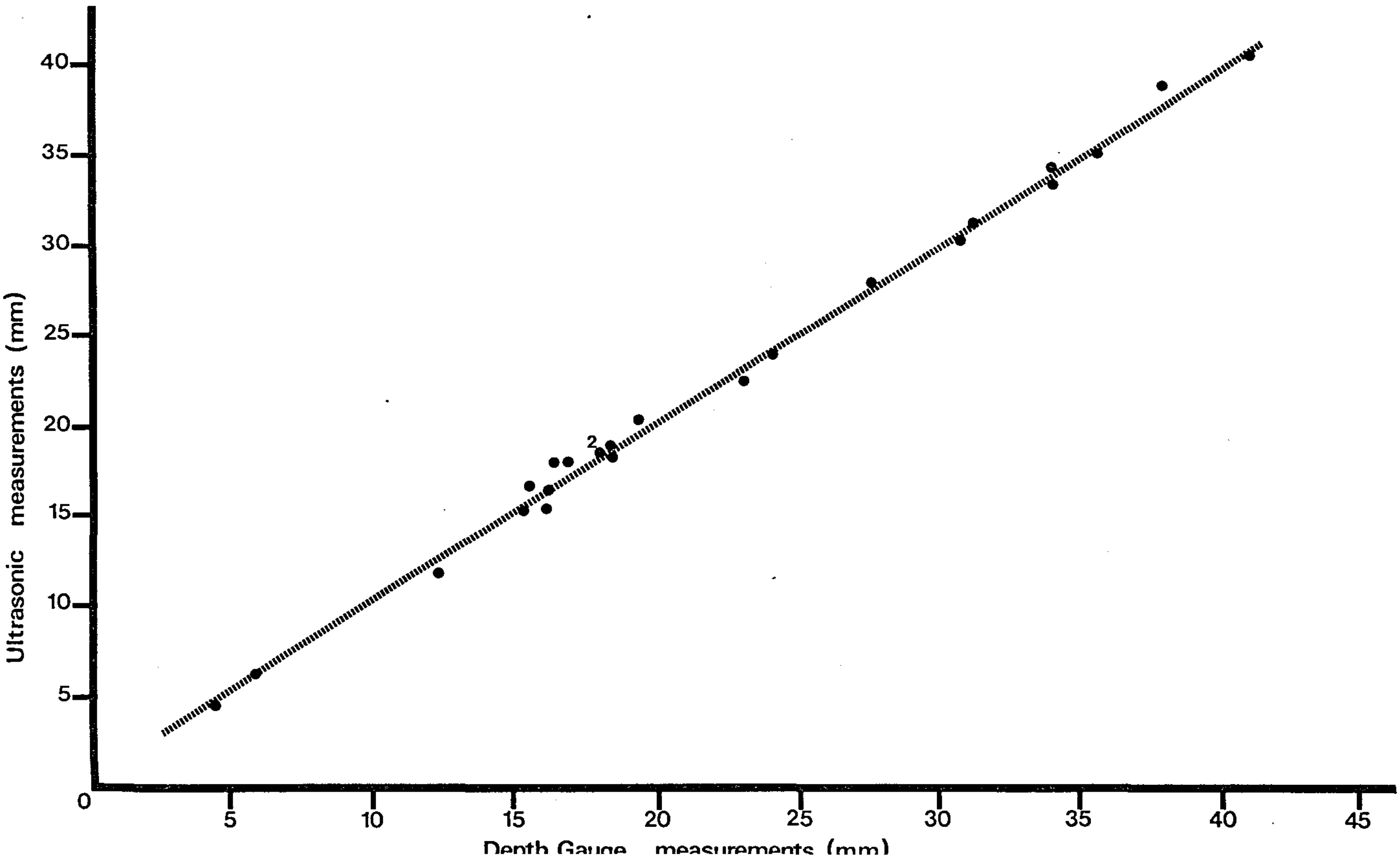
The ultrasonic and depth gauge measurements are shown graphically in figure 3.1, along with the least squares line for the data. The means, standard deviations and ranges of the data are shown in table 3.1.

Technique	Mean (mm)	Standard Deviation (mm)	Range (mm)
Ultrasound	22.3	9.8	4.5 40.8
Depth Gauge	22.3	9.9	4.4 41.2

Table 3.1. The means, standard deviations and ranges of ultrasonic and depth gauge measurements on cadaverous tissue

The repeated ultrasonic and depth gauge measurements allows the calculation of the within observer reliability of the two measurement techniques. This is defined statistically as the standard error of measurement (S. Meas.), and this is obtained by calculating the standard deviation of the differences between the two measurements and dividing this value by the square root of two. Ninety-five percent. of the measurements will then be within two S. Meas. of the true but unknown value. When this calculation was applied to the repeated measurements in this study the S. Meas. for the ultrasonic technique was found to be 0.18 millimetres, and for the depth gauge technique 0.30 millimetres.

Figure 3·1 THE REGRESSION LINE FOR THE ULTRASONIC AND DEPTH GAUGE MEASUREMENTS



The correlation coefficient between the two techniques was high ($r = 0.998$, $p < .001$). Perhaps the most meaningful statistical analysis that can be performed on these data is to express the intercept and gradient of the regression line fitted to the data points. As has been previously mentioned, the ideal relationship is described by the equation $Y = 0 + X$, that is, a line of identity. The relationship found for these data are shown in table 3.2.

Intercept (a)	Regression Coefficient (b)	Standard Error of Estimate (mm)
0.42	0.98	0.65

Table 3.2. The intercept, regression coefficient and standard error of estimate of the regression line for the ultrasound and depth gauge measurements.

The appropriate statistical test (Moore and Edwards, 1965), was applied to the data to determine whether the intercept or regression coefficient of the least squares line differed significantly from the ideal values. The results of this test are shown in table 3.3.

	Ideal	Calculated	t-value	significance
Intercept	0	0.42	1.279	n.s.
Regression Coefficient	1	0.98	-1.175	n.s.

n.s. = not significant

Table 3.3. The t-value and significance of the intercept and regression coefficient of the regression line for ultrasonic and depth gauge measurements when tested from 0 and 1 respectively

DISCUSSION

It can be seen from table 3.3 that the least squares line for the data does not differ significantly from a line of identity, thus indicating that the ultrasonic technique can give similar results to depth gauge measurements of subcutaneous adipose tissue over the range of thicknesses used in this study.

Extraneous ultrasonic echoes were recorded in some cases, and on examination of the adipose tissue these echoes could be matched with interfaces within it. In the majority of cases the echoes were produced from connective tissue running through the adipose tissue. These echoes, however, could be successfully removed by electronic 'damping' of the returning ultrasonic echoes which allowed only the strongest echoes to be recorded. It was noted, however, that at many of the sites of measurement, the adipose tissue had become slightly separated from the underlying muscle tissue. This phenomenon, which is probably due to post mortem changes or tissue shrinkage after embalming, produces a small air space between the adipose tissue and the muscle layer. Thus the adipose tissue boundary at a number of sites was one with air. The reflectivity of such an interface is virtually perfect, leading to returning ultrasonic echoes of large amplitude, and thus enhancing the identification of the adipose tissue boundary. It was therefore decided that a natural corollary to this study would be to investigate the ability of ultrasound to measure subcutaneous adipose tissue thickness in vivo.

Nineteen males and twenty-one females aged eighteen to fifty-three served as subjects. Some of the subjects' physical characteristics are shown in table 3.4.

	MALES		FEMALES	
	Mean	Standard Deviation	Mean	Standard Deviation
Age (a)	24.56	9.74	20.71	1.26
Weight (kg)	69.45	5.20	59.85	6.25
Height (m)	1.758	0.070	1.647	0.065

Table 3.4. Some of the subjects physical characteristics.

They had their left leg marked with dermographic pencil at four anatomically defined sites, as shown in table 3.5.

SITE	LOCATION
Anterior Thigh	In the anterior midline at one third subischial (stature minus sitting height) measured up from the lower border of the femoral condyles.
Posterior Thigh	In the posterior midline at the same level as the anterior thigh.
Lateral Calf	At the level of maximum calf circumference in line with the lateral head of the fibula and the lateral malleolus of the tibia.
Medial Calf	At the same level as the lateral calf in line with the medial condyle and medial malleolus of the tibia.

Table 3.5. Sites at which marks were made on the skin.

Small lead markers were attached at the four sites with adhesive tape and then the leg radiographed in two stages. The left calf was positioned anteroposteriorly with the body weight distributed evenly over both feet. The foot was placed so that the coronal plane through the malleoli was parallel to the cassette, and the central vertical axis (object - film distance) at the maximum calf circumference was 100 mm. The left thigh was radiographed with the subject standing astride the cassette stand, with the top edge of the cassette placed as high as possible into the groin. The foot was positioned so that the posterior borders of the femoral condyles were superimposed. The central vertical axis of the thigh at the one third subischial level, as measured up from the distal borders of the left femoral condyles, was positioned at 100 mm from the cassette face.

The positioning of both the calf and the thigh at an object - film distance of 100 mm, in conjunction with a constant anode film distance of 2 m, ensured a constant object enlargement factor, which took into account variation in limb size (Tanner, 1962). This would not be the case if the limb was situated in contact with the cassette.

As a safety precaution all subjects wore gonad protectors (Jones, 1971). The KV and mAs values used in the study varied according to a subjective assessment of limb size, and were in the ranges shown in table 3.6.

Site	KV	mAs
Thigh	66-78	23-28
Calf	62-70	21-23

Table 3.6 - Radiographic exposure factors used in the study.

The radiographs were processed using a forced development technique which involves raising the temperature of the developer (Kodak DX-80) to 22°C and developing by inspection for three to four minutes, and subsequently fixing in Kodak FX-40 for ten minutes. Once the radiographs were dry, the thickness of subcutaneous adipose tissue could be measured at the marked sites using a specially constructed needle point dial reading caliper (Tanner and Whitehouse, 1955), accurate to 0.1 mm over a range of 150 mm. The thickness of subcutaneous adipose tissue at the marked sites was then measured ultrasonically.

RESULTS

Radiographic and ultrasonic comparison.

The mean values and ranges of tissue thickness measured by ultrasound and soft tissue radiography are shown in table 3.7. As the distribution of most skinfold caliper measurements of subcutaneous adipose tissue in a population is skewed (Durnin and Womersley, 1974), the ultrasonic and radiographic measurements of subcutaneous adipose tissue were tested for normality. At all four sites, for both males and females, the ultrasonic and radiographic data were positively skewed, and hence the data was normalized by taking the logarithm of the measurement. Normally distributed data are a prerequisite of regression analysis (Meddis, 1975), which is to be the major statistical treatment of these data.

The regression coefficients, intercepts, standard error of estimates, correlation coefficients (r), and the latter's level of significance for each site, for males and females are shown in table 3.8.

The regression lines for the data are shown, along with a line of identity for each case, in figures 3.2 to 3.5.

The logarithmic transformation of the data does not alter the hypothesis that the ideal relationship between the radiographic and ultrasonic data should be in the form $Y = 0 + 1X$, as explained earlier in the text. Appropriate statistical analysis (Moore and Edwards, 1965) was applied to the data to determine if any of the regression coefficients or intercepts were significantly different from the ideal values of 1 and 0 respectively. The results of these tests are shown in table 3.9.

Figure 3·2. THE REGRESSION LINES FOR THE FEMALE MEDIAL AND LATERAL CALF MEASUREMENTS

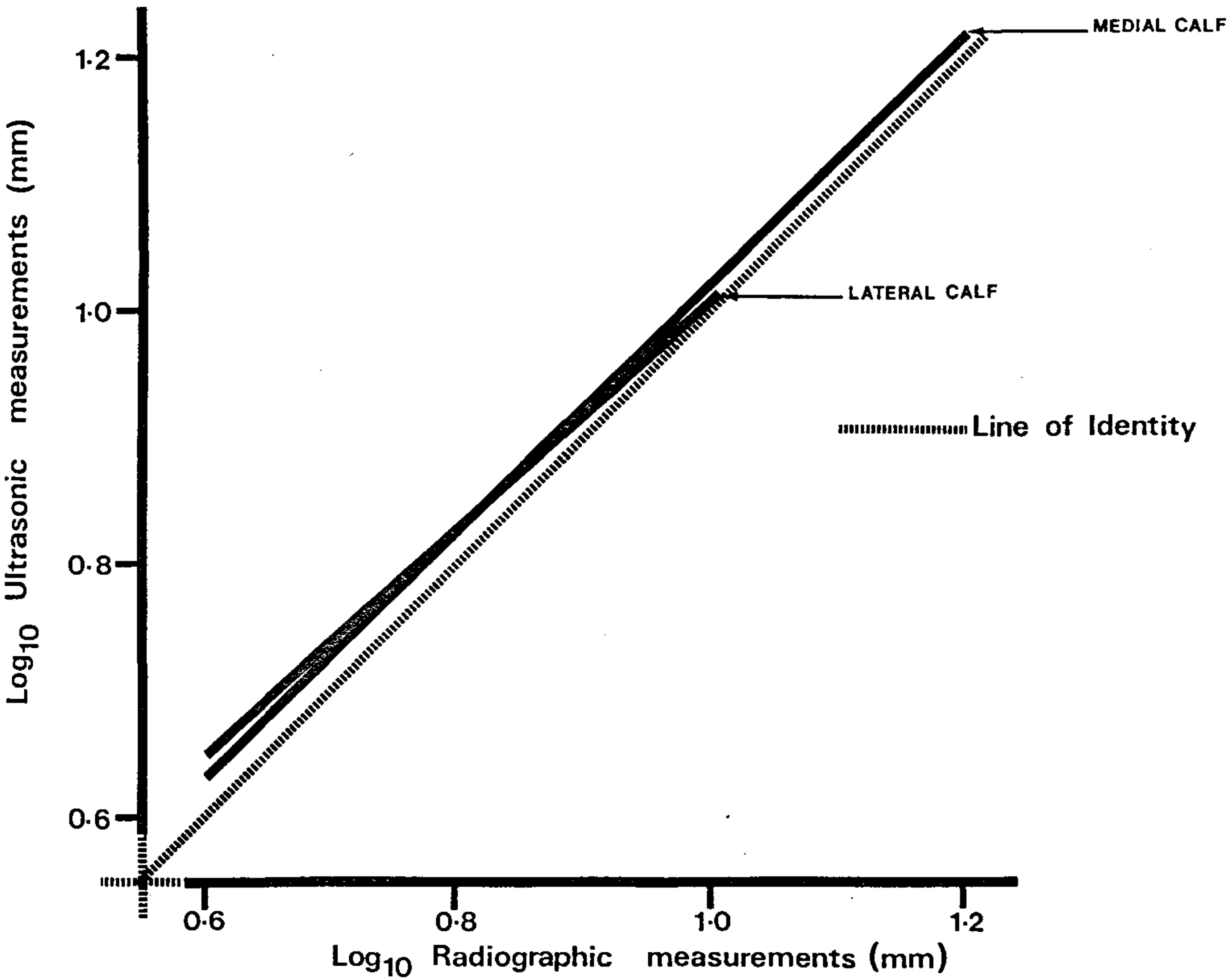


Figure 3·3. THE REGRESSION LINES FOR THE FEMALE ANTERIOR AND POSTERIOR THIGH MEASUREMENTS

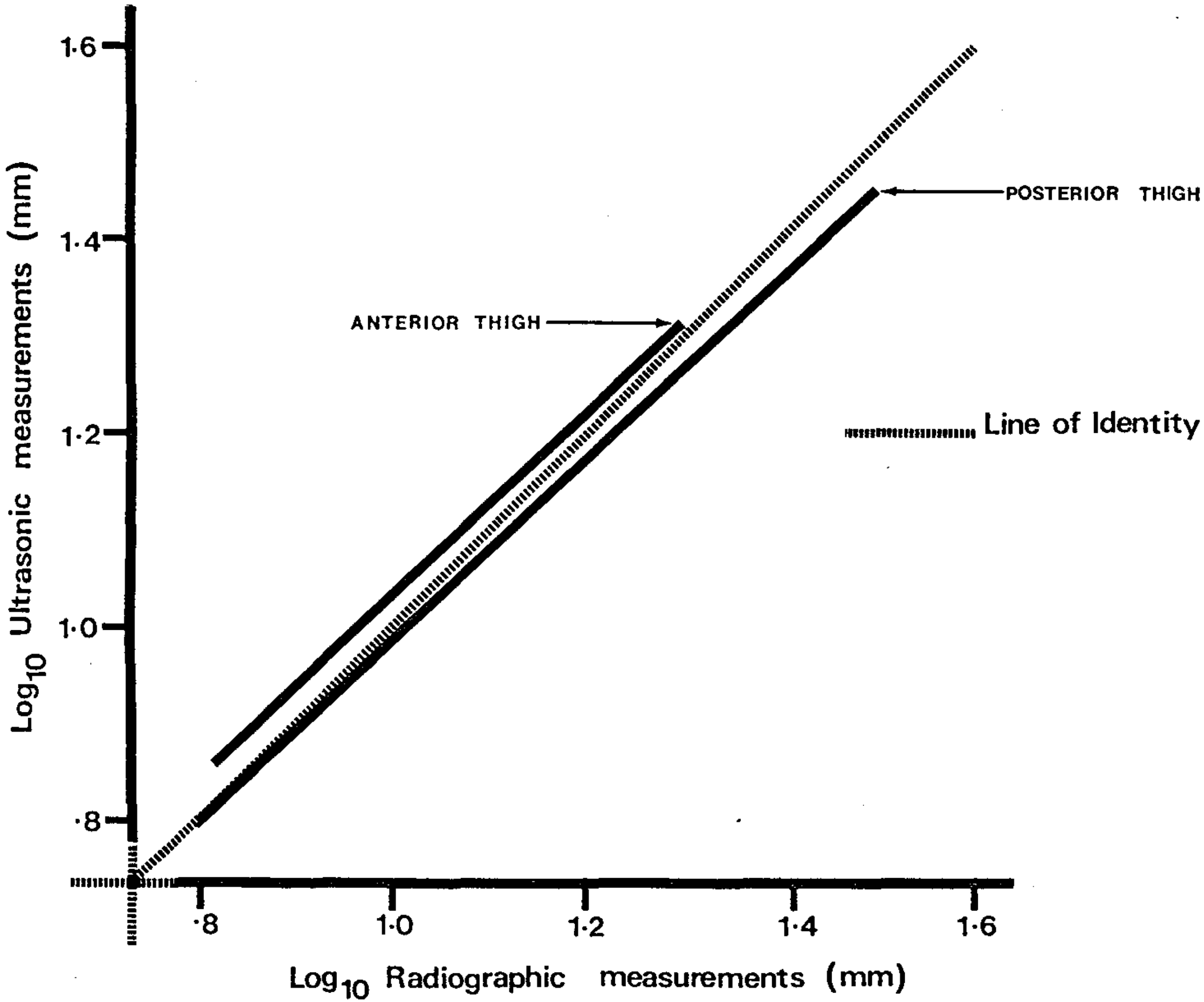


Figure 3·4. THE REGRESSION LINES FOR THE MALE MEDIAL AND LATERAL CALF MEASUREMENTS

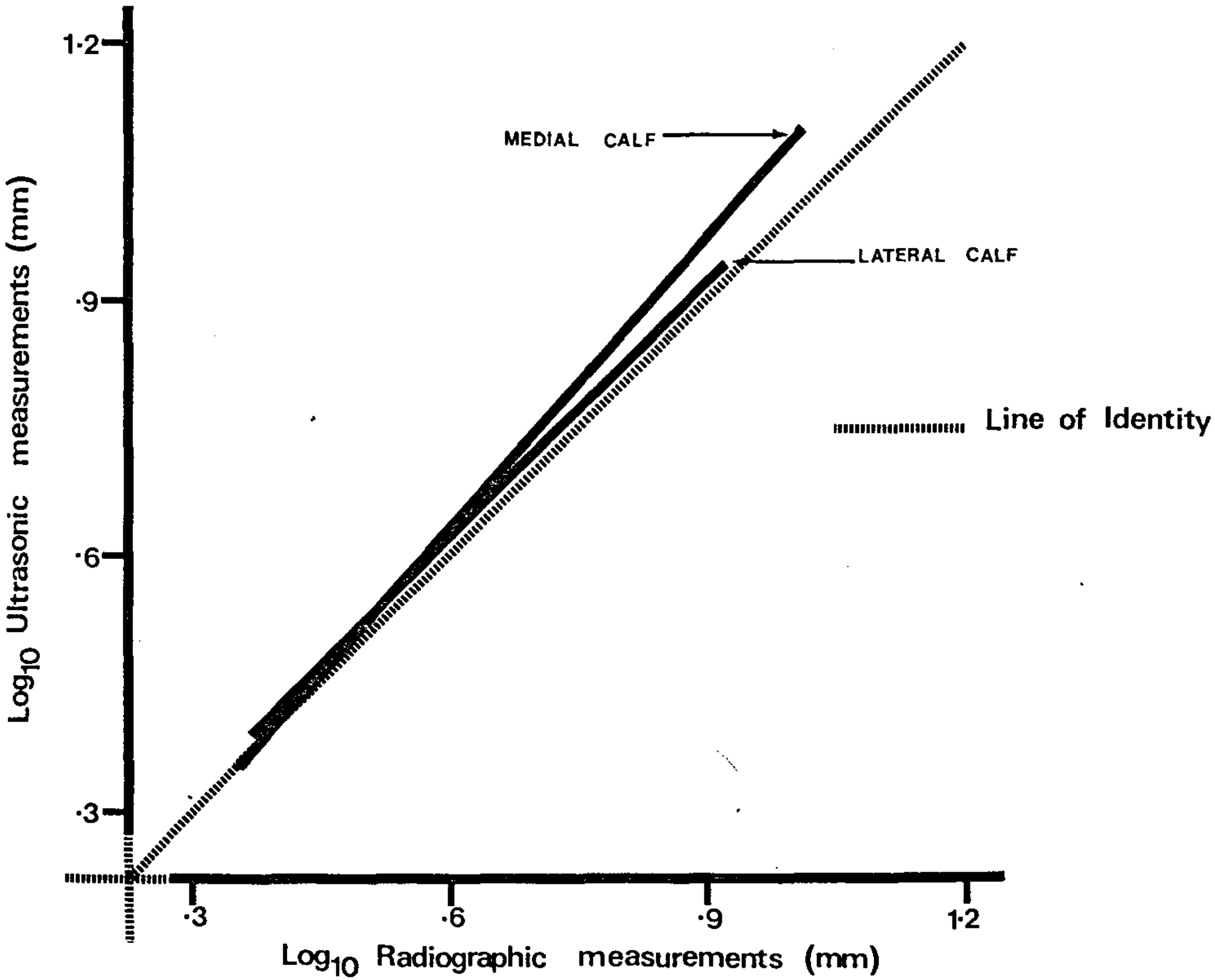
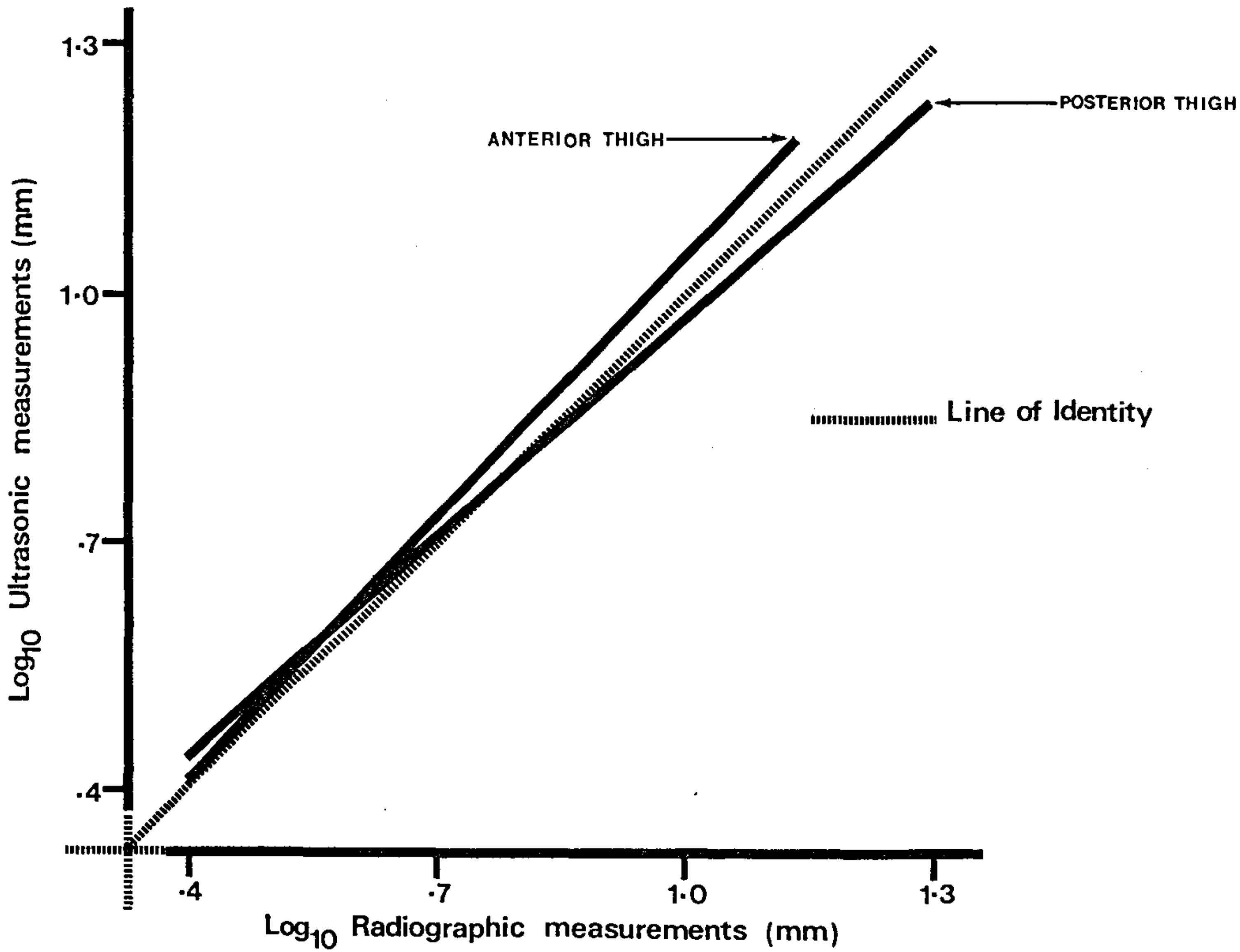


Figure 3·5. THE REGRESSION LINES FOR THE MALE ANTERIOR AND POSTERIOR THIGH MEASUREMENTS



SITE	SEX	ULTRASOUND MEAN (mm)	ULTRASOUND RANGE (mm)	RADIOGRAPHY MEAN (mm)	RADIOGRAPHY RANGE (mm)
ANTERIOR THIGH	M	6.3	2.8 - 13.8	5.8	2.7 - 13.6
	F	13.7	8.5 - 22.1	13.0	8.2 - 19.7
POSTERIOR THIGH	M	7.0	2.9 - 16.6	7.3	2.6 - 16.3
	F	17.9	6.5 - 31.1	19.0	9.6 - 33.4
LATERAL CALF	M	4.2	2.3 - 7.5	3.9	2.6 - 6.9
	F	7.6	3.9 - 10.3	6.8	4.7 - 10.6
MEDIAL CALF	M	4.6	2.2 - 11.1	4.1	2.3 - 8.4
	F	9.7	5.2 - 16.3	9.3	4.2 - 13.7

Table 3.7. The means and ranges of tissue thickness measured by ultrasound and radiography.

SITE	SEX	N	CORRELATION COEFFICIENT	SIGNIFICANCE	INTERCEPT	REGRESSION COEFFICIENT	STANDARD ERROR OF ESTIMATE
ANTERIOR THIGH	M	19	0.88	p<.001	-0.014	1.060	0.091
	F	21	0.88	p<.001	0.095	0.935	0.051
POSTERIOR THIGH	M	19	0.88	p<.001	0.088	0.881	0.097
	F	21	0.91	p<.001	0.054	0.932	0.074
LATERAL CALF	M	19	0.71	p<.001	0.014	1.010	0.108
	F	21	0.92	p<.001	0.088	0.948	0.055
MEDIAL CALF	M	19	0.92	p<.001	-0.057	1.150	0.068
	F	21	0.91	p<.001	0.035	0.993	0.065

Table 3.8. The correlation coefficients and their level of significance, and the intercepts, regression coefficients and the standard error of estimate for the linear regression equations relating ultrasonic measurements to radiographic measurements of subcutaneous adipose tissue thickness.

SITE	SEX	INTERCEPT	T VALUE	SIGNIFICANCE	REGRESSION COEFFICIENT	T VALUE	SIGNIFICANCE
ANTERIOR THIGH	M	-0.014	-0.135	n.s	1.060	0.411	n.s
	F	0.095	0.752	n.s	0.935	-0.575	n.s
POSTERIOR THIGH	M	0.088	0.922	n.s	0.880	-1.044	n.s
	F	0.054	0.426	n.s	0.932	-0.675	n.s
LATERAL CALF	M	0.014	0.097	n.s	1.010	0.045	n.s
	F	0.088	0.791	n.s	0.948	-0.386	n.s
MEDIAL CALF	M	-0.057	0.097	n.s	1.150	0.045	n.s
	F	0.065	0.359	n.s	0.993	-0.063	n.s

Table 3.9. The t values and their significance, for the intercepts and regression coefficients after testing for significant differences from 0 and 1 respectively.

n.s. = not significant

DISCUSSION

By initiating this study it was hoped to determine whether the relationship between ultrasonic measures of subcutaneous adipose tissue and radiographic measures was sufficiently close to the theoretically ideal 1 : 1 relationship to conclude that ultrasound could measure the thickness of subcutaneous adipose tissue with an accuracy comparable to measurements taken from a soft-tissue radiograph, and thereby provide a non-hazardous, accurate measure of adipose tissue thickness.

It can be seen from table 3.8 and figures 3.2 to 3.5 that, for both males and females the regression coefficients and intercepts of the least squares lines at all sites were close to the ideal values. The statistical analysis summarized in table 3.9 reveals that the regression coefficients and intercepts were not only close to the ideal values but, indeed, were not significantly different from them.

This study used four sites, all on the leg, and, therefore, even with the close relationship found between the two measurement techniques at these sites caution must be exercised in infering that the results would be repeated if different sites had been used. However, the fact that over a range of radiographically determined tissue thicknesses of 2.3 to 33.4 mm ultrasonic measurements showed close agreement indicates that to infer that ultrasound could be used to assess subcutaneous adipose tissue thickness at other bodily sites, with confidence, is feasible.

It has been reported (Haymes et al,1977), that multiple ultrasonic echoes occur frequently at trunk sites, and that clear ultrasonic echoes are more easily obtained on the leg than on the trunk (Morgan, 1978). This evidence could suggest that a similar study to the one just described needs to be completed using other bodily sites, before a general statement on the ability of ultrasound to measure subcutaneous adipose tissue thickness could be supported. However, multiple echoes were observed quite frequently in this study, especially at the posterior thigh site in females, and indeed, discrete interfaces within the adipose tissue at this site could be visualized on many of the soft-tissue radiographs. These extraneous echoes could be removed quite successfully by electronic 'damping' of the returning ultrasonic echo. At the posterior thigh site in the female subjects the difference between the mean ultrasonic and mean radiographic measurement is the largest produced by this study, being 1.1 mm. This could indicate that in some cases, however, an extraneous ultasonic echo had been mistaken for the fat-muscle interface echo.

Compression of the subcutaneous adipose layer by the transducer, as described by Whittingham (1962), was not a problem in this study. Care was taken that the transducer merely rested on the skin surface and that no extra pressure was exerted by the operator. The effect of any consistant and significant transducer pressure on the skin is likely to be seen in the values for the intercepts for the least squares lines for these data. Negative intercepts, significantly different from 0 would tend to indicate a constant and significant transducer pressure. Negative intercepts occurred at only two sites, the male anterior thigh, and the male medial calf, and, as has been shown, neither of these intercepts are significantly different from 0.

CONCLUSION

The initial hypothesis that if ultrasound is capable of measuring the thickness of subcutaneous adipose tissue accuratly the expected relationship between radiographic and ultrasonic measurements of adipose tissue thickness should be 1 to 1 ratio can be supported by the results of this study. The experience gained in carrying out this investigation leads to the conclusion that the statements of Ramsden et al (1967), and Booth et al(1966), regarding the care and expertise required in obtaining accurate ultrasonic measures should be amplified. The extrapolation of the results obtained on the four leg sites in this study, to other bodily sites cannot be totally endorsed, however, with the appropriate care and expertise it is suggested that acceptable measures of subcutaneous adipose tissue thickness could be obtained at most bodily sites.

CHAPTER IV

Introduction.

Within the equation produced by Skerlj et al (1953) to calculate the SFM, a most important component is the calculation of mean subcutaneous adipose tissue thickness. Although the thickness of subcutaneous adipose tissue can be measured in a number of ways, the important factor in calculating a representative value for the mean thickness of subcutaneous adipose tissue over the entire body's surface, is, undoubtedly, at which body sites should measurements be taken.

Numerous workers (Pascale, Grossman, Sloane and Frankel, 1956; Fletcher, 1962; Sloan, 1967; Durnin and Womersley, 1974; Katch, Behnke and Katch, 1979; Himes, Roche and Webb, 1980) have related measurements of subcutaneous adipose tissue at different body sites to body density and body fatness. However, studies concerned with the relationship of measurements of subcutaneous adipose tissue at individual sites to the mean subcutaneous adipose tissue thickness are scant.

Edwards (1950) used engineers vernier calipers to measure skinfolds at fifty-three sites, in two groups of women totalling a sample size of one hundred and thirty-eight. The women were divided into the two groups on the basis of whether they had had children or not. Using the measurements obtained at these sites, Edwards was able to show that the thickness of subcutaneous adipose tissue varied over the body's surface in a pattern which remained constant over a weight range of fifty to eighty kg.

Lewis, Masterton and Ferres (1958) measured skinfold thicknesses using engineers calipers at thirty-six body sites on twenty-two men in order to assess changes in subcutaneous adipose tissue over a period of two years. The thirty-six measurements were reduced to six which these workers believed could represent changes in subcutaneous adipose tissue distribution with an accuracy comparable to using thirty-six measurements. The criteria for selection of the six sites were "practicability, anatomical representativeness, sensitivity to change in fat deposition and reproducibility."

In order to estimate or predict the mean subcutaneous adipose tissue thickness over the entire body's surface, work similar in method to that of both Edwards (1950) and Lewis et al (1958) must be undertaken.

The underlying principle of the following work is that the more measurements of subcutaneous adipose tissue thickness taken, the closer the mean value of these measurements is to the true mean over the entire body's surface.

It was decided that ultrasonic measurements of subcutaneous adipose tissue thickness would be made at sixty body sites. The choice of measuring sixty sites was relatively arbitary, however, there were a number of guidelines which were looked at when chosing this number.

It was hoped that by measuring sixty sites the entire range of thicknesses of subcutaneous adipose tissue would be sampled. Moreover, from previous experience, it was calculated that to measure sixty sites would take approximately two and a half hours per subject, and thus any greater number would begin to infringe on the subject's time and the experimenter's concentration.

The choice of body sites was then made. The body was divided into four component parts: the head and neck, the arms, the legs, and the trunk. Data were available (Wilkinson et al, 1982) on the surface area of these four divisions in fifteen young female adults. The proportion that each of the areas contributed to the total surface area was calculated for each individual and the mean values found. These were 0.084, 0.182, 0.369 and 0.365 respectively for the head and neck, arm, trunk, and legs. Therefore, it was decided that the number of sites of measurement on each of the component parts of the body should be in a similar proportion, relative to the total number of sites. Thus the sixty sites were made up of five on the head and neck, eleven on the arm, and twenty-two each on the trunk and legs. The individual sites of measurement were decided on, a full list of these with a description of each is shown in Appendix A.

Method

Thirty subjects, sixteen females and fourteen males, participated in this part of the study. Some of the subjects' physical characteristics are shown in table 4.1.

		MALES		FEMALES	
Variable	Unit	Mean	Standard Deviation	Mean	Standard Deviation
Age	a	21.0	1.3	21.8	1.6
Height	m	1.783	0.062	1.669	0.064
Weight	kg	72.06	9.03	56.71	6.41

Table 4.1. The means and standard deviations of some of the subjects' physical characteristics.

The sixty sites of measurement were carefully marked on the skin with dermographic pencil. Where applicable measurements were made on the left hand side of the body. Ultrasonic measurements of subcutaneous adipose tissue (plus dermis) were then made at each site. The individual thicknesses were not calculated at the time of measurement but were determined at a later time using the ultra-violet paper recordings from the ultrasonoscope.

Results

Analysis of the data

The mean of the sixty measurements was calculated. There are a number of ways in which the data could be analysed. Stepwise multiple regression analysis could be applied to the data to determine the individual site or sites from the sixty which could best predict the mean of sixty. However, bearing in mind the large number of predictor variables in relation to the sample size this option could produce spurious results.

It was decided initially to restrict the analysis to a subset of twelve sites which have been shown to have a strong relationship to total body fat as assessed from body density (Davies, Jones and Norgan 1983). If a suitable method of predicting the mean sixty sites could not be found using these twelve sites the analysis would then be expanded to include more individual sites.

The prediction of the mean of sixty measurements involves the use of regression analysis, and so the measurements obtained at the twelve sites and the mean of sixty sites were tested for normality. A number of sites showed a non- Gaussian distribution, so these data were then normalized by taking the logarithm of the measurement.

Initially two methods of regression analysis were used on the twelve sites. Each site was individually regressed against the mean of sixty. Then every combination of two individual sites within the twelve were summed and this value regressed against the mean of sixty. This was repeated using the sum of all combinations of three and four individual sites.

Multiple regression analysis was carried out using all combinations of two, three and four sites. This analysis yielded over three thousand regression equations for consideration. The analysis was carried out for males and females separately. The statistical computer package 'MINITAB' (Ryan, Joiner and Ryan 1981) was used to carry out the regression analysis. The equations that yielded the lowest standard error of estimate and highest multiple correlation coefficients for the males and females are shown in figure 4.1.

Due to the low standard error of estimate and high multiple correlation coefficients of these equations, no further analysis was carried out.

FEMALES

Mean Subcutaneous Adipose Tissue Thickness = 1.31 + 0.0754 Anterior Thigh (mm) + 0.128 Posterior Thigh (mm) + 0.277 Abdomen (mm) + 0.0854 Triceps (mm)

S.E.E. $\mp$ 0.37 mm R = 0.98

MALES

Mean Subcutaneous Adipose Tissue Thickness = -2.63 + 0.266 Chin (mm) + 4.01 $\log_{10}$ Medial Calf (mm) + 3.76 $\log_{10}$ Abdomen (mm) + 0.144 Triceps (mm)

S.E.E. $\mp$ 0.44 mm R = 0.98

Figure 4.1. Equations relating measurements of subcutaneous adipose tissue at individual sites to mean subcutaneous adipose tissue thickness.

Discussion

The two equations interestingly both contain the abdomen and triceps sites. Lohman (1981) reported that both these sites are very often included in regression equations which relate skinfold caliper measurements of subcutaneous adipose tissue to body density, and indeed these sites appear in the equations produced by Brozek and Keys (1951), Edwards and Whyte (1962) and Katch and McArdle (1973), to name but a few. This might indicate a strong relationship between subcutaneous fatness and total body fatness, which has been suggested by some workers (Chein, Peng, Chen, Huang, Fang 1975), and in effect is the basis of all equations relating subcutaneous adipose measures to total body fat.

The female equation includes both thigh sites which may reflect the large adipose tissue deposits which are usually found on the thigh in females (Satwanti, Singh, Bharadwaj, 1980; Brown and Jones 1977). In the males the inclusion of the chin and medial calf sites are less readily explainable. These sites are not normally measured by other workers, although skinfold caliper measurements of the medial calf site have been shown to be highly correlated with total body adipose tissue mass, as measured in twenty-five cadavers by Martin, Drinkwater, Clarys and Ross (1981).

The sites used in both equations conform to the 'factors of fatness' produced by factor analysis by Badora (1975). This worker showed that for females three factors of fatness were apparent: trunk, lower limbs, and upper limbs; while for the males the factors were - trunk and limbs, the third factor being unidentified. Badora also states that inter-individual variability in the distribution of subcutaneous adipose tissue is greater in males than in females, which could explain the higher standard error of estimate for the male equation.

The two equations produced show high multiple correlation coefficients (R) and low standard errors of estimates, both indicative of a good predictive equation, and although total validation of any predictive equation is dependent on its applicability to another group, it is possible that the equations will produce an estimate of mean subcutaneous adipose tissue thickness which is more representative than has been previously produced, and is close to the true, but unknown, value.

CHAPTER V

Introduction

Having validated the ultrasonic technique and produced equations relating the mean subcutaneous adipose tissue at sixty body sites to a subset of body sites in previous chapters, it is now possible to use this information to calculate the subcutaneous fat mass (SFM). To calculate the proportion of fat situated subcutaneously (PFSS) using these measures of SFM also requires knowledge of total body fat mass.

Densitometry

By measuring body density the percentage of fat in man can be estimated. The method of measuring body density used in this study involved the use of a volumetric tank. Density is defined as mass divided by volume. Body mass can easily be measured using calibrated scales, and the body volume is then measured by utilizing the fact that, the volume of water a body displaces is equal to the volume of the body. The volumetric tank used in this study is shown in figure 5.1. The apparatus consists of a cylindrical tank which has an internal height of 1.88 metres, and a diameter of 0.6 metres. The tank is filled with water at $37^{o}C \mp 2^{o}C$ from a one hundred and thirteen litre domestic copper cylinder fitted with an immersion heater. The main tank is connected via a corrugated rubber hose to a graduated glass side arm, shown in figure 5.2. Detailed description of the construction of the apparatus has been reported elsewhere (Jones, 1972).

Essentially, if the reading on the side arm is recorded before the subject enters the tank, and again when the subject is in the tank, the volume of water that the subject has displaced, and hence body volume, can be calculated, providing one knows the relationship between changes in the main tank volume and side arm readings. Duplicate measurements of body volume using this apparatus have been reported to have a mean difference of 26.2 ml (Jones, 1972).

The volume of the body has to be corrected for the volume of air within the lungs of the subject at the time of measurement. This is done in two stages. Firstly, the residual volume of the lungs is measured

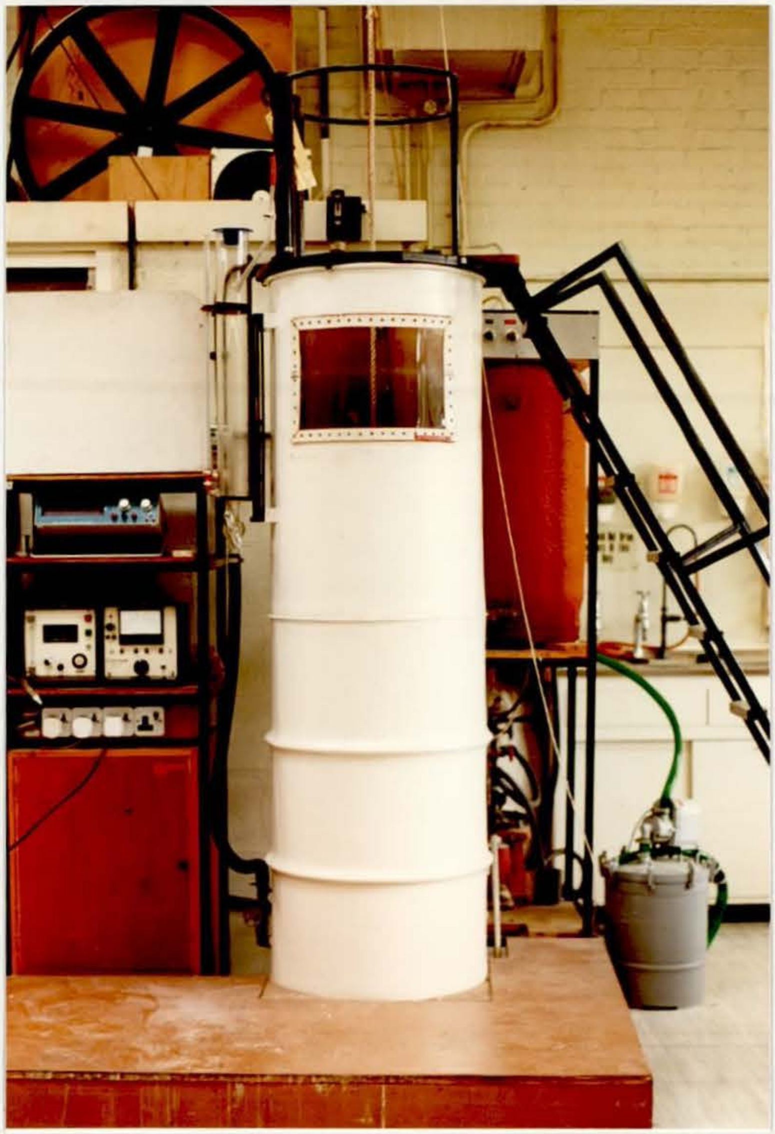


Figure 5.1. The volumetric tank.

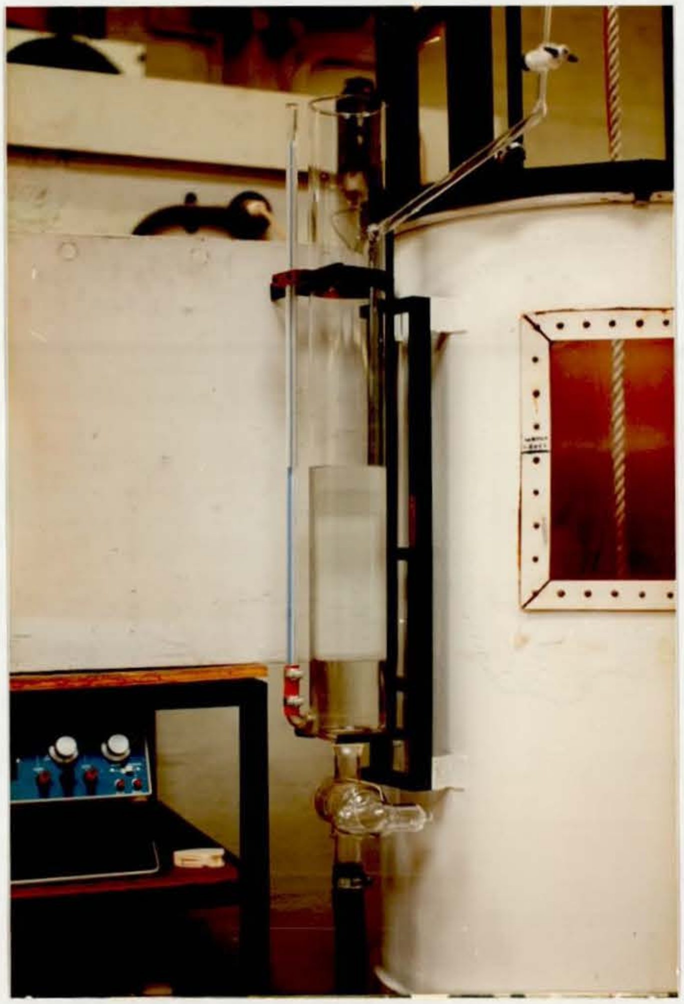


Figure 5.2. Glass side arm of the volumetric tank.

using a modification (Durnin and Rahaman, 1967) of the three breath nitrogen dilution technique (Rahn, Fenn and Otis, 1949). Secondly, as body volume is calculated with the subject having fully inspired, the vital capacity must be measured.

Once these procedures have been completed, the body volume can be calculated as being total water displacement minus the sum of vital capacity, residual volume and the volume of gas in the gastrointestinal tract. As the latter value cannot be easily measured, it is estimated to be 0.1 litre in all cases (Buskirk 1969). The percentage of body weight which is fat can then be estimated using the equation of Siri (1956), which is shown in figure 5.3.

$$\% \text{ fat} = \left(\frac{4.95}{\text{Body Density}} - 4.5 \right) \times 100$$

Figure 5.3. Siri's Equation.

Calculation of SFM and PFSS

It was decided to calculate the SFM, and hence PFSS, by a number of methods. Twelve skinfold caliper and ultrasonic measurements were to be made so that the PFSS calculated using these measurements could be compared with the values obtained by Brown and Jones (1977) and Marfell-Jones (1977).

Methods

Twenty-five females and twenty-one males served as subjects.

1.) Densitometry

Prior to this work the volumetric tank was calibrated by adding water at 37°C, one litre at a time, from a measuring cylinder, to the main tank and recording the reading on the side arm. This enabled a regression equation to be produced, in the form $Y = a + bx$, which related the change in side arm reading to the change in volume in the main tank.

On arrival in the laboratory the experimental procedure of measuring body volume was explained to the subject. Once familiar with the technique the subject was asked to void the bladder, and then to have a shower. While this was being done, the valves to and from the tank were closed and the tank isolated from the heating elements. The reading on the side arm was recorded, along with room temperature, water temperature and barometric pressure. Once the subjects had showered they were asked to step onto calibrated scales (Herbert and Sons, London), and weighed to the nearest 0.05 kg. The reading on the side arm was then read again to check that no further settling of the water level had occurred, and the subject then entered the tank, and a safety harness was attached. The subject was then given a few minutes to become accustomed to the unusual environment.

Once the subject was relaxed, the first measurement of residual volume was taken. A four litre anaesthetic bag was evacuated of air using a vacuum pump, and the bag then 'washed out' with 100 % oxygen and evacuated again, before being filled with three litres of 100 % oxygen, using a calibrated gas syringe. The subject wore a noseclip, and was asked to submerge in the water until the vertex of the skull was just below the surface of the water, while breathing atmospheric air through a mouthpiece and snorkel. After a few seconds, the subject was asked to expire fully, and when this was complete to signal by raising a finger. Immediately this signal was given the snorkel airway was diverted, by means of a valve at the top of the snorkel, to the anaesthetic bag containing the three litres of oxygen. The subjects then followed a procedure, previously explained to them, of inspiring and expiring three times during a period of nine seconds. After the final expiration the valve at the top of the snorkel was closed and the subject redirected to breathing atmospheric air, and was told to come up out of the water. The contents of the anaesthetic bag were then analysed for oxygen and carbon dioxide percentages using a Taylor Servomex oxygen analyser and an ADC carbon dioxide analyser. Both pieces of equipment were calibrated before use. From these percentages the percentage of nitrogen in the bag can be calculated. Normally, the whole procedure was repeated three times to obtain a mean value, however, if the oxygen and carbon dioxide percentages were not consistent, or the time taken to complete the series of inspirations and expirations was less than eight seconds or more than ten seconds, the process was repeated until satisfactory results were obtained. A period of ten minutes was allowed between measurements of residual volume to allow the gaseous composition of the lungs to return to normal. The residual volume was then calculated using the equation (Rahn, et al 1949) shown in figure 5.4. In the intervening time between residual volume measurements the rest of the experimental procedure was carried out.

The vital capacity of the subject was measured by connecting the subject, via a mouthpiece and rubber hose, to an eight litre water trap spirometer (Goddart Expirograph). The subject was asked to submerge in the water to the same level as for the residual volume measurements. The subject breathed atmospheric air for a few seconds, and the respiratory movements were observed through the window in the tank. At an appropriate moment the subject was told to inspire maximally, and at the same time the subject was connected to the spirometer by way of a valve. When maximum inspiration had occurred the subject then expired maximally. When this was complete the valve was closed and the subject was returned to breathing atmospheric air, and told to come up out of the water. This procedure was repeated three times in order to obtain a maximum value, with a period of rest given between each measurement. The subject's vital capacity can then be read off from the paper output of the spirometer, and corrected to BTPS.

To measure the body volume the subject was first asked to ensure that no air was trapped in the swimming costume, and was than asked to stand in the volumetric tank with the water level just below the chin, until the water level settled. The subject was asked to fully inspire and then, holding the breath, submerge in the water, to the same level as in previous procedures, and told to hold the breath for as long as possible,

$$RV = \left(V_s \cdot \frac{f'N - n}{fN - f'N} \times \frac{B - pH_2O}{B - 47} \times \frac{310}{273 + t} \right) - D.S.$$

Where

V_s = Volume of gas in anaesthetic bag (3 litres)

$f'N$ = Nitrogen percentage after rebreathing.

n = Original nitrogen percentage in anaesthetic bag (0.5 %).

fN = Alveolar nitrogen percentage (80 %)

B = Barometric pressure.

pH_2O = Tension of water vapour in anaesthetic bag.

t = Temperature of gas in anaesthetic bag.

D.S. = Volume of dead space in snorkel and valve (0.17 litre).

Figure 5.4. The calculation of residual volume.

or until told to surface. About ten seconds were allowed for the water level in the side arm to stabilize, and then the connection between the side arm and the tank was disconnected by turning a stopcock. The subject was then told to surface. The subject was asked to repeat this procedure a number of times, the stopcock connecting the side arm to the main tank being opened when the subject was submerged, until the water level in the side arm remained constant. When this had been achieved the subject left the tank, and the reading on the side arm was recorded. Body density was then calculated using a computer program written in Basic on a Pet Commodore microcomputer.

After the subject was dry the next part of the experiment could begin.

2.) Skinfold Caliper Measurements

The twelve sites of measurement were marked, where applicable, on the left hand side of the body with dermographic pencil. The sites of measurement are described in table 5.1. The majority of measurements were made using Harpenden electronic readout (HERO) calipers (Jones, Marshall and Branson, 1979), although some of the later measurements were taken with a development of the HERO calipers known as HEROICS (Jones and West, 1983). In twenty-four subjects (twenty female, four male) a repeated measurement was made at a single site chosen at random so that the standard error of measurement of the technique could be expressed.

3.) Ultrasonic Measurements

At the same sites of measurement the thickness of subcutaneous adipose tissue was measured ultrasonically, as described in Chapter 2. Again repeated measurements were made on twenty-four subjects so that the standard error of measurement of the technique could be expressed.

SITE	LOCATION
Chin	Under the mandible, with the fold extending from chin to neck.
Biceps	With the arm resting supinated, over the belly of the muscle at a level midway between the tip of the acromion and the lateral epicondyle of the humerus.
Triceps	With the arm resting supinated, midway between the tip of the acromion and the lateral epicondyle of the humerus, and directly in line with the olecranon process.
Subscapular	Under the inferior angle of the scapula.
Suprailiac	Approximately 1 cm above, and 2 cm medial to, the anterior superior iliac spine.
Side	Midway between the axilla and the iliac crest in the midaxillary line.
Waist	Midway between the tenth rib and the iliac crest in the midaxillary line.
Abdomen	Vertical fold at the level of the umbilicus in the mammillary line.
Anterior Thigh	In anterior midline, at one-third subischial (stature minus sitting height), measured up from the lower border of the femoral condyles.
Posterior Thigh	In posterior midline, at the same level as anterior thigh.
Lateral Calf	At the level of maximum calf circumference, in line with the lateral head of the fibula and the lateral malleolus of the tibia.
Medial Calf	At the same level as lateral calf, in line with the medial condyle and the medial malleolus of the tibia.

Table 5.1. Location of the twelve sites of measurement.

4.) Body Surface Area Measurements

Body surface area was estimated in two ways. The equation of Dubois and Dubois (1916) was used, and also the equation produced by Wilkinson et al (1982). These two equations are shown in figure 5.5.

5.) Mean Subcutaneous Adipose Tissue Thickness

The mean thickness of subcutaneous adipose tissue was estimated from the twelve corrected skinfold caliper measurements and ultrasonic measurements for the males, and from eleven measurements in the females, following the work of Marfell-Jones (1977) and Brown and Jones (1977) respectively. The ratios used to correct the skinfold caliper measurements to a single layer of uncompressed adipose tissue are shown in table 5.2. Once the mean thickness had been calculated 1.7 mm was subtracted from this to represent dermis thickness (Brown and Jones, 1977).

The mean subcutaneous adipose tissue thickness of sixty sites was estimated using the equations produced in Chapter 4, and again 1.7 mm subtracted to represent dermis thickness.

6.) Calculation Of SFM And PFSS

Subcutaneous fat mass was calculated in a number of different ways for each subject. For each calculation the proportion of fat in adipose tissue was taken as 0.8, after the work of Baker (1969) and Garrow (1974); and the density of fat as 0.9×10^3 kgm^{-3} (Findanza, Keys and Anderson, 1953). Different combinations of body surface area equation and mean subcutaneous adipose tissue thickness calculation were used to assess SFM. The different equations are shown in figures 5.6 and 5.7. Once the SFM was determined the PFSS was calculated by dividing the SFM by the total body fat mass (proportion of body weight as fat multiplied by body weight).

Dubois and Dubois 1916

$$S.A. = \text{Weight } 0.425 \text{ (kg)} \quad X \quad \text{Stature } 0.725 \text{ (m)} \quad X \quad 71.84$$

Wilkinson et al 1982

$$S.A. = 0.327 + 0.0071 \text{ Weight (kg)} + 0.0292 \text{ Upper Calf Circumference (cm)}$$

$$S.E.E. = \mp 0.0524 \text{ m}^2.$$

Figure 5.5. The two equations used to estimate body surface area.

SITE	FEMALE	MALE
Chin	1.20 : 1	1.20 : 1
Biceps	1.27 : 1	1.20 : 1
Triceps	-	1.51 : 1
Subscapular	1.40 : 1	1.48 : 1
Side	1.35 : 1	1.50 : 1
Waist	1.07 : 1	1.34 : 1
Abdomen	1.08 : 1	1.32 : 1
Suprailiac	1.09 : 1	1.37 : 1
Anterior Thigh	1.84 : 1	1.47 : 1
Posterior Thigh	1.45 : 1	1.54 : 1
Lateral Calf	2.09 : 1	1.58 : 1
Medial Calf	1.77 : 1	1.45 : 1

Table 5.2. The ratios used to convert skinfold caliper measurements to a single layer of uncompressed adipose tissue.

									Abbreviation
1.)	SFM =	Mean subcutaneous adipose tissue thickness. (Ultrasonically measured from 11 or 12 body sites).	X	Body Surface Area (Dubois and Dubois 1916)	X	0.9	X	0.8	U.D.11 or U.D.12
2.)	SFM =	Mean subcutaneous adipose tissue thickness. (Ultrasonically measured from 11 or 12 body sites.)	X	Body Surface Area (Wilkinson et al 1982)	X	0.9	X	0.8	U.W.11 or U.W.12
3.)	SFM =	Mean subcutaneous adipose tissue thickness. (Corrected skinfold caliper measurements at 11 or 12 body sites.)	X	Body Surface Area (Dubois and Dubois 1916)	X	0.9	X	0.8	S.D.11 or S.D.12

Figure 5.6. Three of the equations used to calculate SFM.

								Abbreviation
4.) SFM =	Mean subcutaneous adipose tissue thickness. (Corrected skinfold caliper measurements at 11 or 12 body sites.)	X	Body Surface Area (Wilkinson et al 1982)	X	0.9	X	0.8	S.W.11 or S.W.12
5.) SFM =	Mean subcutaneous adipose tissue thickness. (Calculated using predictive equations produced in Chapter 4.)	X	Body Surface Area (Dubois and Dubois 1916)	X	0.9	X	0.8	60D
6.) SFM =	Mean subcutaneous adipose tissue thickness. (Calculated using predictive equations produced in Chapter 4.)	X	Body Surface Area (Wilkinson et al 1982)	X	0.9	X	0.8	60W

Figure 5.7. Three of the equations used to calculate SFM.

Results

Some of the subjects' physical characteristics are shown in table 5.3.

1.) Densitometry

The calibration of the volumetric tank yielded the regression equation:

$$Y = -0.0066 + 2.88X$$

$$r = 1.0 \qquad S.E.E. \mp 0.12 \text{ litres}$$

Y = Change in volume in the volumetric tank in litres.

X = Difference between the initial and final reading on the side arm in centimetres.

The means, standard deviations and ranges for the vital capacities and residual volumes of the subjects are shown in table 5.4. All these values are within the normal ranges described by Cotes (1979).

	MALES			FEMALES		
	Mean	Standard Deviation	Range	Mean	Standard Deviation	Range
Vital Capacity (litres)	4.760	0.585	3.560—5.970	3.320	0.375	2.710—3.930
Residual Volume (litres)	1.165	0.255	0.725—1.480	0.965	0.295	0.540—1.635

Table 5.4. The means, standard deviations and ranges of the subjects' vital capacities and residual volumes measured underwater, **corrected to BTPS**

		MALES			FEMALES		
Variable	Unit	Mean	Standard Deviation	Range	Mean	Standard Deviation	Range
Age	a	21.0	1.4	19.033—25.515	20.8	1.7	18.625—24.912
Height	m	1.781	0.063	1.685—1.892	1.665	0.054	1.511—1.762
Weight	kg	70.68	7.71	58.7—93.5	58.21	6.35	43.85—71.45

Table 5.3. Some of the subjects' physical characteristics.

The means, standard deviations and ranges of the body density measurements are shown in table 5.5, along with the derived percentage fat and fat mass values.

		MALES			FEMALES		
Variable	Unit	Mean	Standard Deviation	Range	Mean	Standard Deviation	Range
Body Density	$\times 10^3$ kg/m^3	1.064	0.011	1.043—1.085	1.043	0.011	1.025—1.068
Fat as Body Weight	%	15.2	4.8	6.1—24.7	24.7	4.8	13.7—32.9
Fat Mass	kg	10.7	4.4	3.9—22.5	14.4	3.1	6.0—20.6

Table 5.5. The means, standard deviations and ranges of the body density measurements, and the derived percentage fat and fat mass values.

2.) Skinfold Caliper Measurements

The mean skinfold caliper measurement at each site are shown graphically in figures 5.8 and 5.9. The repeated skinfold caliper measurements yielded a standard error of measurement of 0.3 mm. This figure is favourably comparable to values reported by Lohman (1981) for a single site intra-examiner error, and is in agreement with the accuracy which should be obtained by a trained individual, using Harpenden calipers, as reported by Tanner (1959), regarding repeated measurements at the triceps and subscapular sites.

3.) Ultrasonic Measurements

The mean ultrasonic measurements at each site are shown graphically in figures 5.8 and 5.9. The repeated ultrasonic measurements yielded a standard error of measurement of 0.46 mm. The correlation coefficient for the repeated measurements was 0.95, which compares favourably to values produced in other works (Bullen et al, 1965; Haymes et al, 1976).

4.) Body Surface Area Measurements.

The means, standard deviations and ranges for the body surface areas found from the two predictive equations are shown in table 5.6.

	MALES			FEMALES		
Equation	Mean (m^2)	Standard Deviation (m^2)	Range (m^2)	Mean (m^2)	Standard Deviation (m^2)	Range (m^2)
Dubois and Dubois (1916)	1.88	0.11	1.71—2.13	1.61	0.11	1.42—1.85
Wilkinson et al (1982)	1.80	0.10	1.66—2.08	1.68	0.09	1.46—1.83

Table 5.6. The means, standard deviations and ranges for the body surface areas found from the equations of Dubois and Dubois (1916) and Wilkinson et al (1982).

Related t-tests and sign tests were applied to these data to determine whether the mean values calculated by the two equations were significantly different from each other, and also to determine whether the equations produced values that were significantly higher or lower from each other. The mean values were significantly different ($t = 3.27$ $p < .001$, females; $t = -6.07$, $p < .001$, males). The sign test revealed the

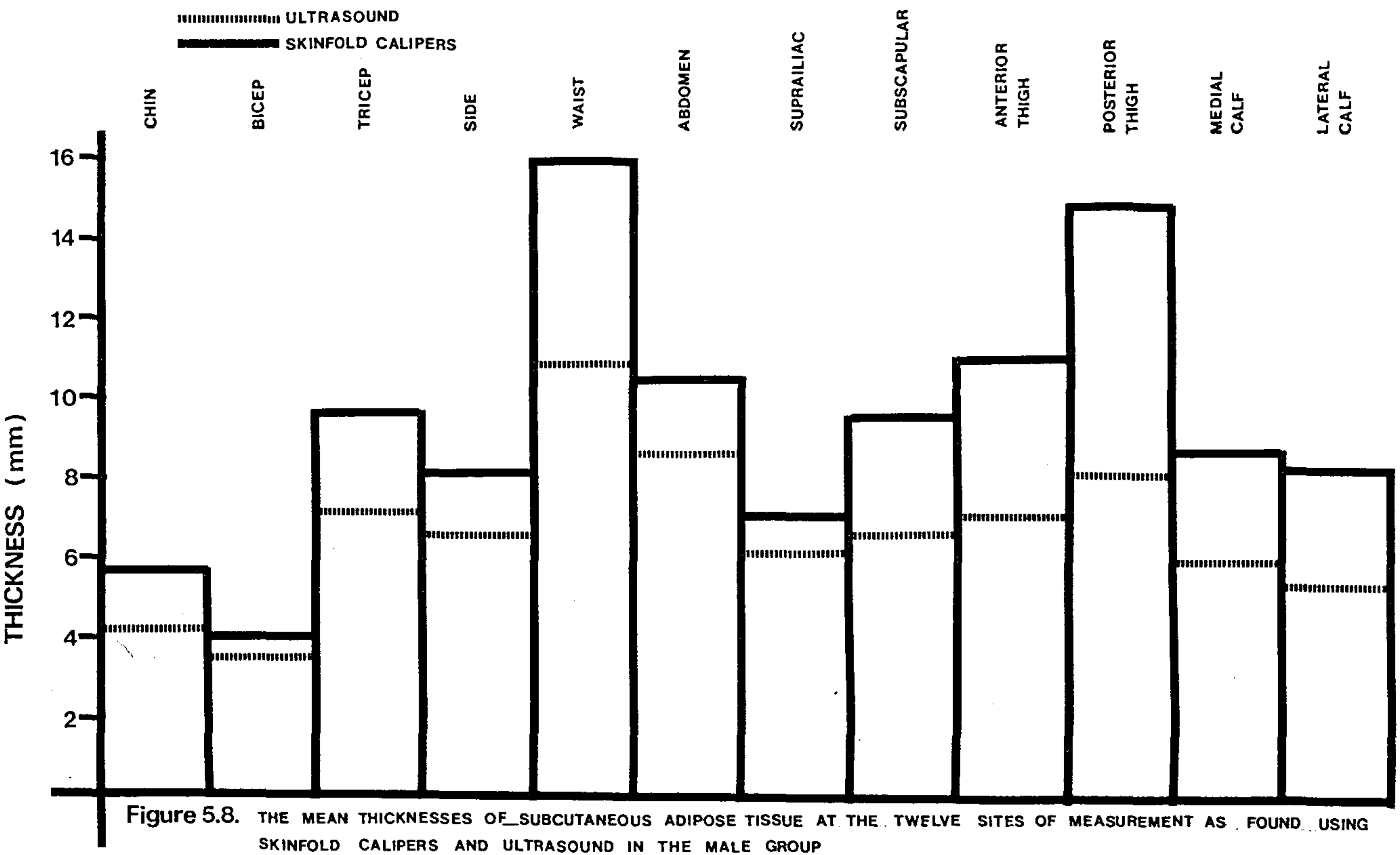


Figure 5.8. THE MEAN THICKNESSES OF SUBCUTANEOUS ADIPOSE TISSUE AT THE TWELVE SITES OF MEASUREMENT AS FOUND USING SKINFOLD CALIPERS AND ULTRASOUND IN THE MALE GROUP

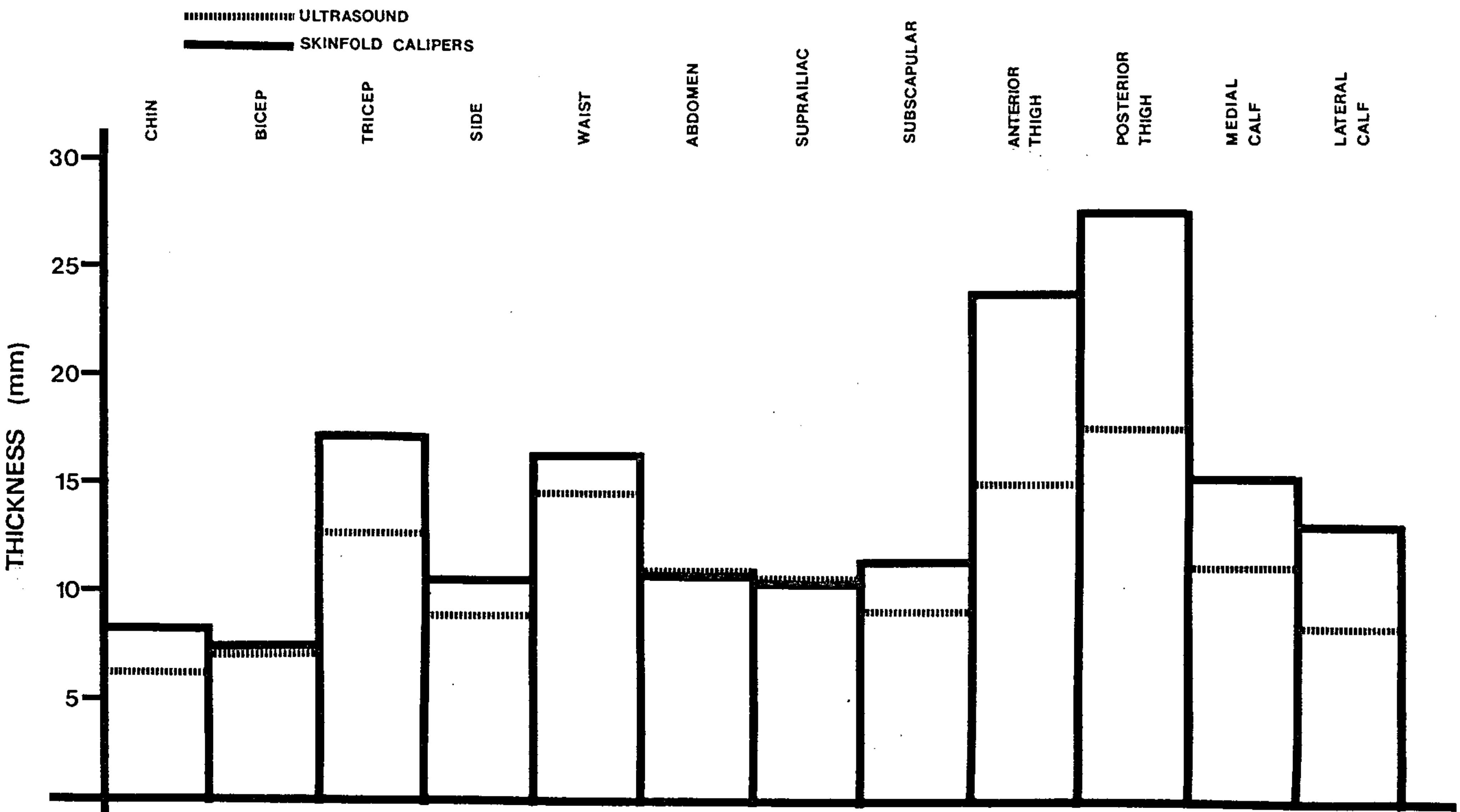


Figure 5.9. THE MEAN THICKNESSES OF SUBCUTANEOUS ADIPOSE TISSUE AT THE TWELVE SITES OF MEASUREMENT AS FOUND USING SKINFOLD CALIPERS AND ULTRASOUND IN THE FEMALE GROUP.

interesting results that for the female group the equation of Dubois and Dubois (1916) consistently produced lower body surface area values than the equation of Wilkinson et al (1982), ($p < .025$), while the opposite was found for the male group ($p < .002$).

5.) Mean Subcutaneous Adipose Tissue Thickness

The means, standard deviations and ranges of the mean subcutaneous adipose tissue thickness, as calculated using corrected skinfold caliper measurements and ultrasonic measurements and the predictive equations produced in Chapter 4, are shown in table 5.7.

	MALES			FEMALES		
	Mean (mm)	Standard Deviation (mm)	Range (mm)	Mean (mm)	Standard Deviation (mm)	Range (mm)
Skinfold Calipers	4.9	2.4	1.1 10.3	8.4	2.2	4.4 12.0
Ultrasound	4.9	2.5	1.9 10.0	9.2	2.1	5.0 12.7
Predictive Equation	4.7	1.9	1.2 8.3	7.1	1.7	3.7 10.4

Table 5.7. The means, standard deviations and ranges of the mean subcutaneous adipose tissue thickness, as calculated using corrected skinfold caliper measurements and ultrasonic measurements and the predictive equations produced in Chapter 4.

Related t-tests and sign tests were applied to these data, the results are shown in table 5.8. This table shows that for the male group the ultrasonic mean and corrected skinfold caliper measurements mean were not significantly different. As the ratios used to correct the skinfold caliper readings were taken from another study this indicates a certain degree of consistency in the compressibility of skinfolds, at the measured sites, in young male adults. This cannot be said of the female group,

	MALES		FEMALES	
	t-Test t value and level of significance	Sign Test Significance and direction	t-Test t value and level of significance	Sign Test Significance and direction
Skinfold Calipers versus Ultrasound	$t = 0.04$ n.s.	n.s.	$t = 3.22$ $p < .01$	n.s.
Skinfold Calipers versus Predictive Equation	$t = 3.75$ $p < .005$	Predictive Equation $<$ Skinfold Calipers $p < .02$	$t = 4.22$ $p < .005$	Predictive Equation $<$ Skinfold Calipers $p < .02$
Predictive Equation versus Ultrasound	$t = 5.65$ $p < .005$	Predictive Equation $<$ Ultrasound $p < .02$	$t = 11.37$ $p < .005$	Predictive Equation $<$ Ultrasound $p < .02$

n.s. = not significant

Table 5.8. The results of the t-tests and sign tests between the mean subcutaneous adipose tissue thicknesses calculated from the corrected skinfold caliper measurements, the ultrasonic measurements and the predictive equations produced in Chapter 4. The direction of the consistent difference between the measurements is shown when significant.

where the mean of the corrected skinfold caliper measurements was significantly different to the ultrasound mean, although the sign test reveals that the direction of the difference between the measurements is not significant in either direction. The measurements shown graphically in figure 5.9 allow the calculation of new female correction ratios which are shown in table 5.9.

Site	Correction Factors	Site	Correction Factors
Chin	1.35 : 1	Abdomen	.98 : 1
Biceps	1.06 : 1	Suprailiac	1 : 1
Triceps	1.34 : 1	Anterior Thigh	1.57 : 1
Subscapular	1.28 : 1	Posterior Thigh	1.58 : 1
Side	1.20 : 1	Lateral Calf	1.58 : 1
Waist	1.13 : 1	Medial Calf	1.38 : 1

Table 5.9. Correction factors to convert a skinfold caliper measurement to a single layer of uncompressed adipose tissue for females.

The fact that for both males and females the predicted mean of sixty sites was significantly different, and significantly consistently lower than both the ultrasonic and corrected skinfold caliper means, suggests that using ultrasound or corrected skinfold caliper measurements at the eleven or twelve body sites in the SFM equation will produce high values of SFM, which will be reflected in the PFSS values.

6.) Subcutaneous Fat Mass

The SFM was calculated using the equations shown in figures 5.6 and 5.7. The means, standard deviations and ranges for the calculated

SFM values are shown in tables 5.10 and 5.11. As can be seen the SFM values for the females were consistently higher than for the males, which reflects the higher values of percentage fat and fat mass shown in table 5.5. Related t-tests and sign tests were applied to the data, the results are shown in tables 5.12 to 5.15.

SFM calculation for the males

As can be seen from table 5.10 the SFM calculated by the equation using twelve body sites are very similar, ranging from 6.47 to 6.74 kg. However, from table 5.12 it can be seen that some of the mean values are significantly different from others. Considering the identical mean values of mean subcutaneous adipose tissue thickness calculated from the ultrasonic and corrected skinfold caliper measurements shown in table 5.7, it is not suprising that the SFM, SD12 versus UD12, and UW12 versus SW12 are not significantly different. The UW12 versus UD12 and SD12 versus SW12 calculations are significantly different due to the difference in surface area found by the Dubois and Dubois (1916) and Wilkinson et al (1982) equations. This would lead one to believe that the SW12 versus UD12 and UW12 versus SD12 calculations should also be significantly different, whereas it can be seen that this is not the case. These results can be explained as follows : although the two surface area equations will tend to make the SFM values different, the mean subcutaneous adipose tissue thickness found by using ultrasound and corrected skinfold caliper measurements have no significant directional difference, and therefore will tend to produce higher values in some individuals, and lower values in others, and so 'smooth' the differences caused by the surface area equations.

The significant difference between the 60D and 60W SFM values and all other methods of assessing SFM is due to the lower mean subcutaneous adipose tissue thickness which was found using the predictive equations.

The sign tests mirror the t-test results, with the exception of the 60D versus SW12 calculation where there was no significant consistent directional difference between the two SFM calculations. The predictive equation used in the 60D calculation has tended to reduce the mean subcutaneous adipose tissue thickness, and hence SFM, while

SFM Equation	Mean (kg)	Standard Deviation (kg)	Range (kg)
U.D.12	6.72	3.59	2.43–15.83
U.W.12	6.47	3.53	2.26–15.45
S.D.12	6.74	3.54	1.44–15.87
S.W.12	6.50	3.48	1.34–15.49
60D	5.45	2.83	1.52–12.65
60W	5.24	2.77	1.41–12.35

Table 5.10. The means, standard deviations and ranges for the SFM values found using the different equations in the male group.

SFM Equation	Mean (kg)	Standard Deviation (kg)	Range (kg)
U.D.11	10.86	2.65	5.46—14.87
U.W.11	11.12	2.75	5.63—15.45
S.D.11	9.97	2.74	5.26—14.59
S.W.11	10.22	2.83	5.14—15.06
60D	8.42	2.03	4.63—11.89
60W	8.62	2.14	4.73—12.67

Table 5.11. The means, standard deviations and ranges for the SFM values found using the different equations in the female group.

the Dubois equation raised the SFM in comparison to the Wilkinson equation and this caused variations in the direction of difference between the SFM calculated by the two techniques.

SFM calculation for the females

There is a wider range in the mean values of SFM in the female group produced using the eleven body sites than was found for the males where twelve sites were used, as can be seen from tables 5.10 and 5.11.

Table 5.13 shows that the mean SFM value produced by all the equations are significantly different from each other. The sign tests shown in table 5.15 disclose interesting results. The SFM values found using the 60D and 60W equations are always significantly less than the values produced by the other equations. The fact that for the female group the equation of Dubois and Dubois (1916) produces surface area values which are significantly consistently less than the values produced by the equation of Wilkinson et al (1982) is reflected in the results that the UD11 equation produces significantly consistently lower values of SFM than the UW11 equation, and the same directional difference is found with SD11 versus SW11 and 60D versus 60W. This is the reverse of that which occurred for the male group.

The non significant results in table 5.15 can be explained by the non significant directional differences between the mean subcutaneous adipose tissue thickness calculated using ultrasound and corrected skinfold caliper measurements, for the SW11 versus UD11 and UW11 versus SD11 calculations, as this non-directional difference will have the effect of 'smoothing' the directional differences induced by the surface area equations of Dubois and Dubois (1916) and Wilkinson et al (1982). Whereas for the UD11 versus SD11 and SW11 versus UW11 the non significant results are due simply to the non significant directional differences between the mean subcutaneous adipose tissue thickness calculated using ultrasound and corrected skinfold caliper measurements.

	U.W.12	S.D.12	S.W.12	60D	60W
U.D.12	p <.001	n.s.	n.s.	p <.001	p <.001
U.W.12	-	n.s.	n.s.	p <.001	p <.001
S.D.12		-	p <.001	p <.001	p <.001
S.W.12			-	p <.02	p <.01
60D				-	p <.001

n.s. = not significant

Table 5.12. The results of the t-tests comparing the mean SFM values found using the different SFM equations for the male group.

	U.W.11	S.D.11	S.W.11	60D	60W
U.D.11	p <.01	p <.01	p <.05	p <.001	p <.001
D.W.11	-	p <.001	p <.01	p <.001	p <.001
S.D.11		-	p <.01	p <.001	p <.01
S.W.11			-	p <.001	p <.001
60D				-	p <.01

Table 5.13. The results of the t-tests comparing the mean SFM values found using the different SFM equations for the female group.

	U.W.12	S.D.12	S.W.12	60D	60W
U.D.12	p <.02 U.W.12<U.D.12	n.s.	n.s.	p <.02 60D<U.D.12	p <.02 60W<U.D.12
U.W.12	-	n.s.	n.s.	p <.02 60D<U.W.12	p <.02 60W<U.W.12
S.D.12		-	p <.02 S.W.12<S.D.12	p <.02 60D<S.D.12	p <.02 60W<S.D.12
S.W.12			-	n.s.	p <.02 60W<S.W.12
60D				-	p <.02 60D<60W

n.s. = not significant

Table 5.14. The results of the sign tests comparing the SFM values found using the different SFM equations for the male group.

	U.W.11	S.D.11	S.W.11	60D	60W
U.D.11	$p < .05$ U.D.11 < U.W.11	n.s.	n.s.	$p < .02$ 60D < U.D.11	$p < .02$ 60W < U.D.11
U.W.11	-	n.s.	n.s.	$p < .02$ 60D < U.W.11	$p < .02$ 60W < U.W.11
S.D.11		-	$p < .05$ S.D.11 < S.W.11	$p < .02$ 60D < S.D.11	$p < .02$ 60W < S.D.11
S.W.11			-	$p < .02$ 60D < S.W.11	$p < .02$ 60W < S.W.11
60D				-	$p < .05$ 60D < 60W

n.s. = not significant

Table 5.15. The results of the sign tests comparing the SFM values found using the different SFM equations for the female group.

7.) PFSS Calculations

The means, standard deviations and ranges of the PFSS found using the different methods of SFM calculation, and the fat mass calculated by body density, are shown in tables 5.16 and 5.17. Related t-tests were applied to the data, the results of these tests being shown in figures 5.18 and 5.19.

These results show that the differences in the mean PFSS values are very similar to the values produced by the SFM tests. This would be expected. A reduction in the level of probability at which the difference between the means occur is apparent in a number of the tests, with the S.W.11 versus U.D.11 for the female group being reduced to such an extent that the mean PFSS values found using these equations are no longer significantly different at the 5 % level. Because of the nature of the test, sign test results will be equivalent to those found for the SFM values, and the results shown in tables 5.18 and 5.19 can be explained by the same criteria as was expounded for the SFM differences. The PFSS values of greater than one found as the maximum values from the S.D.11 and S.W.11 calculations for the females in table 5.17 should be noted - this point will be discussed later.

SFM Equation	Mean	Standard Deviation	Range
U.D.12	0.605	0.162	0.396—0.948
U.W.12	0.580	0.158	0.376—0.932
S.D.12	0.600	0.154	0.344—0.810
S.W.12	0.576	0.154	0.321—0.796
60D	0.495	0.153	0.312—0.827
60W	0.474	0.146	0.297—0.769

Table 5.16. The means, standard deviations and ranges of the PFSS calculated using the different SFM equations for the male group.

SFM Equation	Mean	Standard Deviation	Range
U.D.11	0.756	0.105	0.504—0.965
U.W.11	0.773	0.105	0.493—0.945
S.D.11	0.701	0.164	0.387—1.122
S.W.11	0.718	0.167	0.399—1.158
60W	0.589	0.096	0.429—0.776
60D	0.603	0.099	0.419—0.801

Table 5.17. The means, standard deviations and ranges of the PFSS calculated using the different SFM equations for the female group.

	U.W.12	S.D.12	S.W.12	60D	60W
U.D.12	$p < .001$	n.s.	n.s.	$p < .001$	$p < .001$
U.W.12	-	n.s.	n.s.	$p < .001$	$p < .001$
S.D.12		-	$p < .001$	$p < .001$	$p < .001$
S.W.12			-	$p < .01$	$p < .001$
60D				-	$p < .001$

n.s. = not significant

Table 5.18. The results of the t-tests comparing the mean PFSS values found using the different SFM equations for the male group.

	U.W.11	S.D.11	S.W.11	60D	60W
U.D.11	p <.01	p <.02	n.s.	p <.001	p <.001
U.W.11	-	p <.01	p <.02	p <.001	p <.001
S.D.11		-	p <.01	p <.001	p <.01
S.W.11			-	p <.001	p <.001
60D				-	p <.01

n.s. = not significant

Table 5.19. The results of the t-tests comparing the mean PFSS values found using the different SFM equations for the female group.

8.) The Relationship Between Internal And Subcutaneous Fat

If the subcutaneous fat mass and total fat mass are known the quantity of internal fat can be calculated by subtraction. The quantity of internal fat was thus calculated using the different SFM values, and the means, standard deviations and ranges of the values are shown in tables 5.20 and 5.21. The internal fat mass (IFM) values were correlated with subcutaneous fat mass, and the correlation coefficients are shown in table 5.22. The very low correlation coefficients, none of which are significant at the $p < .05$ level, is indicative of a poor linear relationship between SFM and IFM. Graphical relationships were produced of the SFM and IFM values and no curvilinear or other relationship could be seen. A typical relationship is shown in figure 5.10.

The negative values of internal fat, shown in table 5.21, for the minimum value calculated by the S.D.11 and S.W.11 SFM equations should be noted - this point will be discussed later.

	Mean (kg)	Standard Deviation (kg)	Range (kg)
U.D.12	4.21	2.31	0.77—9.42
U.W.12	4.46	2.31	1.01—9.76
S.D.12	4.19	1.89	1.79—7.31
S.W.12	4.43	1.91	1.86—7.53
60D	5.48	2.74	1.91—11.45
60W	5.69	2.72	2.17—11.70

Table 5.20. The means, standard deviations and ranges of the internal fat masses found using the different SFM equations in conjunction with total body fat mass as assessed from body density, in the male group.

	Mean (kg)	Standard Deviation (kg)	Range (kg)
U.D.11	3.57	1.67	0.49—7.00
U.W.11	3.31	1.59	0.33—6.89
S.D.11	4.45	2.39	-0.73—8.33
S.W.11	4.21	2.35	-0.94—8.45
60D	6.01	2.03	1.33—10.80
60W	5.80	1.99	1.18—10.46

Table 5.21. The means, standard deviations and ranges of the internal fat masses found using the different SFM equations in conjunction with total body fat mass as assessed from body density, in the female group.

SFM Calculation	Males Correlation coefficient and level of significance	Females Correlation coefficient and level of significance
U.D.12/11	0.050 n.s.	-0.006 n.s.
U.W.12/11	0.076 n.s.	-0.041 n.s.
S.D.12/11	0.218 n.s.	-0.269 n.s.
S.W.12/11	0.242 n.s.	-0.285 n.s.
60D	0.263 n.s.	0.176 n.s.
60W	0.224 n.s.	0.138 n.s.

n.s. = not significant

Table 5.22. Correlation coefficients and their levels of significance relating values of IFM and SFM, both calculated using the different SFM equations.

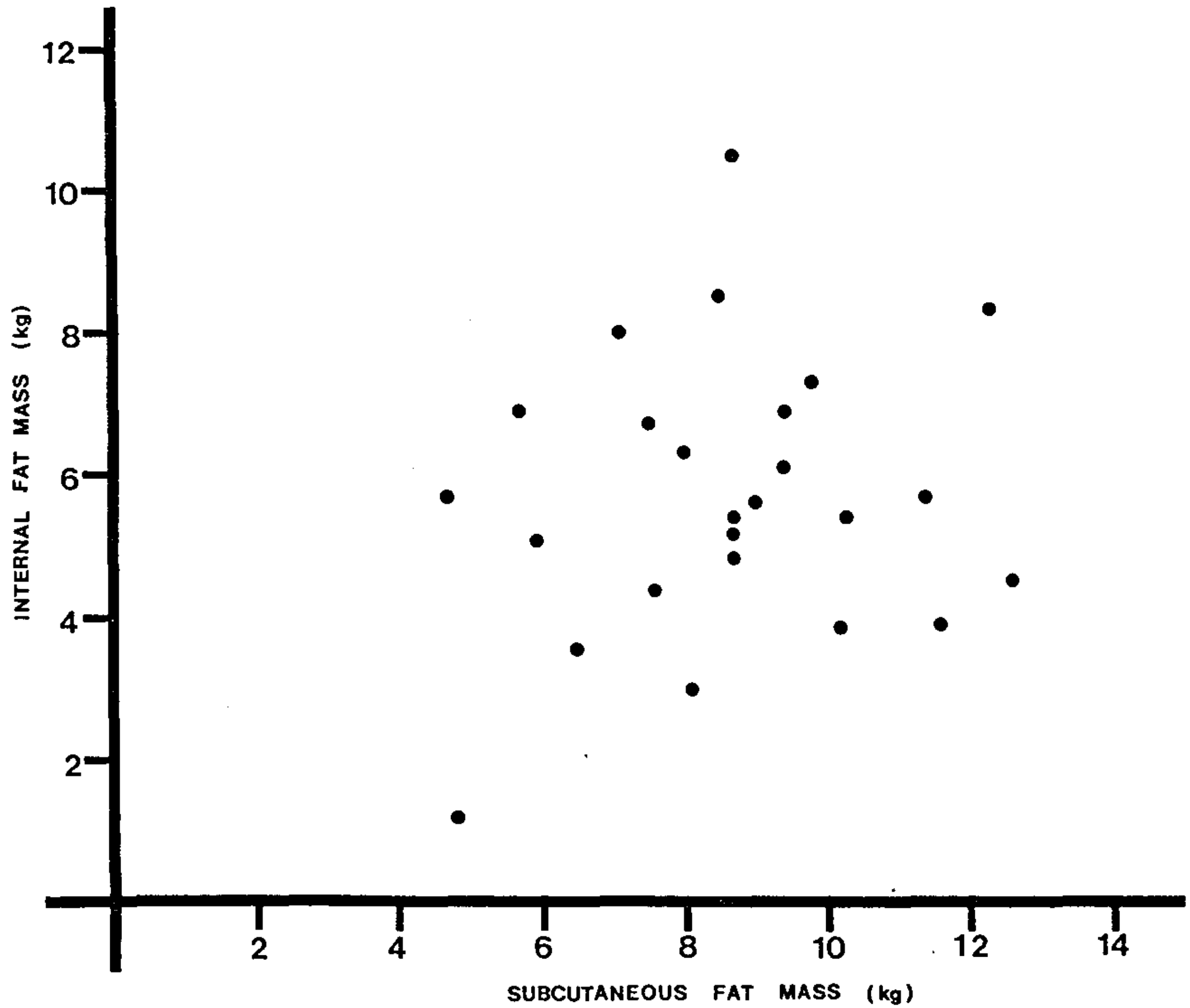


Figure 5.10. A TYPICAL RELATIONSHIP BETWEEN SUBCUTANEOUS FAT MASS AND INTERNAL FAT MASS. (FEMALE GROUP, SFM CALCULATED FROM THE W.60 EQUATION)

DISCUSSION

1.) Densitometry

Throughout this research much emphasis has been placed on the errors that could occur in the calculation of the PFSS due to differing methods and techniques of measuring SFM. An equally important factor in the PFSS calculation is the knowledge of total body fat. In this research total body fat has been calculated from body density, using the equation of Siri (1956). This equation, like all equations relating body density to fatness, has as an essential component in its derivation, the constancy of the fat-free mass and the fat mass, in terms of their constituent components. In recent years this constancy, especially of the fat-free mass, has been questioned by a number of workers (Womersley, Durnin, Boddy and Mahaffy, 1976; Brown and Jones, 1977; Martin et al, 1981), and there is some evidence which suggests that the composition of the fat-free mass alters with age, sex, physical activity and ethnic origin (Jones et al, 1976; Jones and Corlett, 1980).

Some workers (Jones and Corlett, 1980) believe that changes in the mineral content of the skeleton is likely to have the greatest affect on body density determination, whilst others (Lohman, 1981) believe that variation in body water makes the largest contribution to errors.

Jones and Corlett (1980) showed that up to a 15 % difference in the predicted fat mass for a mean body weight of 55.3 kg could occur due to differences in bone mineralization between ethnic groups, whereas Lohman (1981) states that the variability in body density,due to variations in the fat-free mass composition, leads to an error in estimating percentage fat of 2.7 %. Accepting this figure as correct, due to the nature of the equation for calculating PFSS, that is, SFM divided by total body fat mass, the 2.7 % error becomes exacerbated and could lead to differences of approximately 0.18 in the calculation of the PFSS based on data used in this research.

Research into the variability of the fat-free mass and the fat mass continues, and no doubt, in time, a much clearer picture of the affects and variations that inconsistencies in body constituents has on body composition techniques will appear, however, the possible variations and their affect on the calculation of total body fat mass is acknowledged and appreciated within the scope of this research.

2.) Skinfold Caliper Measurements

The profiles of the distribution of subcutaneous adipose tissue, as shown in figures 5.8 and 5.9, were similar to those found by other workers (Sloan, 1967; Badora, 1975; Brown and Jones, 1977), with the females having large skinfold measurements on the thigh, triceps and waist, whilst the males have a more 'central' distribution with large skinfold values at the waist and abdomen, as well as the thigh. The females have larger mean skinfold values at all sites than do the males.

The correction factors used to convert the skinfold caliper measurements to a single layer of uncompressed adipose tissue proved to have different results for the male and female groups. For the males the correction factors worked well when employed to calculate mean subcutaneous adipose tissue thickness, although at individual sites some of the corrected skinfold caliper measurements differed quite considerably from the ultrasonic measurements. However, similar success was not achieved within the female group when the skinfold correction factors were applied.

Although the correction factors shown in table 5.2 and table 5.9 have a relatively small range (0.98 to 1.58 for the males; 1.07 to 2.09 for the females), the range of ratios of skinfold caliper measurements to direct measurements of subcutaneous adipose tissue, in this case measured ultrasonically, from which correction factors are derived, is much larger (0.53 to 3.14 for the females; 0.49 to 3.76 for the males). This indicates a large variability of skinfold compressibility, even within a relatively homogenous group. Ratios greater than two, as found in this research, could suggest that when skinfolds were lifted to be measured, extra underlying tissue was included in the fold, such as superficial muscle tissue.

3.) Ultrasonic Measurements

The ultrasonic measurements of subcutaneous adipose tissue were more easily obtained on the limb sites than at other sites, with multiple echoes being visualized, especially at the chin and suprailiac sites. This may be indicative of the relatively complex underlying anatomy at these two sites. The thin platysma muscle, and the mylohyoid muscle with its midline raphe at the chin site could account for some of the multiple echoes at this site, whilst at the suprailiac site the aponeurosis of the external oblique muscle, and the sheath of the rectus abdominis muscle, as well as the tendinous intersection of this muscle, could give rise to a number of extraneous ultrasonic echoes.

At the abdomen and suprailiac sites in the female group the mean ultrasonic measurement was slightly larger than the mean skinfold caliper measurement, this result could be due to either a high degree of skinfold compressibility at these sites, or error in the skinfold caliper or ultrasonic measurement. The multiple echoes found at the suprailiac site, as mentioned previously, could lend weight to the argument that the wrong ultrasonic echo was identified as being the adipose tissue-muscle interface. However, skinfold compressibility, based on comparisons of skinfold caliper and radiographic measurements, at these two sites, has been shown to be very high in other works (Brown and Jones, 1977).

4.) Body Surface Area Measurements

The almost traditional use, in many areas of human biology, of the equation of Dubois and Dubois (1916) for estimating body surface area should be questioned. The equation produced by these workers was based on direct surface area measurements on twelve subjects, who are unlikely to be representative of the normal population. This statement can be justified by referring to the information regarding the subjects, used by Dubois and Dubois in the construction of their equation, shown in Appendix B. This information makes interesting, if not disturbing, reading in the light of the widespread use of the equation.

It has been shown (Wilkinson et al, 1982) that the equation for estimating body surface area using body weight and upper calf circumference has a smaller standard error of estimate than the equation of Dubois and

Dubois (1916), and therefore, to suggest that the more recent equation is more accurate is quite feasible. However, it should be remembered that the equation produced by Wilkinson et al (1982) was based on a female group, and although these workers suggest that the equation is also applicable to males the fact that the two equations show different results in the direction of the consistent difference between body surface area measurements when applied to males and females could question this statement.

5.) Mean Subcutaneous Adipose Tissue Thickness

Some error will be introduced into this calculation by the assumption that 1.7 mm represents mean dermis thickness (Brown and Jones, 1977). Although the thickness of the dermis has been measured ultrasonically (Alexander and Miller, 1979), in this research the skin-adipose tissue interface could not be successfully identified due to multiple reflection artifacts, produced from the gel used to couple the transducer to the skin, and also the width of the 'main bang' produced from the piezoelectric crystal within the transducer.

Varying values for the thickness of the dermis have been reported in the literature, the variance is often related to the body site at which the thickness had been measured. The value of 1.7 mm used by Brown and Jones (1977) was based on radiographic measurement of dermis thickness. Although the value of 1.7 mm might not be totally accurate it does at least allow comparison of results with other work (Brown and Jones, 1977; Marfell-Jones, 1977).

6.) Subcutaneous Fat Mass Calculations

It is impossible to determine absolutely which of the SFM equations used in this study produces the most accurate results. The only way in which this could be done would be by direct measurement of SFM by dissection on cadaverous material, and relating the true SFM obtained in this way to the estimates produced by the different SFM equations. Cadaverous material was available on which this could be carried out, however, for a number of reasons, several of the requirements of the SFM equations might be inaccurately determined using the cadaverous material available. The equations produced in Chapter 4 are likely to be valid only for the age range from which they were derived, and the application of these equations to cadavers whose ages at death ranged between sixty-five and ninety years old would be inappropriate. Moreover, post mortem changes in adipose tissue would make skinfold caliper measurements difficult to assess, and would certainly alter skinfold compressibility. This, in addition to the population specific skinfold correction factors required in some of the SFM equations, would again probably cause inaccuracies in the SFM calculation. However, the accuracy of the calculation of the SFM is dependent on the accuracy of the measurements used in the SFM equations. The predictive equations produced in Chapter 4 are likely to produce values for the mean subcutaneous adipose tissue thickness which are closer to the true mean than either the corrected skinfold caliper measurements or ultrasonic measurements at eleven or twelve sites. This will be so if we accept that the more measurements of subcutaneous adipose tissue thickness taken over the entire body's surface the closer the mean of these values is to the true, but unknown, value.

Bearing in mind the discussion relating to body surface area measurements, it is suggested that the most accurate SFM for the females is that found by the equation utilizing the predictive equations and the body surface area equation of Wilkinson et al (1982), that is, the 60W equation. Whereas for the male group the most accurate SFM is probably found from the equations using the predictive equations and the body surface area equations of either Dubois and Dubois (1916) or Wilkinson et al (1982), that is, either 60D or 60W.

7.) The Calculation of the Proportion of Fat Situated Subcutaneously

The most accurate PFSS values are dependent on the SFM calculations and therefore the same suggestions as to the most accurate SFM calculations can be applied to the PFSS calculations.

It is interesting to note the large range of PFSS values produced by using the different SFM equations, especially the PFSS value of greater than one shown as the maximum value calculated using the S.D.11 and S.W.11 SFM equations in the females. This result is obviously impossible, and is indicative of an inaccurately high value of SFM or low value of total body fat, or a combination of both of these occurences.

One of the most important implications which the large range of PFSS values could have is regarding predictive equations relating measurements of subcutaneous adipose tissue to body density, and thus the percentage of the body which is fat. Agreat many of these equations have been produced, and each equation assumes that for a given level of 'fatness' the PFSS is constant.

It has been suggested that variations in skinfold compressibility could partially account for the relatively large standard errors of estimates of predictive equations relating skinfold caliper measurements of subcutaneous adipose tissue to body density, and indeed, the use of the ultrasonic technique for measuring subcutaneous adipose tissue thickness has been shown to lower the standard error of estimate (Jones, Davies, and Norgan, 1983). However, the possible non-constant PFSS at different levels of fatness could also account for a large amount of the error found using predictive equations.

In an attempt to illustrate the variation of the PFSS found at a given level of fatness, the range of PFSS values found in this study within a narrow band of body density values are shown in table 5.23. In each case the PFSS is calculated using the 60W SFM equation. Each body density range is equivalent to approximately 4 % body fat using Siri's equation.

As can be seen there is indeed a large variation in the PFSS values found within each body density group.

Some of the most often used predictive equations relating skinfold caliper measurements to body density were produced by Durnin and Womersley (1974). These workers found that regression lines relating the logarithm of four skinfold caliper measurements summed ($\log\Sigma 4$) to body density had different intercepts and gradients for the sexes and for different age ranges.

The difference in position of the regression lines between the sexes was such that, for the same $\log\Sigma 4$ value females had a lower body density, and thus higher percentage body fat, than males.

MALES			FEMALES		
Body Density Range x $10^3 kgm^{-3}$	Number of subjects	Range of PFSS	Body Density Range x $10^3 kgm^{-3}$	Number of subjects	Range of PFSS
1.041—1.050	3	0.325—0.549	1.021—1.030	3	0.499—0.600
1.051—1.060	2	0.427—0.702	1.031—1.040	7	0.454—0.749
1.061—1.070	11	0.297—0.769	1.041—1.050	9	0.419—0.725
1.071—1.080	3	0.302—0.706	1.051—1.060	4	0.472—0.644
1.081—1.090	2	0.362—0.570	1.061—1.070	2	0.454—0.801

Table 5.23. The range of PFSS values found within different body density ranges.

One of the explanations offered by these workers for this difference was that males carried a higher proportion of their body fat subcutaneously than do females. This hypothesis was not supported by the data presented in this thesis, and by other work (Allen et al, 1956; Brown and Jones, 1977; Marfell-Jones, 1977); whilst the results of other work (Pitts, 1956; Forbes and Amirhakimi, 1970) indeed support this theory.

One piece of work cited by Durnin and Womersley (1974) which would support their hypothesis was the post mortem analysis of total body fat, muscle and bone content carried out by Alexander (1964). Durnin and Womersley (1974) reported that the work of Alexander (1964) yielded the results that the PFSS in males was 0.2, and in females 0.1. However, careful analysis of the work of Alexander (1964) showed that, in fact, the data provided in that publication produced values for the PFSS of approximately 0.84 in the males and 0.91 in the females. Clearly Durnin and Womersley (1974) have misinterpreted Alexander's data.

Thus the majority of work in this field indicates that females carry a higher proportion of their body fat subcutaneously than do males, and so one should look to the other possible explanations for the difference in position of the regression lines.

Another possible explanation offered was that variations in skinfold compressibility between the sexes could account for the change in position of the regression lines. For this explanation to be feasible males must have greater skinfold compressibility than females. Again, this was not supported by this research, where, at all measured sites females showed a greater degree of skinfold compressibility than males.

Comparison of PFSS Values Obtained In This Study With Those Obtained In Other Works

Reference to table 1.1 and table 1.2 will aid in the comparison of PFSS values found in this study, and those found in other works. In the male group, the mean PFSS values found by the 60D and 60W SFM equations were lower than the individual group mean values, and total group mean value, found by Jones et al (1976) and Marfell-Jones (1977), with one exception. This was that the 60D PFSS value was higher than the value found for the eighteen sedentary subjects studied by Marfell-Jones (1977). The total group mean PFSS value found by Marfell-Jones (1977) of 0.521 was significantly different ($p < .05$) from the value of 0.495 found in this research using the same SFM calculation (S.D.12), that is, corrected skinfold caliper measurements and the body surface area equation of Dubois and Dubois (1916). However, the 60D and 60W PFSS values, found in the present research, of 0.495 and 0.474 respectively, were not significantly different from the mean PFSS value of 0.521 for the entire group of subjects used by Marfell-Jones (1977).

The large difference between the mean PFSS values found in this research and that of Allen et al (1956) can be partially explained by the lack of correction of skinfold caliper measurements for skinfold compressibility, and the different equation used to estimate total body fat percentage from body density, employed by these workers. The mean PFSS value of the male subjects studied by Allen et al (1956) can be calculated to be 0.259. Treatment of the data obtained in this present research by the same techniques as used by Allen et al (1956) yielded a mean PFSS of 0.297. The now much smaller difference between the values could be due to differing sites at which measurements of subcutaneous adipose tissue thickness were made.

In the female group the 60W mean PFSS value of 0.603 was not significantly different from the mean PFSS value of 0.653 found by Brown and Jones (1977) for their entire group of subjects. The mean PFSS value found in this present research using the same SFM equation as Brown and Jones (1977) (S.D.11), was 0.701. This was not significantly different to the value of 0.653 found by Brown and Jones (1977).

As for the male group, the large difference between the mean values of PFSS found in this present research for the female group, and those of Allen et al (1956) can be partially explained by the different method of calculating SFM. Treatment of the data obtained in this present research with the same techniques as used by Allen et al (1956) yielded a mean PFSS of 0.33. This value is now much closer to the mean PFSS value found by Allen et al (1956) of 0.47.

The mean value for the PFSS found by Skerlj et al (1953), for the age range of eighteen to thirty years, of 0.264 is obviously very different to the mean value of the PFSS found in this present research. However, again, treatment of the present data by the same techniques as employed by Skerlj yielded a PFSS of 0.23. The major contributing factor to the reduction of the PFSS being the inclusion of 0.42 in the SFM equation used by Skerlj et al to represent the proportion of fat in adipose tissue.

8.) The Relationship Between Internal And Subcutaneous Fat

The negative results for the value of internal fat shown in table 5.21 are the result of the PFSS values being greater than one, as shown in table 5.17.

The non-significant correlation coefficients shown in table 5.22, and the typical relationship between subcutaneous fat mass and internal fat mass shown in figure 5.10, revealed that the relationship between the two fat stores, if one exists, is not apparent. A similar conclusion was reached by Martin et al (1981). These workers completely dissected the body fat in twelve male cadavers, and concluded that there was 'no simple relationship' between the two stores. Similar results have been found in other work (Jones and Burkinshaw, 1983).

The use of computerized tomography in body composition studies might aid in the determination of the variability of the relationship between internal and external adipose tissue stores. Indeed, Borkan, Gerzof, Robbins, Hults, Silbert and Silbert (1982) used this technique and showed figures which highlighted the variability between subcutaneous adipose tissue and internal adipose tissue in the abdomen. It is thought by some workers (Borkan, Hults, Cardarelli and Burrows, 1982) that the intraabdominal adipose store is one of the largest in the body. These workers point to the experience of anthropometrists who state that a large abdomen and large abdominal skinfolds do not always occur together. This observation could be a simple example of the variability between internal and subcutaneous adiposity. The variability in quantity and distribution of internal adipose tissue is cited by Borkan, Hults, et al (1982) as one of the reasons why measures of subcutaneous adiposity cannot accurately predict total body fat. The large variation in both the PFSS previously discussed in this research, and the internal fat masses, shown in tables 5.20 and 5.21, lends weight to this argument.

It is doubtful if equations predicting total body fatness will ever satisfy the needs and aspirations of workers within the ambit of body composition studies. This is because of biological variations in man, and the inability of predictive equations to fully accommodate this variability.

However, if accuracy is to be improved greater account must be taken of the variability of the PFSS, and the possible relationship between internal and external adipose stores must be studied further.

Not only will the PFSS vary between individuals but, as has been shown in this thesis, it will also vary depending on the technique used in its calculation. Studies in this field should be standardized in the methods of assessment of SFM and total body fat. The possible non-constancy of the fat-free mass and fat mass, briefly discussed earlier, will always produce error and variability in the estimation of total body fat from body density, and so other techniques might be needed in the assessment of total body fat. The use of A-mode ultrasound, as used in this work, in conjunction with two dimensional ultrasound and computerized tomography, could lead to new and more accurate measures of total body fatness, which will assist in the assessment of the body composition of man, and enhance studies relating the changes in composition in health and disease.

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APPENDIX A

SITE	DEFINITION
HEAD AND NECK	
1) Chin	Approximately three centimetres from the menton, under the mandible.
2) Cheek	Beneath the temple at the level of the nostrils.
3) Posterior Neck	In the posterior midline at the level of cervical six.
4) Lateral Neck	In the lateral midline at the level of cervical six.
5) Mandible	At the angle of the mandible over the masseter.
LOWER LIMB	
1) Greater Trochanter	Immediately over the greater trochanter.
2) Anterior Thigh	In the anterior midline at one-third subischial (stature minus sitting height) height measured up from the lower border of the femoral condyles.
3) Posterior Thigh	In the posterior midline at one-third subischial height measured up from the lower border of the femoral condyles.
4) Medial Thigh	In the medial midline at one-third subischial height measured up from the lower border of the femoral condyles.
5) Lateral Thigh	In the lateral midline at one-third subischial height measured up from the lower border of the femoral condyles.
6) Middle Anterior Thigh	In the anterior midline at one-fifth subischial height measured up from the lower border of the femoral condyles.

7) Middle Posterior Thigh	In the posterior midline at one-fifth subischial height measured up from the lower border of the femoral condyles.
8) Middle Medial Thigh	In the medial midline at one-fifth subischial height measured up from the lower border of the femoral condyles.
9) Middle Lateral Thigh	In the lateral midline at one-fifth subischial height measured up from the lower border of the femoral condyles.
10) Inferior Anterior Thigh	In the anterior midline at one-ninth subischial height measured up from the lower border of the femoral condyles.
11) Inferior Posterior Thigh	In the posterior midline at one-ninth subischial height measured up from the lower border of the femoral condyles.
12) Inferior Medial Thigh	In the medial midline at one-ninth subischial height measured up from the lower border of the femoral condyles.
13) Inferior Lateral Thigh	In the lateral midline at one-ninth subischial height measured up from the lower border of the femoral condyles.
14) Anterior Knee	In the anterior midline over the patella.
15) Posterior Knee	Over the lateral head of the gastrocnemius, medial to the insertion of biceps femoris.
16) Anterior Maximum Calf	In the anterior midline at the level of maximum calf circumference.
17) Posterior Maximum Calf	In the posterior midline at the level of maximum calf circumference.
18) Medial Maximum Calf	In the medial midline at the level of maximum calf circumference.
19) Lateral Maximum Calf	In the lateral midline at the level of maximum calf circumference.
20) Medial Minimum Ankle	In the medial midline at the level of minimum ankle circumference.

21) Lateral Minimum Ankle	In the lateral midline at the level of minimum ankle circumference.
22) Foot	Five centimetres behind the third metatarsal - phalanges joint.

UPPER LIMB

1) Biceps	With the arm resting supinated, over the belly of the muscle at a level midway between the tip of the acromion and the lateral epicondyle of the humerus.
2) Triceps	With the arm resting supinated, midway between the tip of the acromion and the lateral epicondyle of the humerus, and directly in line with the olecranon process.
3) Elbow	In the anterior midline two centimetres proximal from the crease of the elbow.
4) Olecranon	In the posterior midline two centimetres proximal from the olecranon process.
5) Superior Triceps	In the posterior midline one-quarter of the distance between the tip of the acromion and the lateral epicondyle of the humerus, measured down from the tip of the acromion.
6) Superior Posterior Forearm	In the posterior midline one-quarter of the distance between the lateral epicondyle of the humerus and the head of the radius, measured from the lateral epicondyle of the humerus.
7) Inferior Posterior Forearm	In the posterior midline three-quarters of the distance between the lateral epicondyle of the humerus and the head of the radius, measured from the lateral epicondyle of the humerus.

8) Superior Anterior Forearm	In the anterior midline one-quarter of the distance between the lateral epicondyle of the humerus and the head of the radius, measured from the lateral epicondyle of the humerus.
9) Inferior Anterior Forearm	In the anterior midline three-quarters of the distance between the lateral epicondyle of the humerus and the head of the radius, measured from the lateral epicondyle of the humerus.
10) Medial Forearm	In the medial midline half-way between the medial epicondyle of the humerus and the head of the ulna.
11) Hand	A point level with the joint of the first metacarpal and the proximal phalanx and the third phalanx.

TRUNK

1) Abdomen	A point level with the umbilicus in the mammillary line.
2) Side	Midway between the axilla and the iliac crest in the midaxillary line.
3) Waist	Midway between the tenth rib and the iliac crest in the midaxillary line.
4) Suprailiac	Approximately one centimetre above, and two centimetres medial to, the anterior superior iliac spine.
5) Chest	Five centimetres below the sternoclavicular joint.
6) Thorax	Below the twelfth rib in the midaxillary line.
7) Suprapubic	A point two centimetres below the umbilicus.
8) Epigastric	A point two centimetres lateral to a point midway between the umbilicus and the xiphoid.

9) Midclavicular	Over the clavicle, in the middle of the clavicle.
10) Upper Chest	Over the manubrium of the sternum.
11) Lower Chest	Over the ninth rib in the midclavicular line.
12) Xiphoid	A point two centimetres lateral and one centimetre below the xiphoid.
13) Subscapular	Under the inferior angle of the scapula.
14) Spine of the Scapula	Over the spine of the scapula in a line up from the inferior angle of the scapula.
15) Back	A point midway between the inferior angle of the scapula and the iliac crest.
16) Pelvis	A point two centimetres above the iliac crest in line with the inferior angle of the scapula.
17) Cervical	Three centimetres lateral to the spinous process of the seventh cervical vertebra.
18) Superior Thoracic	Three centimetres lateral to the spinous process of the fourth thoracic vertebra.
19) Middle Thoracic	Three centimetres lateral to the spinous process of the seventh thoracic vertebra.
20) Inferior Thoracic	Three centimetres lateral to the spinous process of the twelfth thoracic vertebra.
21) Lumbar	Three centimetres lateral to the spinous process of the fifth lumbar vertebra.
22) Shoulder	Six centimetres from the tip of the acromion measured towards the neck.

APPENDIX B

Subjects For The Dubois and Dubois (1916) Surface Area Equation

Case No.	Age Years	Sex	Weight (kg)	Height (m)	Comment
1	36	M	24.2	1.105	Cretin, physical development of eight year old child.
2	21	M	64.0	1.643	Measured three-and-a-half months after severe typhoid infection.
3	22	M	64.0	1.780	Tall, thin, little subcutaneous fat.
4	32	M	74.49	1.792	Tall, average build.
5	-	F	93.0	1.497	Very short and fat.
6	18	M	45.25	1.718	Tall, thin, emaciated from diabetes. Went eleven days practically without food, had only whisky. Had "invisible" edema.
7	1.75	F	6.27	0.732	Measured two hours after death. Had rachitis, large epiphyses at wrists, pigeon-breasted, chest narrow and deep anterio-posteriorly.
8	12.8	M	32.74	1.415	Well formed, no signs of puberty.
9	26	F	57.62	1.648	Sculptor's model; well-proportioned.
10	21.5	M	63.0	1.842	Unusually tall, thin. On reduction diet just prior to measurement.
11	43	M	-	-	Both legs amputated in accident five years previously; stumps atrophied. Face, arms and trunk were fat.
12	34	M	-	-	Legs amputated when six years old, developed crab-like form with powerful arms. Had stroke with spastic paralysis of right side