BODY COMPOSITION STUDIES USING BIOELECTRIC IMPEDANCE MEASUREMENTS

A Dissertation submitted to
THE UNIVERSITY OF SURREY
in partial fulfillment of the degree of
MASTER OF SCIENCE
in
MEDICAL PHYSICS

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September 1989

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ABSTRACT

A simple non-invasive and inexpensive technique is required so that the gross composition of the body may be determined easily. Skinfold thickness measurements, despite their limitations have been established as a standard field method for estimating total body density and, therefore, body fat and fat-free mass. This method was used as a reference one in order to validate bioelectric impedance measurements (both of total body and segmental) in estimating fat-free mass. Total body impedance and the impedance along the limbs (especially the arm) proved to be good predictors of fat-free mass. Experiments have also been made to determine the variability of impedance measurements. Finally, body mass index mass/height^2, was studied with reference to the skinfold thickness, to examine whether it could be regarded as an index of obesity.

ACKNOWLEDGEMENTS

I acknowledge the State Scholarship Foundation (SSF, Athens, Greece) for the granting of a scholarship, enabling me to study for the Master of Science degree in Medical Physics.

I would especially like to thank my supervisor, Nicholas M. Spyrou, for his help and encouragement. Eric Worpe for his in valuable aid with the experimental part of the project, and Jenny Oduko for reading this dissertation and making useful comments.

My gratitude must be extended to the volunteers who offered their services with patience and interest.

CONTENTS

1. INTRODUCTION

The gross composition of the body has been described in a variety of ways, one of them being in terms of its major functional constituents: water, fat and fat-free residue. Assessment of human gross body composition is of considerable importance as it may influence morbidity and mortality, alter the effectiveness of drugs and anaesthetics [1], give a good indication of the nutritional status of the individual, help in the management of post-operative patients or simply reveal the actual physical fitness of some special groups, such as obese or formerly obese people or athletes. Although a variety of methods for assessing body composition are available, the majority is limited to the research or clinical laboratory because they are relatively expensive, invasive, require highly trained and experienced operators or the equipment is not portable.

However, there are techniques that have been developed for field use, skinfold thickness measurements being one of the most popular and well-established, so as it is often regarded as a standard method for obtaining the total body density [1] and, therefore, estimating body fat and fat-free mass [2]. However, it has its own limitations as it cannot be applied to obese people, because a fold of skin can not be raised and measured with accuracy, formerly obese or elderly people, because of the great variation in the subcutaneous tissue compressibility, or diseased people (such as in oedematous conditions), because of the discomfort imposed on the patient.

During the past few years bioelectric impedance measurements have become an alternative field technique for total body composition studies. The method is based on the fact that fat-free (ionic) tissues conduct a low frequency (l – 800 kHz) alternating current better than adipose (non-ionic) tissues. According to Ohm’s Law (i.e., the impedance of a conductor to an electric current is proportional to the length of the conductor and inversely proportional to its cross-sectional area), the conducting volume of the human body, its fat-free mass, can be estimated from $(\text{stature})^2$ divided by the total body impedance, measured by electrodes placed at wrist and ankle [3,4]. Many studies have been made on total body bioelectric impedance measurements [3, 5, 6, 7, 8, 9, 10] and it has been shown that there exists a significant correlation between the estimates of body composition from this method and those from other standard methods, such as densitometry or skinfold thickness measurements. However, little information has been published about the effect that various factors could have on the total body impedance [11]. Furthermore, it is questionable whether segmental bioelectric impedance measurements could also predict total body composition [12].

Besides the above techniques, there has also been considerable and increasing interest in the identification of an index of obesity, or body mass index, that is independent of stature and reflects body composition. It was proposed that $[\text{mass}/(\text{height})^2]$ is the best index of total body fat [l3]. However, there has been a lot of dispute about whether this body mass index can predict body fat, or if it is more an indicator of body size rather than body constituents [14, 15].

The aims of this project are:

(i) to determine whether there is any significant correlation between the body mass index and the body fat as predicted from skinfold measurements,

(ii) to correlate the estimated fat-free mass from skinfold measurements with bioelectric impedance measurements along different parts of the body and point out which part, if any, would be best to use for body composition studies using impedance measurements, and

(iii) to investigate the variation of impedance measurements (total body and segmental) with various physiological factors, such as the respiratory cycle, contraction or extension of muscles, position of the body and whether the subject has had a drink or a meal prior to measurement.

2. BACKGROUND

2.1. The Gross Composition of the Body

The composition of the body can be described in different ways, ranging from a complete list of its elemental constituents to a description of the proportions of its specific organs. Between these extremes, there is the compartmental view of the human body: water, fat and fat-free residue (i.e. protein and mineral). Although this is considerably more abstract than the traditional view of tissues and organs, it seems capable of yielding new insights concerning the range of values for various elements and the nature of the variation with respect to several factors, such as age, sex, genetics, environment and nutritional habits, as well as various diseases and metabolic disorders [16].

Water is not present in stored fat and occupies a relatively fixed fraction (73.2%) of the fat-free mass [6]. Therefore, the amount of water relative to total body weight, in normal conditions, is mainly dependent upon the quantity of stored fat and decreases with increasing obesity (however, this is not the same in the presence of oedema or ascites and in extreme obesity or emaciation [17]). Approximately one-fourth of the total body water is extracellular (vascular and interstitial) and the rest is intracellular. The body fat compartment is anhydrous and has a quite constant density of about 0.9 gcm^{-3} (at 36^o C), however, it is the most variable of the body's constituents. The amount of fat varies from 1 or 2 % (which is considered to be the quantity of "essential lipid" to about 50% of total body weight [17]. The fat-free residue (what remains after subtracting total body water and fat) consists mainly of protein and mineral.

There are several areas where the gross body composition is of interest. The amount or rapid changes in body water compartments may often represent the condition or degree of recovery from various diseases, such as different forms of protein-energy malnutrition [10]. Many drugs are distributed only in the non-fat tissues of the body so their dosage should be calculated according to the fat-free mass. On the other hand, some anaesthetics, such as thiopental, have a high affinity for fat; therefore their dosage should be adjusted with respect to body fat. Furthermore, the fat content of the body has high physiological and clinical importance as it may influence morbidity and mortality, the ability to withstand exposure to cold and starvation, while it is associated with illnesses such as anorexia nervosa, dysmenorrhea or decompression sickness [l, 16, 18]. Body composition is also of great importance when studying the special characteristics of specific groups, such as professional athletes or astronauts [7, 8].

Nowadays, there are numerous techniques for assessing human body composition, which span from classical densitometry through bioelectric impedance methods to the high technology of computerized tomography and nuclear magnetic resonance. Despite the large number of methods available, the determination of the gross body composition of a living individual is not always easily performed, nor are the results likely to be particularly accurate [17]. The body consists of a large number of organs and tissues, each with its own chemical and physical composition, and both the quantity and the composition of these organs and tissues vary, within certain limits, from one individual to another. Most of the methods for assessing body composition measure one single variable and attempt to determine body composition by assuming fixed values for all the other variables. The validity of these assumptions determines to a great degree the accuracy with which body composition is measured. To enhance the accuracy of measurement of a single body compartment, a combination of methods should be used.

2.2. Body Mass Index

Measurements of height and weight in adults are reliable, have small technical errors, can be easily obtained and, sometimes, may be the only available measurements for assessing nutritional status. Therefore, several indices (body mass indices) that relate mass (m), and height (h), are commonly calculated, in the form of m^p/h^q, where p and q may either be integers or not, or may also be dependent on other variables such as age or sex. In the past few years, body mass index (m/h^2) has attained great popularity [13] and several studies have been made correlating it with total body fat. However, there are a lot of controversial studies published round this subject [14, 15]. It has been proved that m/h^2 index can be height dependent, for some populations at least, and influenced by body proportions [13], while it is well correlated with the amount of both fat and fat-free mass and, for some age groups it may give a better measure of the amount of fat-free mass than of body f at [14]. Although the body mass index is easy and cheap to obtain with great accuracy, more studies have to be made on whether it is indicative of body composition or not.

2.3. Skinfold Thickness

Skinfold thickness is actually the thickness of a double fold of Skin and subcutaneous adipose tissue at Specific sites on the body. As a considerable proportion of the body fat is stored in the subcutaneous adipose tissue, skinfold thickness can be used to give an estimate of body fat. The method involves raising of a proper fold of skin (which includes subcutaneous adipose tissue but no muscle) and measuring its thickness by applying a caliper. which is designed to exert a constant pressure o f 10 g/mm^2 between its jaws. Different kinds of calipers have been used occasionally, exerting constant pressure over the range of' 2.3 – 26.0 g/mm^2. However, comparative studies [24] have shown that, for results, which can be reproduced with accuracy, the pressure should lie in the range of 9 – 20 g/mm^2 and 10 g/mm^2 has been suggested as a standard value (taking also into account the discomfort produced by the caliper squeezing the fold).

It is possible that a skinfold measurement at only one site of the human body can give a good estimation of the body fat [1]. However, it is more reliable to use the sum of several skinfold thickness measurements at various sites of the body, in order to take into account any possible extreme fat distribution in some individuals. Usually, four sites (subscapular, suprailiac, triceps and biceps) are recommended [l, 21].

The skinfold thickness was found to be highly related to body density [1, 22], though not linearly. It has been shown that if a logarithmic transformation is used for the skinfold thickness (individual or sum), a linear relationship is established between log-transformed skinfolds and body density [1, 2. 22]. Several multiple regression equations for prediction of body density from skinfolds have been derived by statistical analysis of experimental data (correlating skinfold measurements with body density values obtained by other methods, such as the water displacement technique), one of the most commonly used being:

$$\text{density} = a - b \times \log_{10}(\text{skinfold}) \qquad (2.1)$$

where a and b are constants whose value has been calculated by regression analysis of experimental data; tables for a and b are given by Durnin & Womersley [1], for skinfolds measured at various sites and for subjects of both genders and in various age-groups. Assuming that fat and fat-free mass have a constant density for all individuals, the relation of the mass of any body component and the total body density is then given by [2]:

$$\frac{\text{mass of body part}}{\text{total body mass}} \; ? \; \text{proprt. const} \;\; \text{x} \;\; \frac{1}{\text{density}} \; ? \; \text{cnst.} \qquad (2.2)$$

Various equations have been derived for the relation between fat content and body density, depending on the estimated density of fat and fat-free body. One of the most widely used equations is that proposed by Siri in 1961:

$$\text{body fat fraction} = \frac{4.95}{\text{density}} - 4.50 \qquad (2.3)$$

Then, the fat-free mass can be calculated:

$$\text{fat-free mass} = \text{total mass x } (1 - \text{body fat fraction}) \qquad (2.4)$$

Although skinfold thickness is a good estimate of body fat, there are some limitations when it is used for quantitative total body composition studies, because of the assumptions that the thickness of the subcutaneous adipose tissue reflects a constant proportion of the total body fat and that the sites selected for measurement are representative of the fat distribution in the body of the population at large. Therefore, the technique cannot be easily applied with accuracy to extremely obese subjects, as a proper fold can hardly be raised.

However, the method can be easily applied by any person (after some training and experience in the use of the calipers), is cheap and well tolerated by the subjects and has a good precision of within 5% [6]. Thus, skinfold thickness measurement is now regarded as an approved method for estimating body fat and is often used in order to evaluate new methods for assessing body composition.

2.4. Bioelectric Impedance

Bioelectric impedance analysis for estimation of gross body composition is based on the differential conductivity of electrical current in adipose (non-ionic) tissue and fat-free (ionic) tissue. The living organism contains intracellular fluids that behave as electrical conductors and can be regarded as imperfect reactive elements. Application of constant alternating current produces impedance to the spread of the current, which is frequency dependent. At low frequencies (l – 100 kHz), the current mainly passes through the extracellular fluids, where the free charges give rise to a conduction current and a resistance, R, can be measured. At high frequencies (500 – 800 kHz), the current penetrates both extracellular and intracellular fluids, therefore the free and bound charges, respectively, give rise to conduction and displacement currents and an impedance z is produced. one of whose components is the resistance R (due to the conductive fluids) and the other component is the reactance X_C (due to the insulating cell membranes) [5].

$$Z = (R^2 = X_C^2)^{1/2} \tag{2.5}$$

The technique for estimating gross body composition from bioelectric impedance measurements generally involves the induction of an alternating current, of constant intensity, on the subject’s hand and foot and the measurement of the potential difference generated between the sites of. the current induction. Using Ohm’s Law the total body impedance Z is then calculated. The frequency of the alternating current is well above 1 kHz (because frequencies below that value can induce nerve stimulation [23]) and it is generally in the range of 1 – 100 kHz, if only the resistive component of the impedance is required, or in the range of 500 – 800 kHz, when both the resistance and the reactance are to be measured. The intensity of the current varies from several hundreds of µA to 1-2 mA, but it never exceeds several mA, as currents in the mA range can stimulate nerves, muscles or glands [23].

Bioelectric impedance measurements can be used to estimate fat-free mass, as the latter contains virtually all the water and conducting electrolytes in the body, to which the electric conduction is related. The basic principle, upon which the method is based, is that the impedance of a geometrical system is related to the conductor length and cross-sectional area, its configuration and signal frequency [5]. With a relatively constant conductor configuration and a constant signal frequency, the bioelectric impedance is given by:

$$Z = ? \frac{L}{A} \tag{2.6}$$

or

$$Z = ? \frac{L^2}{V} \tag{2.7}$$

where Z = bioelectric impedance,
? = volume resistivity,
L = conductor length,
A = conductor cross-sectional area, and
V = conductor volume.

When a signal frequency in the range of 1 – 100 kHz is used, the reactance component, X_C, of the impedance, Z, is small relative to the resistance, R, therefore the impedance practically equals the resistance [24] and can be substituted by it.

Although there are difficulties in applying this general principle in a system with the complex geometry and bioelectric characteristics such as the human body [23], bioelectric impedance Z can be used as an index of fat-free mass:

$$\text{fat-free mass} = \text{const. x } \frac{(\text{height})^2}{Z} \qquad (2.8)$$

The body fat fraction can then be calculated using equation (2.4) which becomes, using equation (2.8):

$$\text{body fat fract.} = 1 - \text{const. x } \frac{(\text{height})^2}{\text{total mass}} \text{ x } \frac{1}{Z} \qquad (2.9)$$

Fat-free mass is estimated from equations similar to equation (2.8) derived from multiple regression analysis of experimental data, correlating bioelectric impedance measurements with fat-free mass values obtained by some other method (such as densitometry or skinfold thickness measurements). Several equations, with various constants and even some more variables (such as body weight or gender) have been developed by several investigators [3, 7, 8, 9, 24] as well as by impedance monitor manufacturers (as in the case of the four terminal impedance analyzer, Model 101-RJL Systems, Detroit, MI [25]). In all these cases, the estimation of body composition by total body bioelectric impedance proved to show a very good statistical correlation with estimations of body composition made by other standard methods. However, equations derived from regression analysis appear to be significantly sample-dependent, so that they should not be generalized and used in clinical investigation. Thus, there is a need for more studies to be made, in order to find a way to extract body compartment sizes from impedance measurements independently of the population. Furthermore, the technique should be tested and validated not only in normal healthy subjects, but also in various extreme or clinical cases. This has already been made in various groups of athletes, obese subjects, cardiac and pulmonary patients and protein-energy malnourished children, [7, 10, 26]. Additional work is needed on the study of bioelectric impedance measurements for the estimation of gross body composition. Once well established, the method may prove to be invaluable, as it is cheap, non-invasive, can be applied by any investigator without special training or experience, can be used for bed-side studies and is well tolerated by any subject.

3. METHODS OF INVESTIGATION

3.1 Volunteers

Twenty apparently normal and healthy adults, aged 21 – 33 yrs (mean age 24.8 ? 2.9 yrs), fourteen males and six females, participated in this study. They were all volunteers and the whole procedure had been explained thoroughly to them before the experiment took place. They have not been selected for their obesity or another particular characteristic, they were all white, but from various ethnicities, and were all students or staff in the University of Surrey (mainly the Physics Department). Most of them appeared to be involved in some kind of sports activity, approximately 1-2 hours per week, except just a few, who were not at all involved in any sport, and one male, who was doing light 'body-building' exercises 6 hours per week. Every subject completed all measurements on the same day (during working hours) and approximately two or more hours after he/she had had the last meal. The whole measurement procedure lasted about 20 – 30 min for each volunteer.

These twenty volunteers constituted the 'reference group' with respect to age and physical fitness. Apart from this reference group, measurements were taken from two elderly subjects (54 and 50 years old), both rather obese. The measurements taken from these two subjects were not taken into account during the statistical analysis (as the subjects could not be regarded as 'reference' ones) but are shown in the various diagrams plotted, using symbols different from those used for the reference group data.

3.2 Body Mass Index Measurements

The body mass index, m/h^2, where m is the body mass (in kg) and h is the standing height (in m), was calculated using the weight and height of each subject.

The weight was measured using a new analogue bathroom scale (EKS, Sweden), which could measure in the range of 0 – 120 kg and its dial was marked in divisions of 0.5 kg (Figure 1).

FIGURE 1: EKS, Sweden, analogue bathroom scale. Its measuring range is 0-120 kg and the dial is marked in the divisions of 0.5 kg.

Three measurements of the weight were taken for each subject wearing only underclothes, with intervals of 5-7 min between each measurement (during which time height, skinfold thickness and impedance measurements were taken). The bathroom scale was zero adjusted before each measurement and the reading was recorded to the nearest 0.5 kg. Finally, the mean value of the three readings was taken as the actual weight of the subject. It should be mentioned that the measurements were very reproducible, and the variation between the three weight measurements for each subject was often 0.0 or up to 0.5 kg.

The height was measured using a wooden ruler that was mounted on the wall, exactly 1m above the floor (Figure 2). The ruler could measure in the range of 0-1 m, so, considering the 1m distance form the floor, standing height could be

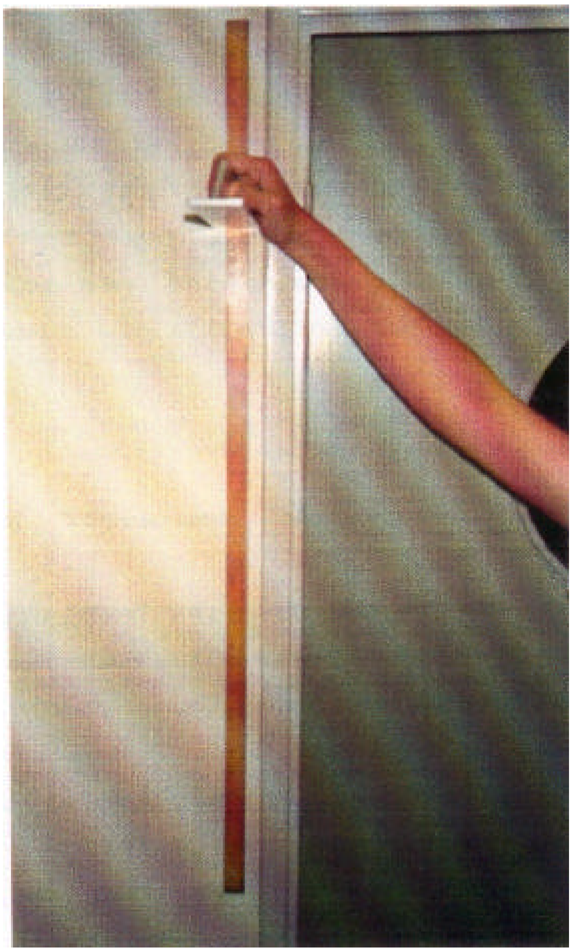


FIGURE 2: The wooden ruler, marked in divisions of 0.001 m, mounted on the wall at 1m distance from the floor (measuring range 1-2 m), and the removable metallic triangle, held as during the measuring procedure.

measured in the range of 1-2 m. The ruler was marked in divisions of 0.001 m and a removable metallic triangle was used to locate the top of the head. Three measurements of standing height were taken for each subject with intervals of 5-7 min between each measurement (during which time weight, skinfold thickness and impedance measurements were taken), with the subject standing barefoot, relaxed, with legs and shoulders against the wall and the spinal column in line with the wooden ruler as viewed from the front of the body. Each reading was recorded to the nearest 0.001 m and, finally, the mean value of the three readings was taken as the actual standing height of the subject. It should be mentioned that the different readings for each subject varied up to 0.006 m, which could be due to the different positioning and the degree of relaxation of the volunteer during the three measurements, although much effort was made to keep the conditions the same.

3.3 Skinfold Thickness Measurements

Skinfold thickness was measured using the Holtain Skinfold Caliper, Holtain Ltd., Crosswell, Crymych, Dyfed SA41 3UF UK (Figure 3), which has been designed to give a constant pressure of 10 gmm^{-2} over its entire operating range (0 – 40 mm). Its dial is marked in divisions of 0.2 mm. The skinfold measurement technique proposed in Anthropometric Standardization Reference Manual [21] was used and each measurement was taken about 4 sec after the pressure of the jaws of the caliper had been released. This was done in order to provide enough time for the compressible subcutaneous tissue to equilibrate but, on the other hand, to avoid the forced extraction of the fluids out of the compressed tissues, which would result in a decrease of the actual skinfold thickness.

FIGURE 3: The Holtain Skinfold Caliper, Holtain Ltd., Crosswell, Crymych, Dyfed SA41 3UF UK. It has been designed to produce a constant pressure of 10 gmm-2 between its jaws over its entire operating range (0-40 mm) and the dial is marked in divisions of 0.2 mm.

Four sites were selected for skinfold measurements on every subject:

i)	subscapular:	just below the inferior angle of the scapula,
ii)	suprailiac:	in the midaxillary line, just superior to the iliac crest,
iii)	triceps:	in the midline of the posterior aspect of the arm, over the triceps muscle, at a point midway between the lateral projection of the acromion process of the scapula and the inferior margin of the olecranon process of the ulna, and
iv)	biceps:	1 cm superior to the line marked for the measurements of triceps skinfold thickness, above

the biceps muscles.

These four sites were chosen because they are recommended [21] as the smallest number of skinfolds representative of body fat. In addition, they are the ones used by Durnin & Womersley [1] to derive the regression equations to estimate total body, density which have been used in this study.

Two sets of the four skinfold thickness measurements were taken for each subject, with an interval of 5-7 min between each set (during which time height, weight and impedance measurements were taken). All the measurements were taken on the right side of the body, where the impedance measurements were also recorded, with the subject standing relaxed. The right side of the body was chosen because it is the most commonly used in relative studies and, more important, it was used by Durnin & Womersley to derive the equations for estimating body density that were used in this study. However, it should be mentioned that most investigators have noticed no statistically significant difference between the skinfold thickness on the right and left side of the body [21] even when a significant difference in the muscle activity between the two sides existed (e.g. in the case of a tennis player [1]).

The reading at each site of measurement was recorded to the nearest 0.2 mm and finally the mean value of the two readings was taken as the actual skinfold thickness at this particular site. It should be mentioned that there was a systematic reading error, as the dial of the calipers was not zero-adjusted (when the jaws of the calipers were in contact between each other, the reading was 0.6 mm), therefore the appropriate correction was made for each measurement.

The frequency distribution of most skinfold measurements in the general population is considerably positively skewed (i.e. with a long tail to the right at higher skinfold thickness values) [22, 20, 27]. Therefore, an appropriate transformation is needed before statistical analysis is applied The transformation proposed by Edwards et al [20], used by most investigators and recommended by the manufactures of the Holtain Skinfold Caliper, used in this experiment, is:

$$\text{transf. skinfold thickness} = \log_{10}(\text{reading in mm} - 1.8) \qquad (3.1)$$

The constant 1.8 mm, which is subtracted from the reading, is an approximation of the double thickness of the skin when it is under pressure of ~10 gmm^{-2}. Using the regression equation proposed by Durnin & Womersley [1] (Eq. 2.1) the total body density was derived from skinfold thickness and the body fat fraction was subsequently estimated using Siri's equation (Eq. 2.3).

3.4 Bioelectric Impedance Measurements

Bioelectric impedance measurements were taken using the Gastric Impedance Monitor, London Medical Electronics, Manor Works, Church St., Cogenhoe, Northampton, NN7 1LS, (Figure 4), on the right side of the body at four sites from-wrist-to-ankle along the arm, along the torso and along the leg. The right side of the body was chosen because skinfold measurements were also taken there, while it was not possible to measure impedance on both sides of the body due to the cost of the disposable electrodes used. The Gastric Impedance Monitor was initially designed for impedance measurements for gastric studies

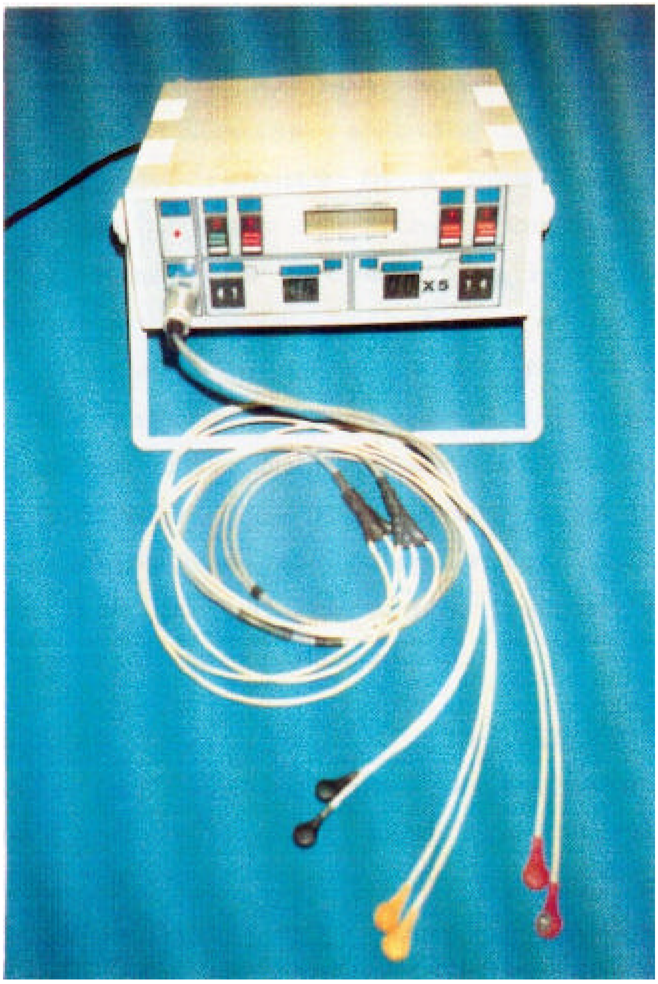


FIGURE 4: The Gastric Impedance Monitor, London Medical Electronics, Manor Works, Church St., Cogenhoe, Northampton, NN7 1LS. Thr red-colored electrodes are the driving ones, the yellow ones are the recording electrodes corresponding to channel 1 (0-99 Ohms), while the black ones are the recording electrodes corresponding to the modified channel 2 (0-600 Ohms).

[28]. It is provided with two driving electrodes that induce an alternating electrical current of 29 kHz frequency and ~1 mA constant intensity. Two recording channels are available. These provide a digital impedance reading in the range of 0-99 O. However, .as the body impedance from- wrist-to-ankle has been reported to be in the range of several hundred Ohms [10], one of the

two channels (channel 2) had to be modified. Because of the short time available the only possible modification was to ‘multiply’ the already existing recording range by 5, which unfortunately results in a loss of accuracy (? 5-6 O). Furthermore, the display of the monitor was not linearly related to the impedance between the recording electrodes for the whole recording range. Therefore, a Decade Resistor (type 5274-5D, H. Tinsley Co. Ltd., London SE25) was used to calibrate both recording channels of the monitor. The calibration curves for both channels (illustrated in Appendix A) were checked every day during the experiment, but no shift was recorded. Therefore, the display for each measurement was converted to the actual recorded impedance value using the calibration curve of each channel.

During impedance measurements, the subject was supine on a couch, relaxed and wearing only underclothes. Care was taken to place the arm well apart from the torso and the legs not touching one another. Self-adhesive, pre-gelled silver/silver chloride ECG electrodes were used (Medicotest, Disposable ECG Electrodes, type R-00-S), 50mm in diameter (actual diameter of the whole electrode ~4 cm), with foam as backing material (Figure 5). A thin layer of electrolyte gel was added to each electrode before application to the skin to ensure good contact. Eight electrodes were put on each subject. Two electrodes (one for inducing the current and the other for recording the potential difference) were placed one beside the other on the dorsal surface of the hand, just above the distal prominence of the radius and the ulna. Two electrodes were placed beside the anterior projection of the acromion process of the scapula and under the clavicle, two more on the abdominal area, just beside the iliac crest, and the last two above the lateral maleoli at the ankle (Figure 6). The impedance measurements from-wrist-to-ankle, along the arm and along the leg, which were estimated to be several hundred Ohms, were

FIGURE 5: The Medicotest Disposable Electrodes, type R-00-S, shown from both surfaces, with the extra layer of the electrolyte gel.

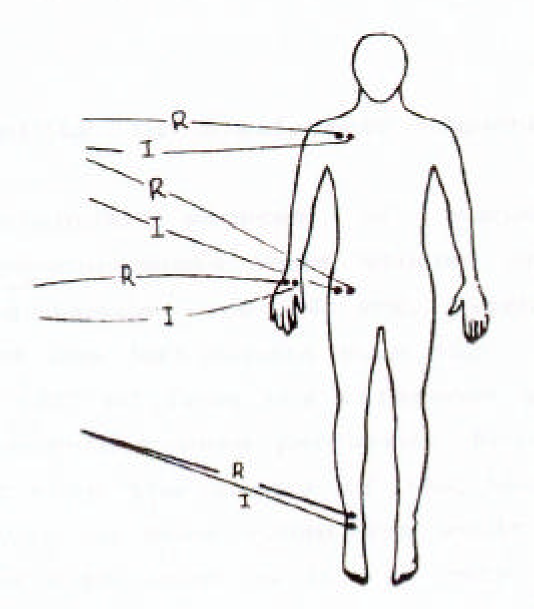


FIGURE 6: Schematic diagram showing the sites where the eight electrodes were put on the right side of the body. I = inducing electrode, R = recording electrode.

recorded using the modified channel 2 (range O-600 O). The impedance measurements along the torso, estimated to be several decades of Ohms, were recorded using channel 1 (range 0-99 O) so as to achieve better accuracy. Two sets of measurements were taken for each subject with an interval of 5-7 min (during which time weight, height and skinfold measurements were taken). After converting each reading to the actual impedance value, using the calibration curves for both channels of the monitor, the mean of the two recorded values was taken as the impedance of that particular part of the body. Finally, the lengths of the arm, torso and leg were measured (between the different pairs of electrodes) to the nearest 0.01 m, using a flexible plastic ruler (Figure 5).

3.5 Variability in Bioelectric Impedance Measurements

Several potential sources of variability in bioelectric impedance measurements were studied on two volunteers, one right-handed female (age 24 yrs. weight 61.3 kg and height 1.681 m) and one left-handed male (age 31 yrs, weight 101.5 kg and height 1.817 m) from the reference group of 20 volunteers. All the experiments were performed during the same day. It is acknowledged that the sample of the two volunteers is rather small however no more volunteers would agree to undergo this part of the experiment, as it was very time consuming, lasting for several hours.

3.5.1. Effect of the Side of the Body (Right or Left)

Eight electrodes were placed on each side of the body (using the same anatomical landmarks as before, § 3.4), taking great care so that the placement was done as symmetrically as possible. Three measurements of bioelectric impedance from-wrist-to-ankle, along the arm, along the torso and along the leg were taken for each subject for both right and left sides. The mean of the three readings was taken as the actual impedance value for the particular part of the body for the particular side.

3.5.2. *Effect of the Respiratory Cycle*

Three impedance measurements were taken from-wrist-to-ankle and along the torso on the right side of the body, in three different parts of the respiratory cycle, with the volunteer in full inspiration, in full expiration and breathing normally in a relaxed position. The mean of the three measurements was then taken as the actual impedance corresponding to each different respiratory situation.

3.5.3. *Effect of Contraction of Muscles*

Three impedance measurements were taken from-wrist-to-ankle and along the arm, on the right side of the body, with the arm relaxed first and then in complete contraction of its muscles The mean of the three values was taken as the actual impedance value corresponding to either relaxation or contraction of arm muscles.

3.5.4. *Effect of the Position of the Body*

Three impedance measurements were taken from-wrist-to-ankle on the right side of the body, with the subject relaxed on a horizontal couch, then with the upper part of the couch raised so as the torso formed an angle of ~45^o with the horizontal and finally with the subject sitting on the couch so as the torso was at right angles to the legs. In all three positions it w as strongly recommended that the volunteer should be as relaxed as possible. The mean of the three measurements was taken as the actual impedance corresponding to each one of the three positions of the body.

3.5.5. Effect of a Drink or Meal prior to Measurement

An impedance measurement was taken from-wrist-to-ankle and along the torso on the right side of the body just before and just after the subject had drunk 1 liter of water. The whole experiment was repeated once more, several hours later. Finally, the impedance from-wrist-to-ankle and along the torso on the right side of the body was measured just before and just after the subject had had a complete meal.

3.6 Statistical Analysis

Statistical analysis (using the correlation coefficient r) was made in order to indicate any possible correlation between the variables measured during the experiment or derived subsequently from the experimental data by calculations.

Therefore, in order to examine if the body mass index can be used as a predictor of body fatness, the values of body mass index for all 20 volunteers were correlated with the values of body fat mass and fat-free mass estimated using the skinfold thickness measurements. The values of fat-free mass were then correlated with the index of fat-free mass as estimated by the impedance measurements along various parts of the body. Comparing the various correlation coefficients of fat-free mass and fat-free mass index as calculated by the impedance measurements of different parts of the body, it would be possible to suggest which part of the body is best to use when measuring bioelectric impedance in order to predict fat-free mass.

The correlation coefficient r is given [29, 30] by:

$$r = \frac{N \sum Xi\,Yi - \left(\sum Xi\right)\left(\sum Yi\right)}{\left[N \sum Xi^{2} - \left(\sum Xi\right)^{2}\right]^{1/2} \left[N \sum Yi^{2} - \left(\sum Yi\right)^{2}\right]^{1/2}} \qquad (3.2)$$

where Xi and Yi are the experimental (direct or derived) values for the pair of variables considered and N is the number of pairs.

The correlation coefficient may range from -1 to +1 and a coefficient of absolute value 1 indicates a perfect correlation between two variables, while a coefficient of zero suggests complete lack of correlation. Varying degrees of correlation are then presented by coefficients ranging from zero to +1 or -1.

To determine whether a value of correlation coefficient is of sufficient magnitude to indicate that the two variables of interest are correlated or it is simply a chance deviation from zero, the ‘null hypothesis’ was used, stating that the population coefficient is equal to zero. Since the specific direction of the difference between the calculated coefficient and the population coefficient (assumed to be zero) is of no concern a two-tailed distribution test was used to test the ‘null hypothesis’. As the number of pairs was N = 20, which is relatively small (N < 30), the Student's - t test was used [29], with N-2 degrees of freedom. The t-value was calculated by:

$$t = r \left[\frac{N - 2}{1 - r^{2}}\right]^{1/2} \qquad (3.3)$$

The critical t-values for N-2 = 18 degrees of freedom and for the preset significance levels (usually 0.05 and 0.01) were obtained from a published table [29], which is reproduced in Appendix C. If the calculated t-value is less than the critical t-value at the 0.05 significance level (considering absolute values of t), the 'null hypothesis' cannot be rejected and the decision is that no significant correlation exists between the two variables. If the calculated t-value exceeds the critical one at the 0.05 level but not the one at the 0.01, there is reasonable evidence (at the 0.05 level) that a real correlation exists between the two variables. In case the calculated t-value exceeds the critical one at the 0.01 level, there is almost conclusive evidence that the correlation coefficient is of sufficient magnitude to indicate a true correlation between the two variables.

To determine if bioelectric impedance measurements are significantly affected by the various factors listed in §3.5, the mean of impedance measurements along any part of the body, taken under normal conditions, was compared to the mean of-impedance measurements along the same part of the body, taken after having altered some factor (such as side of the body or relaxation degree of muscles etc.). The 'null hypothesis' was used for the comparison, stating that the difference between the two means $\overline{X}_1$ and $\overline{X}_2$ is zero. The pooled variance, $S_?^2$, was then calculated:

$$S_?^2 = \frac{\sum (X_1 - \overline{X}_1)^2 + \sum (X_2 - \overline{X}_2)^2}{N_1 + N_2 - 2} \qquad (3.4)$$

where X_1, $\overline{X}_1$ and N_1 are impedance measurements, mean of impedance measurements and number of measurements, respectively, taken under normal conditions, and X_2, $\overline{X}_2$ and N_2 are impedance measurements, mean of impedance measurements and number of measurements, respectively, taken after having altered some factor.

The standard error of differences between means, $S_{\overline{X}_1 ? \overline{X}_2}$, was then calculated:

$$S_{\overline{X}_1 ? \overline{X}_2} \; ? \; ? \frac{S?^2}{N_1} \; ? \; \frac{S?^2}{N_2} ?^{1/2} \qquad (3.5)$$

and finally the t-value was found:

$$t = \frac{\overline{X}_1 - \overline{X}_2}{S_{\overline{X}_1 ? \overline{X}_2}} \qquad (3.6)$$

Considering that the degrees of freedom are N_1+N_2-2 the critical t-value was obtained from the table in Appendix C for the preset significance level. A calculated t-value less than the critical one indicates failure to reject the ‘null hypothesis’, thus no statistically significant (at the preselected significance levels) difference between the two mean values exists and, as a result, the particular factor altered does not affect significantly the impedance measurements. On the other hand, calculated t-value equal to or greater than the critical one indicates a significant (at the preselected significance levels) difference between the two means.

4. RESULTS

Throughout the tabulation of all variables (either directly measured during the experiment or subsequently calculated), each individual volunteer is represented by a serial number from 1 to 20. The same number is always ascribed to the same volunteer throughout the whole presentation of the results and it represents the order in which every volunteer was examined during the experiment.

General descriptive and anthropometric characteristics for all the volunteers are provided in Table 1.

TABLE 1

no.	sex	age (yr)	weight (kg)	height (m)
1	m	24	68.3 ? 0.3	1.822 ? 0.001
2	m	21	74.5 ? 0.0	1.805 ? 0.001
3	m	23	73.8 ? 0.2	1.721 ? 0.002
4	m	24	70.0 ? 0.0	1.729 ? 0.001
5	f	23	59.5 ? 0.0	1.681 ? 0.002
6	m	33	74.3 ? 0.2	1.840 ? 0.002
7	f	24	59.1 ? 0.2	1.716 ? 0.002
8	f	23	52.0 ? 0.0	1.631 ? 0.003
9	m	31	101.5 ? 0.4	1.817 ? 0.002
10	f	24	61.3 ? 0.2	1.681 ? 0.002
11	m	23	73.3 ? 0.2	1.790 ? 0.002
12	f	25	57.0 ? 0.0	1.706 ? 0.001
13	m	24	64.0 ? 0.0	1.799 ? 0.002
14	m	29	72.5 ? 0.0	1.705 ? 0.001
15	f	23	63.7 ? 0.3	1.767 ? 0.001
16	m	24	68.0 ? 0.0	1.796 ? 0.002
17	m	23	73.7 ? 0.2	1.803 ? 0.002
18	m	23	75.7 ? 0.2	1.739 ? 0.002
19	m	27	78.0 ? 0.0	1.779 ? 0.001
20	m	25	66.0 ? 0.0	1.741 ? 0.001

TABLE 1: General descriptive characteristics (sex and age) and means and standard deviations (N=3) for weight and height for 20 volunteers consisting the reference group.

The mean values and standard deviations for the physical characteristics of the group of volunteers as a whole, as well as the range values for each variable presented in Table 1 are provided in Table 2.

TABLE 2		
variable	**range**	**mean ? SD**
age (yr)	21 –33	24.8 ? 2.9
weight (kg)	52.0 – 101.5	69.1 ? 10.1
height (m)	1.631 – 1.840	1.753 ? 0.055

TABLE 2: Range, mean value and standard deviation (N=20) for the physical characteristics (age, weight, and height) of the refence group as whole.

Measurements (mean value and standard deviation of two subsequent measurements) of the skinfold thickness at subscapular, suprailiac, triceps and biceps sites, as well as the sum of all four skinfold thickness measurements, for every volunteer are provided in Table 3.

TABLE 3					
no	**subscapular (mm)**	**suprailiac (mm)**	**triceps (mm)**	**biceps (mm)**	**sum (mm)**
1	9.5 ? 0.1	13.6 ? 0.4	6.3 ? 0.1	4.4 ? 0.0	33.8 ? 0.4
2	9.0 ? 0.0	13.9 ? 0.5	9.3 ? 0.1	3.9 ? 0.0	36.1 ? 0.5
3	11.3 ? 0.1	24.7 ? 0.9	13.2 ? 0.2	6.5 ? 0.1	55.7 ? 0.9
4	15.6 ? 0.2	26.9 ? 0.5	15.2 ? 0.4	8.1 ? 0.3	65.8 ? 0.7
5	23.3 ? 0.7	30.6 ? 0.2	20.8 ? 0.4	6.5 ? 0.3	81.2 ? 0.8
6	9.3 ? 0.3	18.3 ? 0.3	11.0 ? 0.6	3.9 ? 0.1	42.5 ? 0.8
7	13.0 ? 0.4	14.4 ? 0.8	11.6 ? 0.2	5.4 ? 0.3	44.4 ? 1.0
8	10.4 ? 0.2	8.9 ? 0.3	10.8 ? 0.4	6.8 ? 0.4	36.9 ? 0.6
9	28.6 ? 1.0	32.7 ? 0.1	17.1 ? 0.5	10.6 ? 0.2	89.0 ? 1.1
10	8.5 ? 0.1	10.0 ? 0.4	14.9 ? 0.5	6.0 ? 0.2	39.4 ? 0.6
11	12.1 ? 0.5	18.0 ? 0.6	9.1 ? 0.5	6.7 ? 0.0	45.9 ? 1.0
12	13.1 ? 0.1	17.2 ? 0.6	15.3 ? 0.3	7.6 ? 0.5	53.2 ? 0.7
13	9.6 ? 0.0	14.8 ? 0.2	9.9 ? 0.3	5.5 ? 0.2	39.8 ? 0.5
14	10.7 ? 0.1	15.4 ? 0.4	9.8 ? 0.4	5.3 ? 0.3	41.2 ? 0.8
15	11.8 ? 0.2	20.7 ? 1.1	14.4 ? 0.6	7.3 ? 0.5	54.2 ? 1.3
16	9.6 ? 0.0	8.6 ? 0.4	7.3 ? 0.1	4.3 ? 0.3	29.8 ? 0.4
17	11.3 ? 0.1	19.9 ? 1.1	15.8 ? 0.2	9.0 ? 0.1	56.0 ? 1.2
18	7.2 ? 0.0	9.4 ? 0.2	5.6 ? 0.0	2.9 ? 0.4	25.1 ? 0.2
19	10.5 ? 0.1	20.0 ? 0.4	9.7 ? 0.3	4.4 ? 0.1	44.6 ? 0.6
20	8.5 ? 0.1	9.0 ? 0.2	7.2 ? 0.4	5.1 ? 0.4	29.8 ? 0.5

TABLE 3: Skinfold thickness measurements for subscapular, suprailiac, triceps and biceps and the sum of all four skinfold measurements for each volunteer.

The range, mean value and standard deviation for the skinfold measurements at the four selected sites and their sum for the reference group of 20 volunteers are summarized in Table 4. In Table 4, the error (intra-examiner error) in measuring the skinfold thickness for each selected site for skinfold measurements is also provided. The intra-examiner error has been calculated using the method proposed by Jonhston et al (1978) [2], as the standard deviation of the differences between two consecutive measurements by the same investigator divided by the square root of 2.

TABLE 4

variable	**range**	**mean ? SD**	**error**
subscapular (mm)	7.2 – 28.6	12.2 ? 5.0	0.4
suprailiac (mm)	8.6 – 32.7	17.4 ? 6.9	0.4
triceps (mm)	5.6 – 20.8	11.7 ? 3.9	0.3
biceps (mm)	2.9 – 10.6	6.0 ? 1.9	0.2
sum of all (mm)	25.1 – 89.0	47.2 ? 1.6	0.6

TABLE 4: Range, mean and standard deviation, as well as the intra-examiner error for skinfold thickness at the four selected sites and their sum, for the group of 20 volunteers.

The impedance measurements form-wrist-to-ankle, Z_{tot}, along the arm, Z_{arm}, along the torso, Z_{torso}, and along the leg, Z_{leg}, for each volunteer are given in Table 5. The impedance values presented are the mean of two measurements. The calibration curves for channel 1 and 2 of the Gastric Impedance Monitor (Appendix A) were used to transform each value into the actual impedance value, before any calculation for the mean was made. The error given for each impedance value is due to the digitization error of the two channels of the monitor (the digital display was not linearly related to the actual impedance between the recording electrodes for each channel). The sum of the three segmental impedance measurements, Z_{sum}, is also given in Table 5, as well as the difference, ? Z, between the sum of the three segmental impedance measurements and the impedance from-wrist-to-ankle.

TABLE 5						
no	**Z_{tot} (O)**	**Z_{arm} (O)**	**Z_{torso} (O)**	**Z_{leg} (O)**	**Z_{sum} (O)**	**? Z (O)**
1	454 ? 5	214 ? 3	62 ? 1	211 ? 3	487 ? 7	33 ? 8
2	391 ? 4	187 ? 2	42 ? 1	205 ? 3	434 ? 7	43 ? 8
3	378 ? 4	176 ? 2	52 ? 1	196 ? 2	424 ? 7	46 ? 8
4	472 ? 5	231 ? 3	55 ? 1	249 ? 3	535 ? 7	63 ? 8
5	510 ? 5	249 ? 3	60 ? 1	254 ? 3	563 ? 7	53 ? 8
6	440 ? 4	211 ? 3	52 ? 1	245 ? 3	508 ? 7	68 ? 8
7	506 ? 5	272 ? 3	51 ? 1	228 ? 3	551 ? 7	45 ? 8
8	451 ? 4	251 ? 3	40 ? 1	196 ? 2	487 ? 7	36 ? 8
9	368 ? 4	202 ? 3	78 ? 2	199 ? 3	479 ? 7	111 ? 8
10	465 ? 4	234 ? 3	44 ? 1	226 ? 3	504 ? 7	39 ? 8
11	402 ? 4	223 ? 3	46 ? 1	193 ? 2	462 ? 7	60 ? 8
12	593 ? 5	340 ? 4	47 ? 1	257 ? 3	644 ? 7	51 ? 8
13	493 ? 4	255 ? 3	46 ? 1	270 ? 3	571 ? 7	78 ? 8
14	381 ? 4	196 ? 2	47 ? 1	196 ? 3	439 ? 7	58 ? 8
15	498 ? 4	282 ? 3	51 ? 1	228 ? 3	561 ? 7	63 ? 8
16	444 ? 4	234 ? 3	38 ? 1	209 ? 3	481 ? 7	37 ? 8
17	479 ? 4	251 ? 3	55 ? 1	242 ? 3	548 ? 7	69 ? 8
18	317 ? 4	155 ? 2	29 ? 1	165 ? 3	349 ? 7	32 ? 8
19	352 ? 4	171 ? 2	39 ? 1	187 ? 3	397 ? 7	45 ? 8
20	410 ? 4	196 ? 2	38 ? 1	217 ? 3	451 ? 7	41 ? 8

TABLE 5: Impedance measurements form-wrist-to-ankle, along the arm, along the torso, and along the leg, the sum pf the three segmental impedance measurements and the difference between the sum and the impedance from-wrist-to-ankle, for 20 volunteers.

The range, mean value and standard deviation for the impedance measurements for the group of 20 volunteers are summarized in Table 6.

The measurements of the length of arm, l_{arm}, torso, l_{torso}, and leg, l_{leg}, and their sum, l_{sum}, for each volunteer are shown in Table7.

TABLE 6		
variable	**range**	**mean ? SD**
Ztot (O)	317 – 593	440 ? 64
Zarm (O)	155 – 282	226 ? 42
Ztorso (O)	29 – 78	49 ? 10
Zleg (O)	165 - 270	219 ? 27

TABLE 6: Range, mean and standard deviation for impedance measurements along different parts of the body for the group of 20 volunteers.

TABLE 7				
no	**larm (m)**	**ltorso (m)**	**lleg (m)**	**lsum (m)**
1	0.59	0.49	0.90	1.98
2	0.60	0.42	0.98	2.00
3	0.58	0.45	0.88	1.91
4	0.57	0.44	0.90	1.91
5	0.55	0.41	0.92	1.88
6	0.62	0.43	1.01	2.06
7	0.61	0.40	0.94	1.95
8	0.52	0.45	0.82	1.79
9	0.60	0.50	0.96	2.06
10	0.62	0.43	0.93	1.98
11	0.63	0.44	0.94	2.01
12	0.62	0.44	0.88	1.94
13	0.64	0.48	0.96	2.08
14	0.56	0.40	0.88	1.84
15	0.58	0.44	0.95	1.97
16	0.63	0.42	0.97	2.06
17	0.60	0.46	0.97	2.03
18	0.59	0.41	0.91	1.91
19	0.59	0.45	0.98	2.02
20	0.57	0.44	0.94	1.95

TABLE 7: Lengths of the arm, torso and leg and sum for each of the 20 volunteers in the control group.

The body mass index, m/h^2, was calculated for every volunteer and the results are shown in Table 8. The sum of all four skinfold measurements was corrected for the double skin thickness 1.8 mm (under a pressure of 10 gmm^{-2}) and was transformed using the log$_{10}$ transformation. The values of the transformed sum of four skinfold measurements were used to calculate total body density, d, using Equation (2.1). The values of the constants a and b (which are sex and age dependent) were taken from published tables [1] which are reproduced in Appendix B. The body fat fraction for every volunteer was then calculated by the total body density (Equation 2.3). Finally, fat-free mass (Equation 2.4) and fat mass were calculated for each volunteer. The derived values for total body density, fat fraction, fat-free mass and fat mass are given in Table 8, together with the calculated body mass index.

TABLE 8

no	**body mass index**	**density kg-3**	**body fat fraction**	**fat free mass (kg)**	**body fat mass (kg)**
1	20.6	1073.4	0.111	60.7	7.6
2	22.9	1070.8	0.123	65.7	9.2
3	24.9	1056.9	0.184	60.2	13.6
4	23.4	1051.2	0.209	55.4	14.6
5	21.1	1025.8	0.325	40.2	19.3
6	21.9	1057.9	0.179	61.0	13.3
7	20.1	1047.3	0.226	45.7	13.4
8	19.5	1054.5	0.194	41.9	10.1
9	30.6	1038.3	0.267	74.4	17.1
10	21.7	1051.6	0.207	48.6	12.7
11	22.9	1062.6	0.158	61.7	11.6
12	19.6	1040.9	0.255	42.5	14.5
13	19.8	1067.7	0.136	55.3	8.7
14	24.9	1066.4	0.142	62.2	10.3
15	20.4	1040.2	0.259	47.2	16.5
16	21.1	1077.8	0.093	61.7	6.3
17	22.7	1056.3	0.186	59.9	13.8
18	25.0	1084.1	0.066	70.7	5.0
19	24.6	1063.9	0.153	66.1	11.9
20	21.8	1077.8	0.093	59.9	6.1

TABLE 8: Body mass index and total body density, body fat fraction, fat-free mass and body fat mass, as estimated by the skinfold thickness measurements.

4.1 Body Mass Index Measurements

The correlation coefficient between body mass index and fat-free mass (as estimated by skinfold measurements) was found to be r=0.760, which is of sufficient magnitude ($p<0.01$) to indicate a good correlation. However, the correlation coefficient between body mass index and body fat mass was found to be r=0.432, which gives a reasonable evidence ($p<0.05$), though not a conclusive one ($p>0.01$), that there is some correlation between the two variables. The body mass index has been plotted against fat-free mass and body fat mass in Diagrams 1 and 2, respectively.

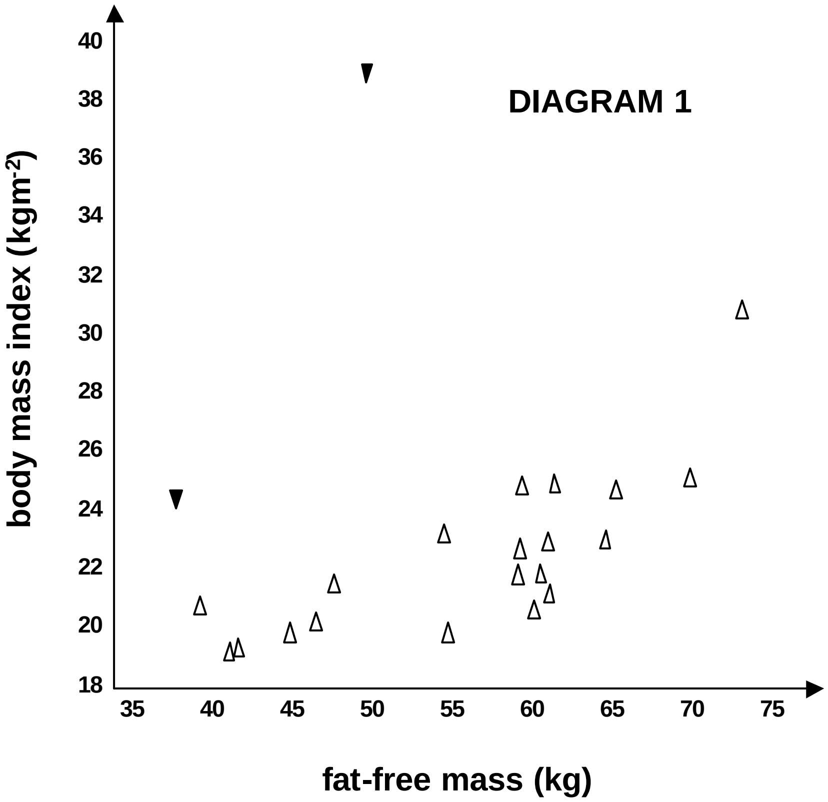


DIAGRAM 1: Body mass index versus fat-free mass (kg), estimated from skinfold measurements. The blank triangles indicate subjects from the reference group, while the black ones the two rather obese, elder subjects.

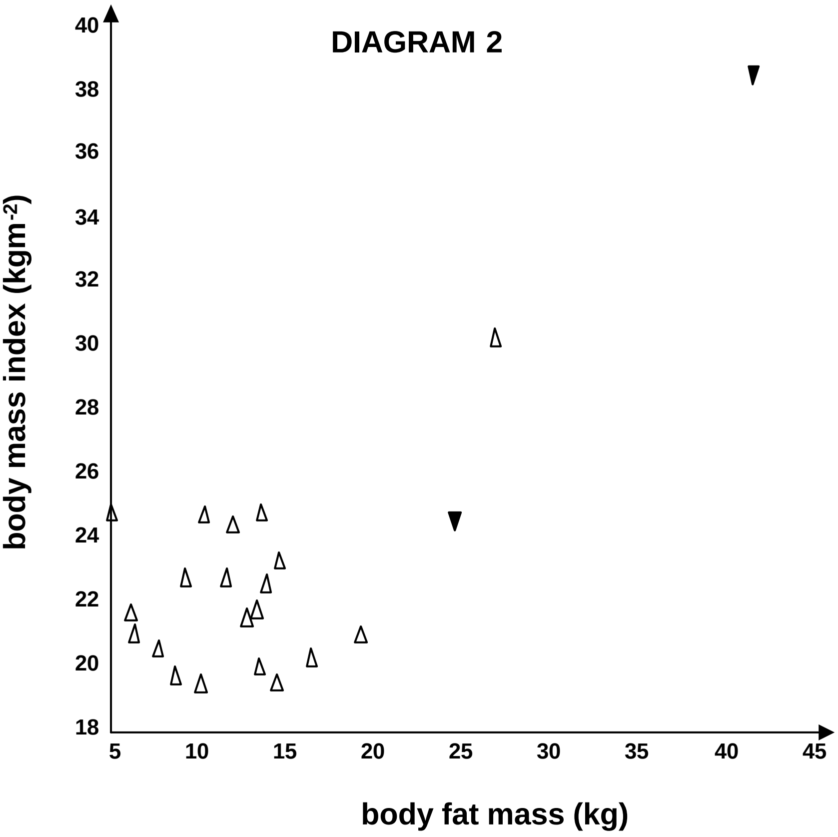


DIAGRAM 2: Body mass index versus body fat mass (kg), as estimated from the skinfold measurements. The blank triangles indicate subjects from the reference group, while the black ones the two rather obese, elder volunteers.

4.2 Bioelectric Impedance Measurements

The correlation coefficient between fat-free mass (as estimated by skinfold thickness measurements) and the fat-free mass index, ht^2/Z_{tot}, was found to be r=0.938, which indicates good correlation ($p<0.001$) between the two variables. Fat-free mass index, calculated using the total body impedance measurements has been plotted against fat-free mass, estimated by skinfold thickness measurements, in Diagram3.

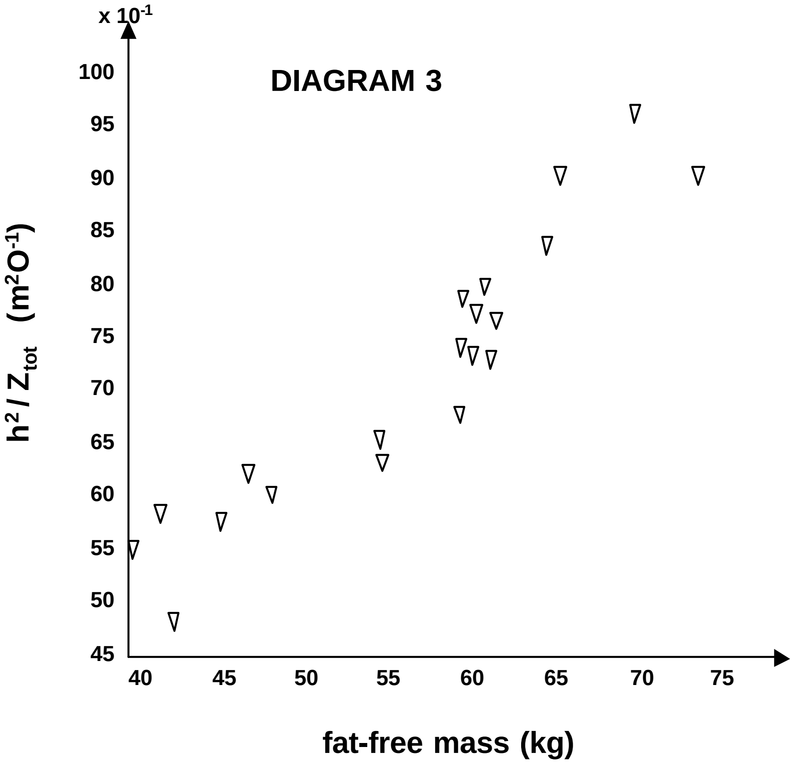


DIAGRAM 3: Fat-free mass index (in m^2O^{-1}), as calculated by total body impedance measurements, versus the fat-free mass (in kg), as estimated from skinfold measurements.

Considering the bioelectric impedance measurements along parts of the body, i.e. along the arm, torso and leg, as well as the lengths of these parts of the body, segmental fat-free mass indices can be calculated, l_i^2/Z_i, where i = arm, torso, or leg. The correlation coefficients between fat-free mass estimated from skinfold thickness measurements and each one of the fat-free mass indices l_i^2/Z_i were found to be:

i) l_{arm}^2/Z_{arm}, r=0.853, which indicates statistically good correlation (p<0.01)

ii) l_{torso}^2/Z_{torso}, r=0.257, which indicates that no statistically significant correlation exists between the two variables, and

iii) l_{leg}^2/Z_{leg}, r=0.787, which indicates statistically good correlation (p<0.01)

When, instead of the height, h, the sum of the segmental lengths, l_{sum}, was used to calculate the fat-free mass index from total body impedance, the correlation coefficient with fat-free mass was found r=0.924, which provides conclusive evidence (p<0.001) of correlation. The sum of segmental fat free mass indices, $? \left(l_i^2 / Z_i \right)$, when correlated with fat-free mass, gave r=0.660, which provides reasonable evidence (p<0.01) that a correlation, though poor, exists.

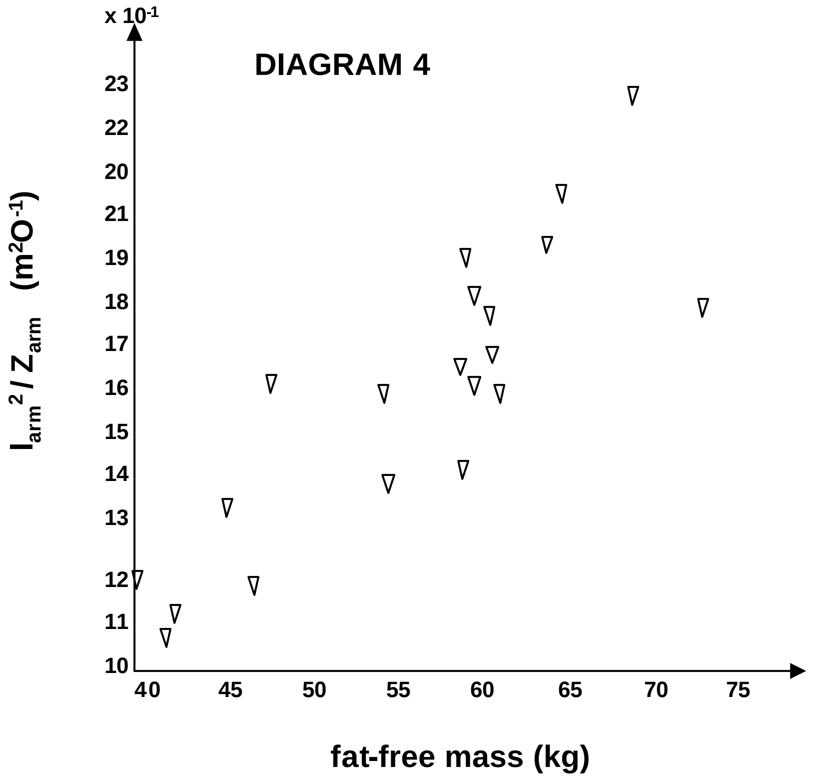
Table 9 summarizes the correlation coefficients found between fat-free mass and the various fat-free mass indices.

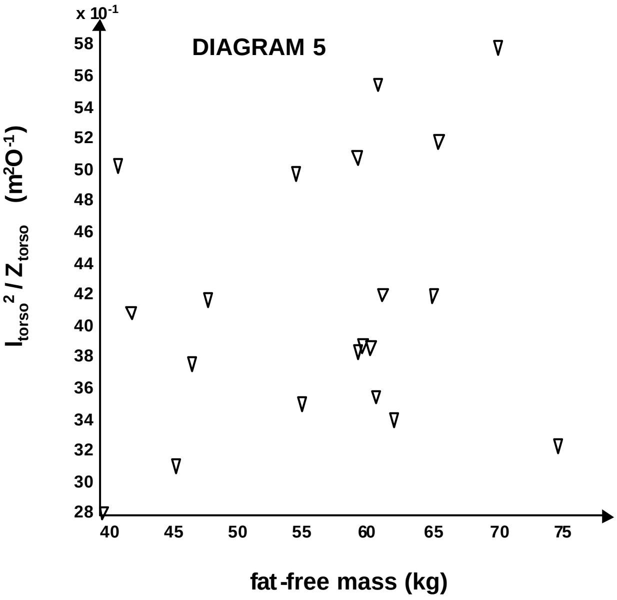
An interesting observation was made when the total impedance, Z_{tot}, was compared with the sum of the three segmental impedance measurements, Z_{sum}, for every individual volunteer. It could be expected that Z_{tot} and Z_{sum} would be approximately of the same value (within the inaccuracy introduced in the experimental procedure). However, as it is obvious from Table 5, Z_{sum} was significantly larger than Z_{tot} for all volunteers. When this difference ? Z was correlated with fat-free mass, the correlation coefficient was found r=0.182, which indicates that there is no significant correlation (p>0.2). However, the correlation coefficient between ? Z and body fat mass was found to be r=0.730, which gives conclusive evidence (p<0.001) that there is a real correlation between the two variables.

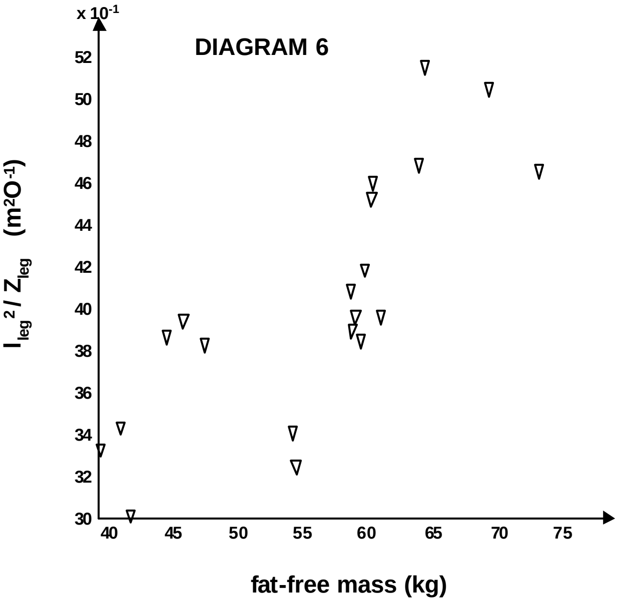
TABLE 9

fat free mass indices	correlation coefficient with fat free mass	
h^2/Z_{tot}	0.938,	$p < 0.001$
l_{arm}^2/Z_{arm}	0.853,	$p < 0.001$
l_{torso}^2/Z_{torso}	0.257,	$p > 0.2$
l_{leg}^2/Z_{leg}	0.787,	$p < 0.001$
$(Sl)^2/Z_{tot}$	0.924,	$p < 0.001$
$S(l_i^2/Z_i)$	0.660,	$0.01 < p < 0.001$

TABLE 9: Correlation coefficients between fat-free mass, estimated by skinfold thickness measurements, and various fat-free mass indices, calculated from bioelectric impedance measurements along different parts of the body.







DIAGRAMS 4, 5, and 6: Fat-free mass indices (m2O-1), calculated from segmental bioelectric impedance measurements along the arm (4), along the torso (5) and along the leg (6), versus fat free mass (kg) as estimated from skinfold measurements.

4.3 Variability in Bioelectric Impedance Measurements

General descriptive characteristics and anthropometric variables for the two volunteers participated in this part of the experiment are given in Table 10.

TABLE 10				
no.	**sex**	**age (yr)**	**weight (kg)**	**height (m)**
1	m	31	101.5 ? 0.4	1.818 ? 0.002
2	f	24	61.3 ? 0.2	1.681 ? 0.002

TABLE 10: General descriptive characteristics (sex and age) and weight and height measurements for both subjects participating in the experiment made to test the variability of impedance measurements.

The mean value of impedance measurements along different parts of the body on the right and on the left side for both volunteers are shown in Table 11.

TABLE 11				
	Volunteer 1		**Volunteer 2**	
imped.	**R-side**	**L-side**	**R-side**	**L-side**
Ztot	399 ? 3	395 ? 3	517 ? 3	518 ? 3
Zarm	208 ? 3	207 ? 3	273 ? 3	275 ? 3
Ztorso	86 ? 1	87 ? 1	50 ? 1	49 ? 1
Zleg	222 ? 3	220 ? 3	242 ? 3	240 ? 3

TABLE 11: The values tabulates are bioelectric impedance measurements (O), along various parts of the body, in the right and on the left side, for both volunteers.

Comparing the impedance measurements on the right side with that on the left for each part of the body and for both volunteers, no statistically significant difference (p>0.2) was found.

The mean value of impedance measurements from-wrist-to-ankle and along the torso on the right side of the body, in three different parts of the respiratory cycle (breathing normally, full inspiration and full expiration) are shown in Table 12.

TABLE 12

	volunteer 1			**volunteer 2**		
imped.	**normal**	**inspiration**	**expiration**	**normal**	**inspiration**	**expiration**
Z_{tot}	399 ? 3	421 ? 3	398 ? 3	517 ? 3	539 ? 3	517 ? 3
Z_{torso}	86 ? 1	95 ? 1	85 ? 1	50 ? 1	54 ? 1	50 ? 1

TABLE 12: Bioelectric impedance measurements (O), from-to-wrist-to-ankle and along the torso, on the right side of the body at different parts of the respiratory cycle.

Comparing the impedance values taken when the subject was breathing normally with those taken at full inspiration, a statistically significant increase ($p<0.01$) in the bioelectric impedance from-wrist-to-ankle and along the torso for both volunteers was quoted during the full inspiration. The increase in the impedance during full inspiration was ~5% for Z_{tot} and ~9% for Z_{torso}. However, no statistically significant difference ($p>0.2$) was found between impedance measurements taken with the subject breathing normally and those taken with the subject at full expiration.

The mean value of impedance measurements from-wrist-to-ankle and along the arm, on the right side of the body, with the arm relaxed and with the arm muscles in full contraction are shown in Table 13.

TABLE 13				
	volunteer 1		volunteer 2	
imped.	**relaxed**	**contracted**	**relaxed**	**contracted**
Z_{tot}	399 ? 3	368 ? 3	517 ? 3	479 ? 3
Z_{arm}	208 ? 3	185 ? 3	273 ? 3	237 ? 3

TABLE 13: Bioelectric impedance measurements (O) from-wrist-to-ankle and along the arm with the arm relaxed or fully contracted.

A statistically significant difference ($p<0.01$) was found between impedance measurements taken with the subject's arm relaxed and contracted. The impedance decreased when the arm was contracted, ~7% of the initial value for Z_{tot} and ~12% for Z_{arm}.

The mean value of impedance measurements from-wrist-to-ankle, on the right side of the body, with the subject lying on a horizontal couch, with the subject lying on a couch so that the torso formed 45^o angle with the legs and with the subject sitting so as the torso formed 90^o angle with the legs, are shown in Table 14.

TABLE 14			
no.	**horizontally**	**torso at 45^o**	**torso at 90^o**
1	399 ? 3	399 ? 3	398 ? 3
2	517 ? 3	516 ? 3	516 ? 3

TABLE 14: Bioelectric impedance measurements (O) from-wrist-to-ankle for different positions of the body.

Comparison of the impedance values taken with the subject lying on a horizontal couch with those taken with the subject lying on the semi-raised couch or sitting on the couch showed no statistically significant difference ($p>0.2$).

The impedance measurements from-wrist-to-ankle and along the torso, on the right side of the body, with the subject fasting and just after the subject had had a drink (1 liter of water) or meal are shown on Table 15.

TABLE 15						
	volunteer 1			**volunteer 2**		
imped.	**fasting**	**after drink**	**after meal**	**fasting**	**after drink**	**after meal**
Z_{tot}	399 ? 3	401 ? 3	402 ? 3	517 ? 3	517 ? 3	516 ? 3
Z_{torso}	86 ? 1	85 ? 1	86 ? 1	50 ? 1	49 ? 1	49 ? 1

TABLE 15: Bioelectric impedance measurements (O) from-wrist-to-ankle and along the torso prior to and just after a drink or meal.

No statistically significant difference ($p>0.2$) was found between impedance measurements form-wrist-to-ankle and along the torso taken prior to or just after the subject had had a drink or a complete meal.

5. DISCUSSION

5.1 Body Mass Index

Body mass index, m/h^2, has often been identified as an index of obesity and considered to reflect aspects of body size and composition [13]. Several studies have been made correlating the body mass index to various anthropometric variables, such as weight, height or circumference of different parts of the body, as well as to skinfold thickness. An optimal criterion to render m/h^2 as an index of obesity has bean considered to be its high correlation with skinfold thickness measured at various sites of the body. Several such correlation coefficients in the range of 0.60-0.80 have been reported [13, 31]. However, in the view of the fact that obesity refers to the proportion of an individual's body weight that is attributed to body fat and since skinfold thickness is not linearly and directly related to body f at mass [l, 17], it would be of greater validity to correlate m/h^2 directly to body fat mass rather than to skinfold thickness.

Such a correlation was attempted during this study. The body fat mass was indirectly calculated from skinfold thickness measurements. There was found to exist a poor correlation ($r = 0.431$, $0.001<p<0.05$) between body mass index and body fat. However, the correlation with the fat-free mass appeared to be considerably stronger ($r = 0.760$, $p<0.001$).

The results listed in Table 8, as well as in Diagram 2, show that quite often individuals with a higher value of body mass index presented a greater adiposity than individuals with a lower body mass index. However, there appeared several subjects with small values of body fat but high m/h^2. The better correlation of m/h^2 with fat-free mass (as it is also shown in Diagram 1) leads to the result that body mass index could he a predictor of fat-free mass but not of body fat, at least in population groups with similar general characteristics (age, weight, height) to the ones of the group of volunteers who participated in this study.

5.2 Skinfold Thickness

Measurements of skinfold thickness have been widely used since 1948 to give an estimate of body fat and body composition by those concerned with nutrition, fat distribution or general anthropometric surveys. During the recent years, it has been established as a standard technique to assess gross body composition.

In this study, skinfold thickness was used to estimate total body density and, thus, calculate body fat and fat-free mass. This was regarded as a standard and approved method to which other methods (body mass index and bioelectric impedance measurements) were compared for evaluation purposes.

However, there are some significant limitations in accepting skinfold measurements as a fully reliable technique to assess body composition. The equation used to derive body density from transformed skinfold thickness (Eq. 2.1), has been experimentally developed using data from a specific population, therefore it could be population dependent [21]. The same consideration applies to the equation used to derive the body fat fraction from total body density (Eq. 2.3) [2]. Considering the fact that the compressibility of subcutaneous tissue varies with gender, age nutritional and health condition (especially with regard to formerly obese subjects or those with oedematous conditions [32]) and that a proper fold cannot be raised in fat or obese people, skinfold measurements are valid only in age-specified groups of normal and healthy subjects (which was generally the case in this project).

During the experiment, the intra-examiner error (i.e. between different measurements) was calculated (Eq. 4.1, Table 4) to be 0.4, 0 .4, 0.3, and 0.2 mm for subscapular, suprailiac, triceps and biceps skinfolds, respectively, which is less than or equal to values usually reported (0.88-1.16, 0.3-l.0, 0.4-0.8, and 0.2-0.6 mm, respectively) by various investigators [21].

5.3 Bioelectric Impedance

Bioelectric impedance measurements as predictors of fat-free mass were evaluated by comparison to fat-free mass values estimated using skinfold thickness measurements. As most composition studies using bioelectric impedance have suggested, it was found that the index h^2/Z_{tot} was highly correlated with fat-free mass (r = 0.938). However, this approach would not be useful in the elderly or in chair- or in bed-patients for whom an accurate measurement of standing height is difficult to obtain. In such cases, segmental impedance measurements combined with the length of the respective segment, if valid, would be of great practical value for the assessment of body composition. Correlation of three segmental indices, l_{arm}^2/Z_{arm}, l_{torso}^2/Z_{torso}, and l_{leg}^2/Z_{leg}, with fat-free mass have shown that impedance measurements along the limbs, especially along the arm, could be used to predict fat-free mass in cases where h^2/Z_{tot} could not be calculated. Although the correlations of l_{arm}^2/Z_{arm} (r = 0.853) and l_{leg}^2/Z_{leg} (r=0.787) with fat-free mass are not so strong as that of h^2/Z_{tot} (r = 0.938), they are still good enough to allow segmental impedance measurements along limbs to be used in body composition studies when h^2/Z_{tot} can not be calculated. On the contrary, the index l_{torso}^2/Z_{torso}, calculated from the impedance along the torso showed no statistically significant correlation with fat-free mass (r = 0.257). This could be due to the complex internal structure of the torso. The electric current may not be conducted in the same simple way as it is conducted in the tube-like limbs, thus producing a complex pathway whose length can not be approximated by the length of the torso, l_{torso}.

When standing height, h, was substituted by the sum of lengths of the three segments, l_{arm} + l_{torso} + l_{leg}, in the index h^2/Z_{tot}, the high correlation with fat-free mass was not significantly reduced (Table 9). Therefore, when standing height is difficult to be measured, the sum of the lengths of arm, torso and leg can be used with total body impedance measurements to estimate fat-free mass.

An interesting observation made during the experiment was that the impedance along the whole body, Z_{tot}, was significantly less than the sum of the three segmental impedance measurements, $Z_{arm} + Z_{torso} + Z_{leg} = Z_{sum}$, for all volunteers. The difference was significantly correlated (r = 0.730) with body fat mass. A possible explanation may be based on the fact that as the current is induced into the body by the surface electrode, it has to be conducted through a part of subcutaneous adipose tissue before it diffuses within the deeper tissues, thus it confronts an additional impedance z due to that extra part of adipose (non-ionic) tissue. This impedance z is included in every body impedance measurement, thus it is included three times in the Z_{sum} while only once in Z_{tot}. To support, or otherwise, this proposed explanation, the difference $Z_{sum}–Z_{tot}$ could be correlated with skinfold thickness measured at the sites where the current is induced into the body, that is pectoral (chest) and abdominal skinfolds, (as the induction of the current at wrist and ankle is common for both Z_{tot} and Z_{sum} measurements).

5.4 Variability in Bioelectric Impedance Measurements

Several factors have been studied as potential sources of variability in bioelectric impedance measurements. It must be mentioned that the experiments were made on an extremely small group (only two volunteers), thus the results should rather be considered more as an indication and not as a statistically significant proof.

One factor that could affect impedance measurements could be the side of body (right or left) where the measurements are taken. However, no significant difference was observed between similar measurements on the opposite sides of the body for both volunteers (one of them being right-handed while the other one left-handed).

The respiratory cycle proved to affect impedance measurements Although no difference was observed when the subject was breathing normally or was in full expiration, during full inspiration an increase of ~5% (from-wrist-to-ankle) and ~9% (along the torso) of the initial value was quoted for impedance measurements. Therefore, care should be taken that the subject does not breathe deeply during impedance measurements. The effect that the respiratory cycle has on the impedance measurements makes the latter a potential method for respiratory monitoring.

Significant variability could also be induced by different degrees of relaxation of muscles. Full contraction of the arm muscles was found to cause a decrease of ~12% (along the arm) and ~7% (from-wrist-to-ankle) of the initial value of impedance measurements.

The position of the body (supine or lying with the torso at 45^o or at 90^o angle to the horizontal) has not been found to affect impedance measurements from-wrist-to-ankle, provided that the subject was always as relaxed as possible.

Finally, no significant difference was found in impedance values from-wrist-to-ankle and along the torso taken prior to or just after the subject had had a drink or meal. This is in accordance with other relevant published data about the accuracy and precision of bioelectric impedance measurements for estimating body composition [33].

Bioelectric impedance measurements were found to be independent of several factors, such as side of the body, position of the body, fasting condition of the subject, while they were significantly affected by full inspiration and muscle contraction. Another potential source of variability could be the accurate placement of the electrodes according to the anatomical landmarks used [11]. However, this factor has not been studied during this experiment, because it would require a large number of the disposable electrodes, raising the total cost of the project.

6. CONCLUSIONS

Body mass index, m/h^2, proposed by several investigators as an obesity index [12, 13], proved to statistically correlate better with fat-free mass than with body fat (both as estimated by skinfold thickness measurements) for the twenty volunteers consisting the reference group. Since m/h^2 appeared to be a better indicator, though rather poor, of fat-free mass rather than of body fat, it would be a misnomer to refer to it as an obesity index, while it would be better to consider it as an indicator of body size rather than body composition. However, during this study, body mass index as a predictor of body fat was evaluated only for normal subjects. It would be interesting to examine if and how this index is related with body fat in obese subjects or even extremely lean ones. If such a study is attempted, body mass index should be correlated with fat-free mass and body fat estimated by a method other than skinfold thickness measurements (such as densitometry or helium dilution method) because it is extremely difficult to raise and measure properly a fold of skin in obese subjects.

The results of the experiments on bioelectric impedance measurements justified the wide use of the impedance from-wrist-to-ankle to produce an index (h^2/Z_{tot}) of the total body fat-free mass, at least for normal, healthy young subjects. This method was again evaluated using as a reference the estimates for body composition from skinfold thickness. As skinfold measurements in body composition studies have their own considerable limitations [6], it would be wise to compare and evaluate impedance measurements also with some other methods for assessing body composition. One good choice would be to estimate fat-free mass by measuring the amount of potassium present in the body (as it is an intracellular cation which is not present in stored fat and it is found in relatively constant concentrations, 2.46 and 2.28 g potassium per kg of fat-free residue mass in men and women, respectively [6]). Total body potassium can be measured non-invasively, by external counting of its radioactive isotope ^{40}K, which emits a characteristic ?-ray at 1.46 MeV and exists in the body at a known natural abundance (0.012%).

Evaluation of segmental impedance measurements (along the arm, torso and leg) revealed that impedance measured along the limbs (especially along the arm) could be a good predictor of the fat-free mass, while impedance along the torso provided an index (l_{torso}^2/Z_{torso}) that did not seem to statistically correlate with fat-free mass. Also, the index $(l_{arm}+l_{torso}+l_{leg})^2/Z_{tot}$ proved to be a very good predictor of fat-free mass. These results indicate that even when the index h^2/Z_{tot} cannot be calculated (because the standing height cannot be measured) segmental length and impedance measurements can provide almost equally valid indices for body composition studies.

The variability in impedance measurements was studied with respect to several factors. It was found that the side of the body (right or left) where the measurements were taken, the position of the body and the fasting condition of the subject (whether he/she had had a drink or meal prior to impedance measurements) did not significantly affect impedance measurements. However, full inspiration and contraction of arm muscles did have a significant effect on impedance measurements. These results should be regarded only as a rough indication, as they were obtained from data selected from an extremely small sample (only two volunteers). Since it is of great importance to know accurately which factors affect any method of investigation and how they affect it, studies upon the variability of impedance measurements should be continued on more volunteers and also considering more potential sources of variability. In particular, the effect of displacement of electrodes should be studied, as it would be easy to misunderstand the anatomical landmarks used to indicate the proper placement of the electrodes in routine clinical investigations. It would also be interesting to examine how much is the variability in measurements between different examiners.

During this study no attempt has been made to derive any regression equations for quantitative estimation of the fat-free mass from bioelectric impedance measurements, because of the relatively small number of volunteers available. Furthermore, it is doubtful whether one more population dependent regression equation added to the already existing number of relevant equations published [3, 7, 8, 9, 24, 22] would be of great value. On the contrary, what seems to be quite promising is a comparative study, where several different reference groups (consisting of normal volunteers but also of obese subjects or any other extreme or peculiar cases such as professional athletes) would be used to evaluate several prediction equations already proposed, in order to indicate the best one for routine clinical studies.

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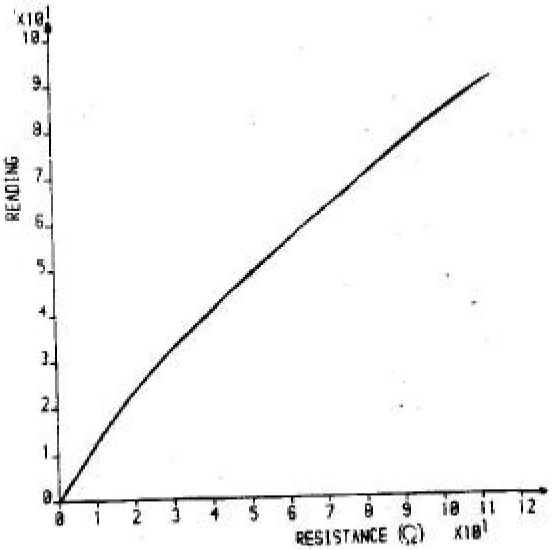
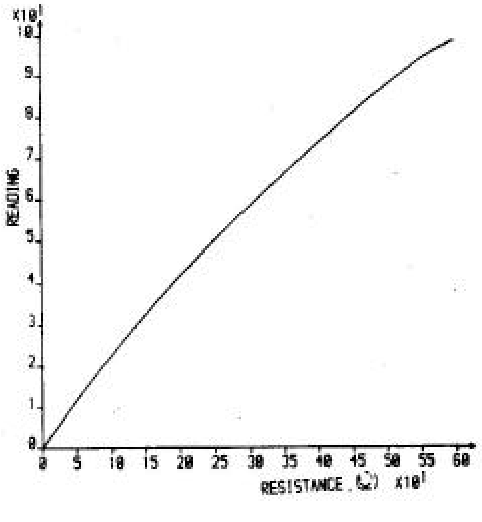
APPENDIX A

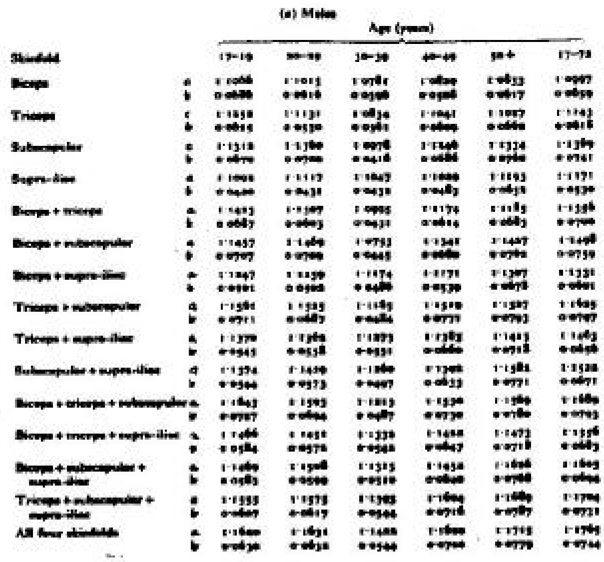


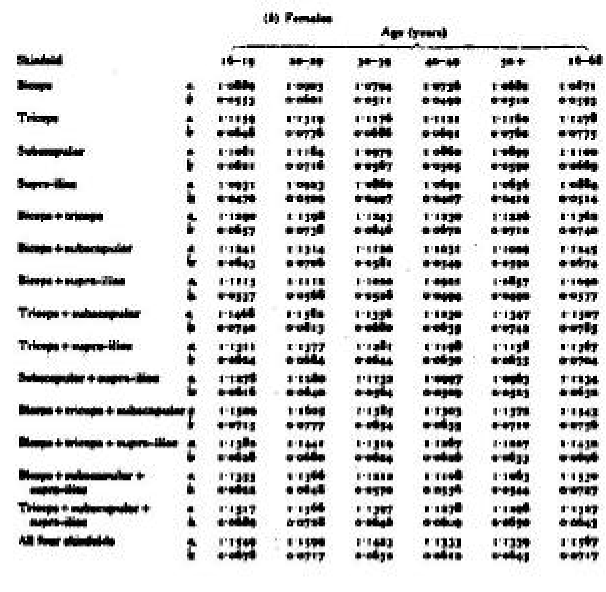
Calibration Curve for Channel 1 of the Gastric Impedance Monitor



Calibration Curve for Channel 2 of the Gastric Impedance Monitor

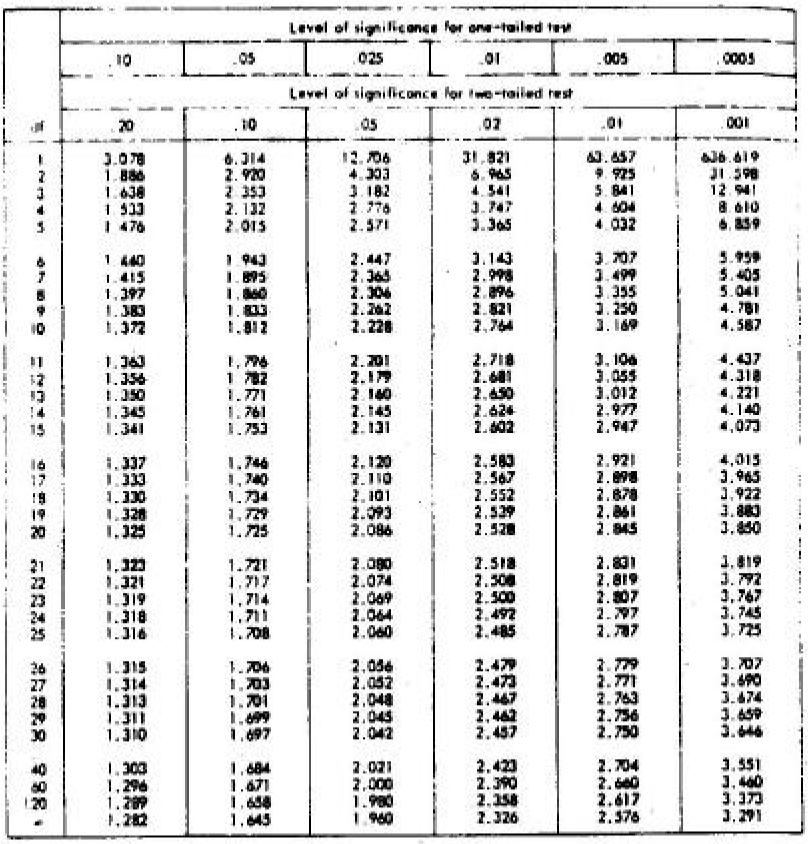
APPENDIX B





Linear regression equations for the estimation of body density x 103 (kg/m3) from the logarithm of the skinfold thickness: density = a – blog10skinfold. Reproduced from: JVGA Durnin and J Womersley, “Body Fat Assessed from Total Body Density and Its Estimation from Skinfold Thickness: Measurements on 481 Men and Women Aged from 16 to 82 Years”, Br. J. Nutr., vol. 32, pp. 7-97, 1974.

APPENDIX C



	Level of significance for one-tailed test					
	.10	.05	.025	.01	.005	.0005
	Level of significance for two-tailed test					
df	.20	.10	.05	.02	.01	.001
1	3.078	6.314	12.706	31.821	63.657	636.619
2	1.886	2.920	4.303	6.965	9.925	31.598
3	1.638	2.353	3.182	4.541	5.841	12.941
4	1.533	2.132	2.776	3.747	4.604	8.610
5	1.476	2.015	2.571	3.365	4.032	6.859
6	1.440	1.943	2.447	3.143	3.707	5.959
7	1.415	1.895	2.365	2.998	3.499	5.405
8	1.397	1.860	2.306	2.896	3.355	5.041
9	1.383	1.833	2.262	2.821	3.250	4.781
10	1.372	1.812	2.228	2.764	3.169	4.587
11	1.363	1.796	2.201	2.718	3.106	4.437
12	1.356	1.782	2.179	2.681	3.055	4.318
13	1.350	1.771	2.160	2.650	3.012	4.221
14	1.345	1.761	2.145	2.624	2.977	4.140
15	1.341	1.753	2.131	2.602	2.947	4.073
16	1.337	1.746	2.120	2.583	2.921	4.015
17	1.333	1.740	2.110	2.567	2.898	3.965
18	1.330	1.734	2.101	2.552	2.878	3.922
19	1.328	1.729	2.093	2.539	2.861	3.883
20	1.325	1.725	2.086	2.528	2.845	3.850
21	1.323	1.721	2.080	2.518	2.831	3.819
22	1.321	1.717	2.074	2.508	2.819	3.792
23	1.319	1.714	2.069	2.500	2.807	3.767
24	1.318	1.711	2.064	2.492	2.797	3.745
25	1.316	1.708	2.060	2.485	2.787	3.725
26	1.315	1.706	2.056	2.479	2.779	3.707
27	1.314	1.703	2.052	2.473	2.771	3.690
28	1.313	1.701	2.048	2.467	2.763	3.674
29	1.311	1.699	2.045	2.462	2.756	3.659
30	1.310	1.697	2.042	2.457	2.750	3.646
40	1.303	1.684	2.021	2.423	2.704	3.551
60	1.296	1.671	2.000	2.390	2.660	3.460
120	1.289	1.658	1.980	2.358	2.617	3.373
∞	1.282	1.645	1.960	2.326	2.576	3.291

Critical values for t, reproduced from: Schefler WC, “Statistics for the Biological Sciences”, Addison-Wesley Publishing Co., Buffalo, 1969.