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HYDRATION LEVELS OF THE BODY AS MEASURED BY BIOELECTRICAL IMPEDANCE

Clayton Lance Grieve

A thesis submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy

Johannesburg, April 1994

ABSTRACT

This study considered the influence of the free electrolytes in the total body water on the electrical properties of the body. Using a model of slightly dehydrated and then rehydrated volunteers, the electrolyte concentration and total body water volume were measured and compared with electrical impedance (Z) measurements of the body.

Pilot investigations performed showed that the ionic concentration and nature of venous blood are significantly related to the impedance the blood poses to a 50kHz, 800μA AC. Calculated correlation coefficients between blood Z, and plasma Na^+, Cl^-, K^+ concentration and osmolarity, ranged from R = 0.74 to R = 0.98. In a second pilot study, the equilibration period for the even distribution of orally ingested tritiated water was 60 minutes. This value was used in the main study, and was not exceeded by more than 15 minutes. The final pilot investigation considered the validity and reliability of the tritiated water dilution method in assessing total body water. A rat model was used for this study, and the results of a direct assessment of total body water by desiccation were compared to results from the isotope dilution technique. The isotope dilution method slightly overestimated the total body water values in the measurement of 12 rats. Despite this overestimation, the results were significantly related to the desiccation values (R = 0.95, α = 0.05).

The main study involved the measurement of total body water volume, blood

ionic concentrations, and electrical impedance in 74 young adult males and females. The total body water volume was measured by means of tritiated water dilution, the ion concentrations and osmolarity were measured by standard laboratory instruments, and the electrical impedance measurements were performed using a commercial 50kHz, 800μA impedance instrument. Whole body, segmental, and blood impedance measurements were performed on subjects in a mildly dehydrated and then rehydrated state. The value of the bio-electrical impedance analysis (BIA) technique, which involves the inclusion of whole-body Z with anthropometric variables in a regression equation to predict body composition, was examined. It was found that the inclusion of Z provided no significant benefit over anthropometry in predicting total body water volume. Height and weight explain a substantial amount of the predictive variance (70% to 94%). Z only accounts for approximately 16% of the variance in the BIA method. Blood impedance and some segmental body impedance measurements do, without the confusion of anthropometry, exhibit a strong relationship to hydration status indicators, namely plasma ionic concentrations and osmolarity. Considering the relative total body water volume, the relationship indicates R = 0.97, R = 0.92 and R = 0.89 for blood, leg and arm impedance measurements against total body water volume, respectively.

Further studies showed that the relationship seen between blood impedance and ionic concentration is true for impedance measured at different frequencies. Furthermore, the mechanisms of muscle contraction also influence the electrical (impedance) properties of the body. Increased contraction of skeletal muscle results in a significant concomitant decrease in the impedance measured over that muscle.

From the results obtained in the pilot investigations, the main study, and the further investigations, it was concluded that Z measurement of the body, as a whole or in various segments, is determined largely by the electrolyte concentration (or lack thereof) in the body fluid compartment.

DECLARATION

I declare that this thesis is my own, unaided work. It is being submitted for the degree of Doctor of Philosophy to the University of the Witwatersrand, Johannesburg. It has not previously been submitted before for any degree or examination to any other University

Clayton Lance Grieve

18 day of OCTOBER, 1994

Dedicated to:

B. J. &
Johann Hattingh

PREFACE

The work presented in this thesis has been an ongoing project for the last six years. Initially, the work started out with the measurement of body composition - fat and fat-free masses in particular - by means of anthropometry and hydrodensitometry. These assessments were performed on a variety of normal samples, ranging in mean ages. The inclusion of the bio-electrical impedance analysis (BIA) technique to measure body composition was a subsequent step. Very soon after beginning work with the BIA method, the inadequacies of the technique were noticed. BIA was not as consistently adequate as much of the literature was implying. These inadequacies resulted in the asking of a series of questions regarding the principles and premises of the method. The questioning, and lack of substantial answers led to the research work presented here.

After a few casual observations and small research studies, it was felt that the electrical impedance properties of the body were mostly, if not wholly, influenced by the conductive compartment of the body - the free electrolytes found in solution in the total body water. To examine the accuracy of this presumption, a research project was initiated. This project considered the fluid and electrolyte nature of the body in relation to hydration status change, and compared these characteristics to impedance measurements of the body. Before starting this project, a few pilot studies were performed. Furthermore, the research project then led to the initiation of further investigations. The purpose of these further investigations was to consider some 'loose ends' that were either not adequately answered, or arose due

to the answers acquired in the research project.

The structure of the presentation of this thesis is slightly unconventional. After providing an introduction to body composition and bio-electrical impedance in Chapter 1, the empirical research is presented in Chapters 2 to 4. This research is presented in the format that begins with the pilot investigations in Chapter 2, continues with a description of the main research project in Chapter 3. Chapter 4 presents the work of the further investigations, and Chapter 5 contains the overall discussion and conclusions in Chapter 5. Some of the methods of measurement and analysis are common in different aspects of the research, and a degree of cross-referencing and referral to other parts of the study is used. Where it was considered necessary, some of the information is repeated.

Acknowledgements

This work would not have been possible without the immense help received from the following people. Mr. Stuart Fripp from the Department of Physics provided invaluable help in the area of impedance instrument design and construction. Professor Noël Cameron initiated the work 6 years ago, and was an encouraging and motivational force throughout. Professor Beverly Kramer, in her own quiet manner, assisted me through the 'low spots' of the research. Professor Johann Hattingh provided the most generous support, both financial and motivational at the beginning of the project - at a time when I was unsure whether the work would be possible at all. Professor Maciej Henneberg, with all his insight, fun, care and dedication to science, was, is, and always will be a tremendous inspiration to me. Finally, to my wife, Cathryn - how you put up with me at times, I will never

know. Thank-you to all these people for being such an important inspirational, motivational and supportive part of my life, while I went through labour and gave birth to this work. Domo arigato gozaimasu.

Publications from this work

- Grieve CL (1991) The validity of using bioelectrical impedance analysis to determine fat-free mass in children. Anat. Soc. SA Newsletter 24:18.
- Grieve CL & Grieve CA (1991) Body asymmetry and body composition estimation by bioelectrical impedance analysis. Anat. Soc. SA Newsletter 24:22.
- Grieve CL. (1992) The use of impedance measurements to indicate various blood parameters relating to hydration status. SA Med. J. 82:194.
- Grieve CL & Henneberg M. (1993) A modified method of hydrodensitometry in young children. Am. J. Clin. Nutr. 58(5): 713-714.
- Grieve CL & Henneberg M. (1994) Fat-free mass of young males determined by electrical impedance and hydrodensitometry: possible conceptual errors. J. Appl. Physiol. (submitted April 1994)
- Grieve CL. (1994) The indication of muscle activity by means of bio-electric impedance measurements. J. Anat. (in press)

Presented at conferences (not listed above)

- Grieve CL & Grieve CA. (1992) Total body water and extracellular water assessed by electrical impedance analysis. PSSA : September (Langebaan)
- Grieve CL. (1993) Total body water assessed by electrical impedance of the blood and body. IUPS : August (Glasgow)

LIST OF ABBREVIATIONS

ρ:	density
$^{18}O_2$:	isotopically labelled oxygen
$^{3}H_2O$:	tritiated water / tritium
A, μA:	amps (measure of current)
AC:	alternating current
anisotropy:	having physical properties that depend on direction
BIA:	bio-electrical impedance analysis
CT:	computerised tomography
D_2O:	heavy water / deuterium
DC:	direct current
dielectric:	insulating property
H_2O:	water
Ht:	height
I:	current (amps)
L:	measure of distance (length)
l:	measure of volume (litres)
NaCl:	sodium chloride
NMR:	nuclear magnetic resonance
R:	resistance (Ω)
STD:	standard deviation
TOBEC:	total body electrical conductivity
v:	coefficient of variation
V:	voltage (volts)
Wt:	weight
Xc:	reactance (Ω)
Z:	impedance (Ω)

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CHAPTER 1

INTRODUCTION

To fully understand the normal and abnormal functioning of the human body, it is necessary to gain a deep awareness of the structure of the body. This relationship between the function and structure of organisms is the basis of many biological and medical sciences. The levels of investigation for the understanding of this relationship differ, depending on the functional knowledge required. The levels include the structure and function of the organism as a whole, the various functional and structural systems that constitute the organism, specific organs within these systems, and the tissues, cells and molecules that comprise the organs.

The study of the structural composition of the body depends on the research methods and techniques used. By definition, the study of body structure is either considered macroscopically (gross anatomy), microscopically (histology and cell biology), or embryologically (developmental biology). Within the confines of these fields, different structural perspectives exist.

1.1) Structural Perspectives of the Human Body

The views with which the bodily structure of the studied organism may be perceived are varied. Some of the more common perspectives are discussed briefly below. A *regional perspective* involves dividing the body into explicitly predefined regional parts, for example the limbs, the abdomen, the trunk or thorax, and the head and neck. Due to its broadly classified nature, this perspective is often used in macroscopic studies of the body. A *systematic perspective* of the body involves the consideration of separate and specific functional systems of the body, for example, the cardiovascular system, the nervous system, and the integumentary system. This perspective may be considered from a macroscopic, a microscopic and an embryological point of view. A *compartmental perspective* is similar in nature to both the regional and systematic perspectives, but relies more on the division of the body into functional compartments. An important example of such a functional compartment are the fluid compartments of the body, which are often considered separately as the intracellular and extracellular fluid compartments. The final perspective to be mentioned is the *compositional perspective* of the body. It is within this perspective that the field of body composition has arisen. The compositional aspects of the body are described and studied, more often than not, as individual entities. Examples of such compositional perspectives of the body include the ionic (Na^+, K^+, etc.), fat, mineral, fluid, bone and muscle composition of the body. It is this perspective - body composition - that forms the basis of the present study.

1.2) The Composition of the Human Body as a Structural Perspective

The study of the detailed composition of the body has fascinated many biological and medical scientists for a long time. McCance and Widdowson (1951) aptly describe this fascination in their introduction to a review paper published on the composition of the body:

> *"Everyone connected with physiology or medicine* (normal and abnormal function of the body) *must at some time or another have wanted information about the composition of the body. How much water does it contain? How much iron? Or calcium? What is the weight of the liver or the volume of the blood? These and similar questions must have been asked over and over again, and the ways in which investigators have attempted to obtain answers to them present good examples of the evolution of measurement in medicine. ... The central problem has always been the composition of living men, women and children in various stages of health and development, and there are probably no two people with the same chemical composition."*

This fascination has not only been recent. As far back as 300BC, scientists such as Hippocrates were concerned with the study and description of body composition. Hippocrates wrote that "the human body contains four constituents, blood yellow bile, black bile, and phlegm. These are the things that make up its constitution and cause its pains and health. Health is primarily the state in which these constituent substances are in the correct proportion to each other, both in strength and quantity, and are well mixed."

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An underlying theme throughout the published body composition studies is the desire to understand the functional mechanisms and their possible clinical application, as related to the structural components of the body (Morgan, 1982). It is this functional driving force that has resulted in the development of the field of body composition study, description and measurement (Woodard & White, 1986; Lukaski, 1987; Brodie, 1988a & 1988b; White *et. al.*, 1991).

Body composition research is a branch of human biology that has three interconnecting areas: the quantification of various body components and their organisational rules, measurement techniques, and biological factors that influence body composition. Due to the initial lack of definition of the field of body composition, many descriptive models have been proposed, each according to the need and interest of the proposing investigator (Garrow, 1982; Mackie *et. al.*, 1989; Elia & Jebb, 1990; Heymsfield *et. al.*, 1990; Wang *et. al.*, 1992).

1.2.1. Body Composition Models

The development of models for the use in body composition studies began in the laboratory of AR Behnke in the early 1940's (Lukaski, 1987). In the five decades that followed, numerous models were proposed, limited only by the technicaland technological ability to perform indirect measurements of specific body components. As an introduction to the field of body composition, some of these models will be discussed briefly.

a) *Two-Compartment Model: Fat and Fat-Free Mass*

Most contemporary methods of body composition measurement are based on the model first proposed by Keys and Brozek (1953) in which the body consists of two chemically distinct compartments, the fat and the fat-free masses. The fat mass is defined as all ether extractable material in the body, and includes adipose tissue (Brozek *et. al.*, 1963). The fat-free mass consists of the remainder of the body, namely the body cell mass, the extracellular fluid, and extracellular solids, all with a theoretically constant chemical composition (Siri, 1956). Figure 1.1 illustrates this model.

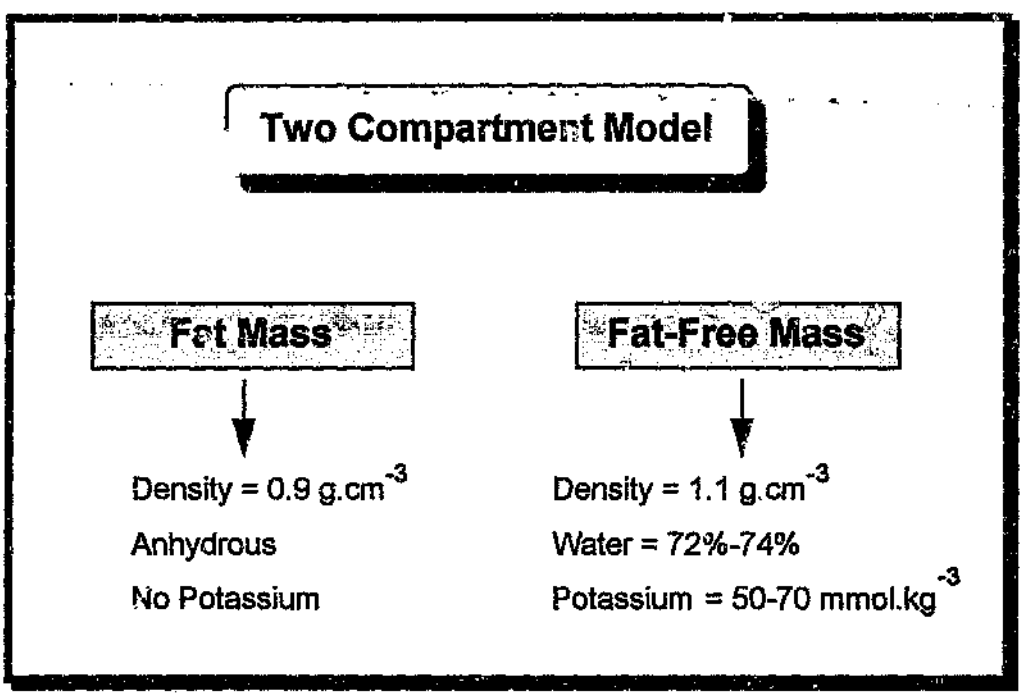


Figure 1.1: Two compartment body composition model

a) *Two-Compartment Model: Fat and Fat-Free Mass*

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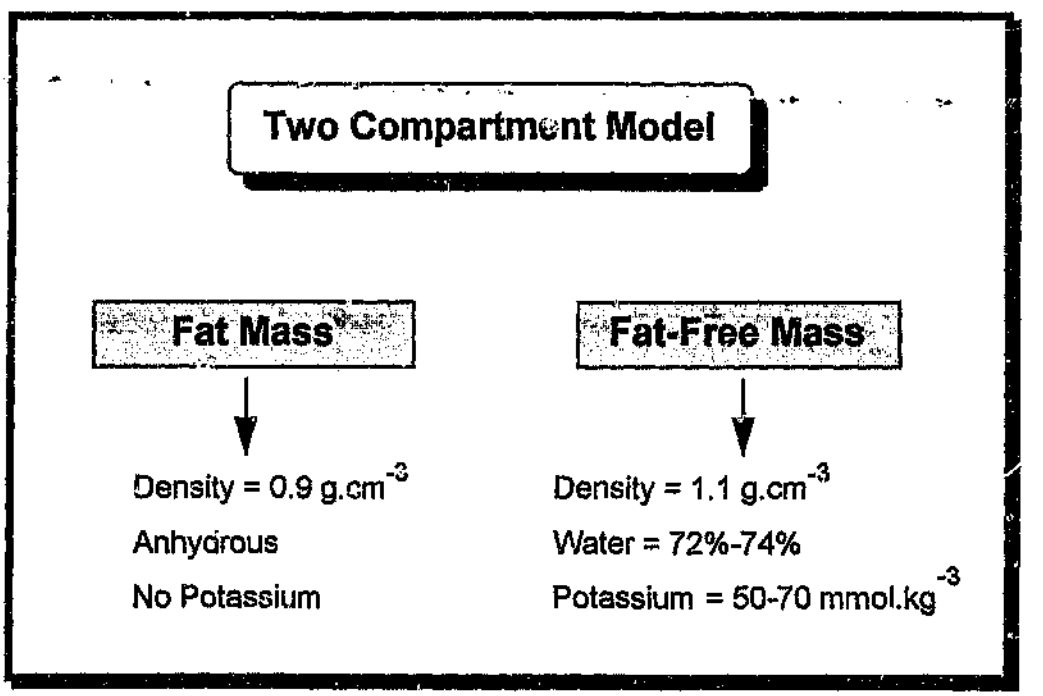


Figure 1.1: Two compartment body composition model

b) *Three-Compartment Model: Muscle, Non-Muscle, and Fat*

Anderson (1963) and later Burkinshaw *et. al.* (1979) subdivided the fat-free mass of the two-compartment model into two components - the muscle and non-muscle compartments. This subdivision was done because of observed physical changes in the muscle compartment. It was seen that the muscle component of the fat-free mass was not of a constant nature, undergoing hypertrophy and wasting due to certain conditions. The non-muscle mass consisted of the skeleton and other extracellular solids which theoretically possessed a more constant nature. The two subdivisions of the fat-free mass, namely the muscle and non-muscle compartments, are often assessed from measurements of total body potassium and nitrogen. This model has not been favourably accepted due to the complexity and expense of measuring the required body potassium and nitrogen content. Furthermore, the current techniques of measuring body potassium and nitrogen yield high standard errors of estimation of between 15% and 20% (Mackie *et. al.*, 1989).

c) *Four-Compartment Model: Protein, Mineral, Water and Fat*

During the development of the two-compartment model, Keys and Brozek (1953) further divided the body into four basic chemical groups; water, protein, mineral and fat. The point of departure of this model from the two-compartment model was the further division of the fat-free mass into its constituent parts of protein, mineral and water. Initially, this was a theoretical division as it was technically not possible to measure the different components of the four-compartment model. Recently, with the advent of techniques such as neutron activation and

photon absorbtiometry, the measurement of at least three of these compartments can be made, thereby allowing the calculation of the fourth compartment (McCarthy *et. al.*, 1980; Peppler & Mazess, 1981; Kyere *et. al.*, 1982; Beddoe *et. al.*, 1984). This four compartment model depends on fewer assumptions regarding the relationships between compartments, and is therefore less subject to biological variation influencing the measurement accuracy (Heymsfield *et. al.*, 1990). Figure 1.2 graphically summarises this model.

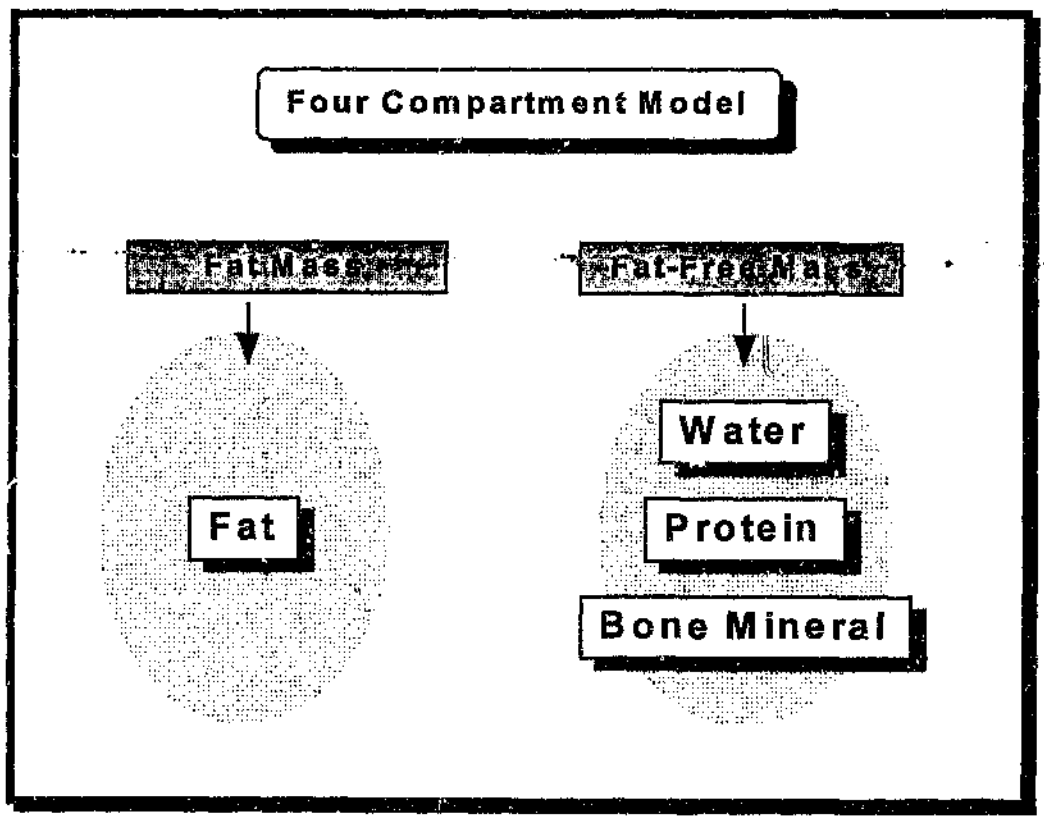


Figure 1.2: Four compartment body composition model

d) *Five-Level Model: Five Levels of Increasing Complexity*

Using a different perspective, Wang *et. al.* (1992) recently organised the field of body composition into five levels of increasing complexity; atomic, molecular, cellular, tissue-system; and whole body (see Figure 1.3). It was postulated that each structural level, and its multiple compartments, is distinct, with certain bio-chemical and physiological connections between levels. The structural levels exist so that the model is consistent and and the organism functions as a whole. Two important aspects of this model are that 1) it does not rely upon measurement techniques for its existence, and 2) the concept of a steady state body composition, in which quantitative associations between compartments at the same or at different levels exist, is entertained.

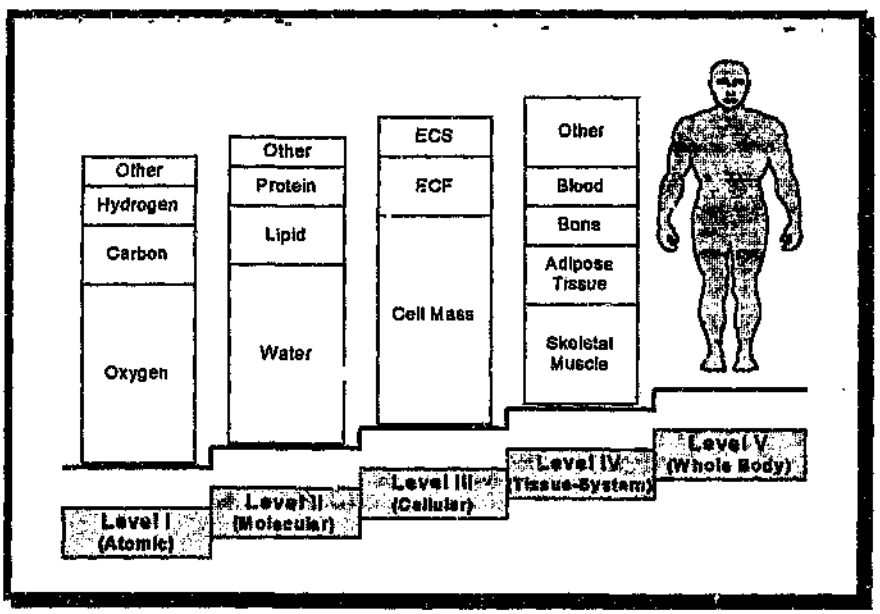


Figure 1.3: Five levels of body composition (ECF and ECS: extracellular fluid and solids, respectively) (after Wang *et. al.*, 1992)

1.2.2. Body Composition Measurement Techniques

An increase in the interest in body composition studies has recently resulted in the rapid development of measurement techniques for scientific and medical use. The direction of this development has relied upon two factors: current technological advances and the requisite model of body composition used. The methods of body composition measurement may be considered from two perspectives; direct measurement and indirect measurement. The direct measurement of human body composition is reserved exclusively for cadaver studies, which provide accurate and complete measurements of the body. This method is often used as a reference method for the basis of indirect methods of measurement, and is of limited value to practical applications. The indirect, and relatively non-invasive techniques of body composition measurement are of far more value to the investigator. There are many such techniques, varying in difficulty, accuracy, and cost, and their development is currently a predominant interest in body composition science (Roche, 1984). As a basis for the understanding of the current trends and practices, a brief summary of some of the contemporary techniques of body composition measurement follows. This summary does not only detail techniques used in the present study, but also highlights areas of current and future research interest. Lukaski (1987) and Brodie (1988a, 1988b) provide a far more detailed review of the various body composition measurement techniques.

a) *Anthropometry*

Anthropometric assessments of body composition have facilitated the measuring of body composition variables outside the laboratory. These methods of measurement are generally easy to perform, requiring minimal training, and are reasonably inexpensive (Cameron, 1978). Anthropometric observations are usually used in regression equations to predict the fat and fat-free masses, utilising the two-compartment model of body composition.

The various types of anthropometric measurements performed in body composition studies include measurements such as bone dimensions, skinfold thickness, and body circumference (Behnke, 1959; Behnke, 1963). The *bone dimensions* are theoretically related to the skeletal mass, and thus the fat-free mass. *Skinfold thickness* measurements assess body composition, in particular the fat mass, by assuming that the thickness of the subcutaneous adipose tissue reflects a constant proportion of the total body fat mass (Lohman, 1981). The fat and the fat-free masses, used in the two-compartment model, are summative and mutually exclusive. In other words, if the two-compartment model is used and either the fat or the fat-free mass is measured and the total body mass is known, the second unmeasured compartment can be simply calculated. Another common anthropometric body composition analysis is *body circumference*. In particular, the measurement of upper arm circumference is often used in conjunction with upper arm skinfold thickness measurements to estimate the cross-sectional fat and muscle areas of the arm (Jelliffe & Jelliffe, 1969; Gurney & Jelliffe, 1973).

b) *Computerised Tomography (CT)*

Computerised tomography is an application of modern radiography, in which regional body composition measurements are performed. Briefly, a CT scanner produces a computer generated image of the distribution of differences in x-ray attenuntion. This attenuation is a result of the differences in the physical density of various tissues. Often, the produced computer image is evaluated with the aid of complex image analysis computer software to yield quantitative contrasts between bone, adipose and fat-free tissues. The obtained two-dimensional image can be related to cross-sectional areas of tissue compartments, and when numerous CT scans are performed, with the thickness of the CT scan slice known, the volume of the tissue compartment can be estimated (Winter & King, 1983).

This method is highly accurate, but prohibitively expensive and not suitable for field studies. The applications of the measurements performed are varied, with different tissue compartments being reasonable accurately estimated. The assessment of these tissue compartments could be a function of the two-, three-, or four-compartment models of body composition.

c) *Densitometry*

Based on the 2-compartment model, and assuming that the fat and the fat-free masses have different and constant densities, a proportion of the fat to fat-free tissue can theoretically be estimated from a measurement of whole body density. The whole body density is calculated from the two measured variables: body mass and body volume, using the relationship

$$\text{Body Density} = \frac{\text{Body Mass (kg)}}{\text{Body Volume } (l^{-3})} \quad \text{.....(1.1)}$$

The measurement of body mass is relatively straightforward, and the measurement of body volume is usually accomplished using the hydrostatic weighing technique, which incorporates Archimede's principle (Katch *et. al.*, 1967; Katch, 1969; Wilmore, 1969; Francis, 1990).

The entire technique of measuring body density, commonly known as hydrodensitometry, has been accepted as a safe, reliable and relatively inexpensive method of measuring the fat and fat-free compartments of the body composition. Hydrodensitometry is often considered to be a standard method used in the validation of other methods.

d) *Electrical Conductance Measurements*

The measurement of the electrical properties of the body has experienced enormous interest during the past decades. There are numerous theories regarding the relationship between the electrical properties of the body and body composition measurements (Harrison & Van Itallie, 1982; Jackson *et. al.*, 1988). Two broad fields of applied electrical measurement of the body exist: electrical impedance measurements and total body electrical conductivity (TOBEC). The former of these two methods is discussed in further detail in Chapter 1.3, and a brief summary of the latter method, TOBEC, is presented below. The similarities of the two methods are that they are both based on the solid state model of electricity, and hence both rely upon the differences in electrical conductivity and the dielectric

properties of the fat and fat-free tissues to estimate body composition (Pethig, 1979).

Unlike electrical impedance measurements which rely on the injection and subsequent measurement of a current in the body, TOBEC is a method that is based on electric current induction. TOBEC operates on the principle that an object placed in an electromagnetic field will disrupt the field. The degree and amount of disruption is dependent on the quantity and quality of the conducting material present (Klish *et. al.*, 1984). It is postulated that the electrolytes present in the fat-free compartment account for almost all the electrical conductivity, which means that the measured TOBEC value should theoretically indicate the fat-free mass (Presta *et. al.*, 1983). This method therefore depends on the two-compartment model for its estimations. Further developments of the TOBEC technology have yielded advanced applications and increased measurement accuracy (Segal *et. al.*, 1985; van Loan & Mayclin, 1987b).

e) *Infra-red Interactance*

A relatively recent method of measuring subcutaneous adipose tissue thickness is the technique using infra-red interactance principles (Conway *et. al.*, 1984). Infra-red interactance is based on the principles of light absorption and reflection using near-infra-red spectroscopy. The degree of infra-red energy absorption is related to the composition of the substance through which the energy is passing, and the particular wavelength of the energy. The amount of absorption is estimated from the amount of resultant transmitted or reflected energy. The data from

these measured estimates are then collected and analysed by computer-run software to indicate fat tissue thickness and mass.

f) *Muscle Metabolites*

Creatinine is the most widely used biochemical marker for the estimation of body muscle mass (Brodie, 1988a). The basis of this method is that creatinine is the only metabolite of creatine. Although other tissues take up metabolised creatine, most of it (98%) is found in skeletal muscle in the form of creatine phosphate (Heymsfield *et. al.*, 1983). The absorbed creatine constantly undergoes spontaneous chemical dehydration, forming creatinine, which is excreted unaltered in the urine. Thus an assay of creatinine excretion in a 24-hour urine collection should theoretically reflect the level of total body creatine and therefore total body muscle mass (Talbot, 1938; Boileau *et. al.*, 1972; Forbes & Bruining, 1976).

The method of using 24-hour urine samples was extended to the measurement of total plasma creatinine (Schutte *et. al.*, 1981). Based on the same principles as the urinary method, total plasma creatinine corrected for plasma volume, also provides a reasonable estimation of muscle mass.

3-methylhistidine is a further muscle metabolite that is found exclusively in myofibrillar protein, and, when catabolised, is excreted in the urine (Young & Munro, 1978). Thus, the collection and measurement of exogenous urinary 3-methylhistidine excretion will also theoretically indicate skeletal muscle mass.

The creatinine and the 3-methylhistidine methods of body composition measurement are both based on the 3-compartment model of body composition, and are

valuable indicators in nutritional assessment. In particular, this assessment theoreticall provides an indication of the amount of muscle protein available for the normal functioning of the body.

g) *Neutron Activation Analysis*

In vivo total-body neutron-activation analysis is a technique allowing for the measurement of the multi-elemental composition of the human body (Cohn & Parr, 1985; Siwek *et. al.*, 1987). Absolute content of calcium, sodium, chloride, phosphorus and nitrogen is safely determined with this method. The technique depends upon the irradiation of a subject with a beam of regulated fast neutrons. Specific atoms in the body capture these neutrons and form unstable isotopes. Whilst reverting to a stable condition, these isotopes emit one or more γ-rays of characteristic energy. The quantity and nature of these γ-rays are then measured. Standard γ spectrographic analysis is then used to identify the nature and abundance of the element, using the characteristic energy levels exibited by individual elements.

Estimations of calcium are often related to the amount of bone mineral found in the 4-compartment body composition model of the body, and nitrogen estimations are related to the ratio of muscle and non-muscle masses in the 3-compartment model.

h) *Nuclear Magnetic Resonance Imaging*

A new, potentially safe and non-invasive method of body composition measurement is nuclear magnetic resonance imaging (NMR). NMR is based on the fact that atomic nuclei absorb or emit electromagnetic waves as a response to particular magnetic fields. Essentially, the nuclei of the body will attempt to align themselves to an external magnetic field which is applied to the body. If a wave of radio frequency is then directed into the body, some nuclei absorb energy from the introduced wave and change their orientation in the magnetic field. If the introduced radio wave is then removed, the activated nuclei emit the radio signal that they absorbed (Lukaski, 1987). This emitted signal is recorded by a computer, which can develop an image of the structure being studied (Koutcher & Burt, 1984).

Being a new technique, and due to its expense and complexity of measurement, NMR has not been used to any great extent in body composition studies. These studies have been limited to the measurement of total body water and water compartments (Hayes *et. al.*, 1982), and the estimation of body fat in baboons (Lewis *et. al.*, 1986).

i) *Photon Absobtiometry*

The attenuation of x-rays or γ-rays through the body can provide some indication of the composition of specific parts of the body. Either using a single energy transmission when two clearly defined tissues are involved, or a dual energy transmission system when variable tissue thicknesses are involved, the principles of this technique are relatively simple. Used mainly in the assessment of the content

and quantity of bone tissue, the bone mineral content is assumed to be directly proportional to the amount of photon energy absorbed by the bone being studied (Lukaski, 1987).

Single photon absorptiometry is used to measure local or regional bone mineral content (Cameron & Sorenson, 1963; Mazess, 1971; Christiansen & Rodbro, 1975). For total body bone mineral content, the dual photon absorbtiometry method is commonly used (Peppler & Mazess, 1981).

j) *Total Body Potassium*

Being largely an intracellular ion found in the fat-free tissue cells, a measure of total body potassium is often used as an estimate of the fat-free mass (Forbes *et. al.*, 1961). The measurement of total body potassium depends on the naturally occurring isotope ^{40}K in non-fat tissue, which is a constant fraction (0.012%) of body potassium (Brodie, 1988a). A whole body radiation counter is connected to a suitable recording device, and is used to count γ radiation at 1.46MeV (Cohn & Palmer, 1974).

k) *Total Body Water*

Total body water being is a functionally important component of the composition of the body, therefore its measurement has caused considerable interest amongst body composition investigators (Andersson, 1977; Sheng & Huggins, 1979). The average human male's body is composed of approximately 60% water,

with the average female's body being approximately 50% (Bradbury, 1973; Hill, 1990). Children and neonates have a much higher hydration level of approximately 85% - which decreases with age (Cheek, 1961; Cheek *et. al.*, 1984). More than 62% of the body water is found in the intracellular compartment, with the remainder in the extracellular compartment. Thus, the total body water is often functionally separated into the intra- and extracellular fluid volumes (Cheek, 1961).

The first methods of measuring total body water were by direct weighing and desiccation of the body and organs. With the advent of isotope measurement techniques in the 1930's, the dilution of various isotopes in the body was used to estimate total body water and the different fluid compartments (McCance & Widdowson, 1951).

Commonly known as the "dilution principle" for measuring body fluid volumes, the method is based on two assumptions: 1) the isotope has the same distribution volume as the body fluid being measured, and 2) it is non-toxic in the amounts used (Pinson, 1952). Essentially, the volume of any fluid compartment of the body can be measured by placing a labelling substance in the compartment, allowing it to disperse evenly throughout the fluid, and then measuring the extent to which the substance has become diluted. In practice, a small known quantity of dye, isotope or other foreign substance is placed in the fluid compartment being measured, and the substance is allowed to disperse throughout the compartment until it becomes mixed in equal concentration in all areas (see Figure 1.4). A sample of the dispersed fluid is removed and the concentration of the substance is analysed chemically, photo-electrically, or by any other appropriate means (Loeppky *et. al.*, 1977; Guyton, 1986).

Using the relationship between concentration and volume, i.e.,

$$C_1V_1 = C_2V_2 \quad \text{.....(1.2)}$$

The volume of the compartment can then be determined from the following basic formula:

$$\text{Compartm Vol} = \frac{\text{quantity of test substance}}{\text{dispersed test subst. concentration}} \quad \text{.....(1.3)}$$

Thus, all one needs to know is 1) the quantity (concentration and volume) of the test substance put into the compartment (C_1V_1), and 2) the concentration of the fluid after dispersion (C_2).

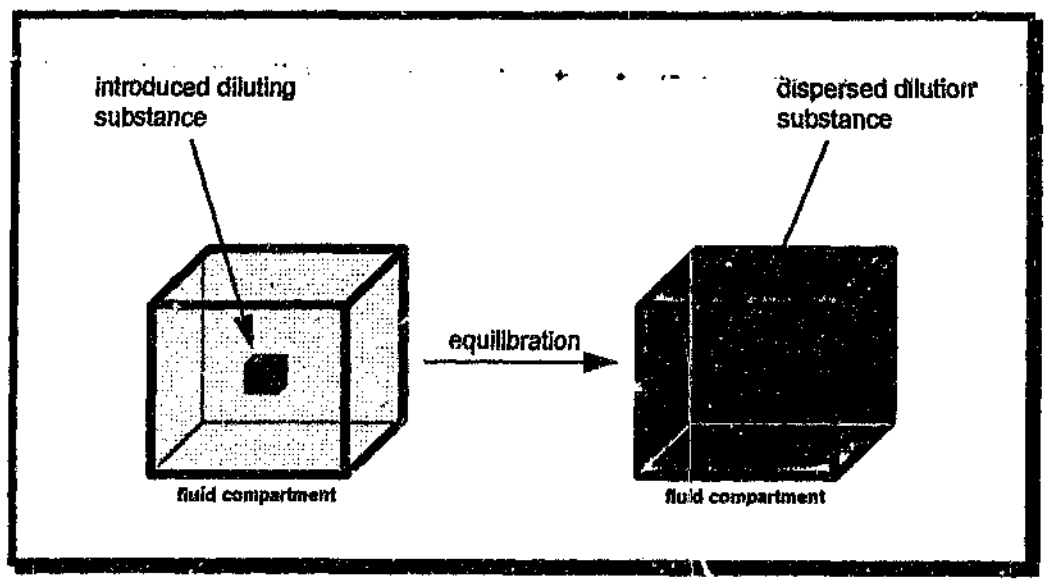


Figure 1.4: Principles of the dilution method (after Guyton, 1986)

Due to the ease of measurement, the relative safety, and the ability of radio-isotopes to diffuse into the measured body fluid volumes, chemical isotopes are popular labelling substances. The isotopes used in total body water measurements include labelled oxygen ($^{18}O_2$), heavy water or deuterium (D_2O), and tritiated water (^3H_2O). The latter of these substances (^3H_2O), is used extensively due to its relatively inexpensive nature and ease of measurement (Prentice, *et. al.*, 1952; Olsson, 1970; Shizgal, 1981; Pierson *et. al.*, 1982; Szeluga *et. al* , 1984)

l) *Ultrasound*

Commercially available ultrasound instruments provide images of tissue configuration and changes in tissue density for a variety of clinical and scientific applications (Leopold, 1980; Schumacher *et. al.*, 1982). Typical ultrasonographic instruments convert electrical energy to a high frequency ultrasonic energy, which is transmitted into the body, by way of a directing probe, as short pulses. Body tissues of different density have different acoustical properties, which cause a partial deflection of ultrasonic energy back to a receiver in the probe. The amount and nature of deflected energy is re-transformed into specific electrical energy, which is graphically depicted as an echo image (Volz & Ostrove, 1984; Stokes & Young, 1986).

Due to the similarity in technology used and the images produced, the ultrasonographic method of studying body composition is used for similar estimations as computerised tomography. Ultrasound is commonly used to assess adipose tissue thickness (Haymes *et. al.*, 1976), and body fat (Borkan *et. al.*, 1982).

1.3) The Electrical Impedance Measurement Technique

During the end of the 19th and the beginning of the current century, human biologists were interested in documenting the electrical properties of the human body as a whole, and at the tissue level (Ackmann & Seitz, 1984). These pioneering investigations may generally be divided into two major categories: 1) studies conducted to investigate fundamental electrical properties of the body, and to correlate these properties with body compartmental and tissue structure, and 2) the electrical measurement of physiologic events. The advances in medical and scientific measurements, such as electroencephalography, electrocardiography, pressure transducer recorders and electromyography, indicate a highly productive and profitable foray into the latter category - electrical measurement of physiological events. The category of investigation based on anatomical study has been no less productive. Anatomists of all natures, which include those interested in describing the composition of the body, have extensively developed and used electrical techniques to image the human body.

One such electrical technique, total body electrical conductivity (TOBEC), has previously been discussed. A second type of electrical measurement of the human body, which considers the dielectric or impedance properties of a body or its tissues, has been of intense interest to investigators for well over a century (Löfgren, 1951; Burger & van Dongen, 1961; Rush *et. al.*, 1963; Pethig, 1987). It is this body composition application, i.e., the measured impedance of an electrical current by a biological structure, which is the basis of the present study, and is further discussed below.

1.3.1. A Brief History of Electrical Impedance

One of the first documented studies of the measurement of biological impedance to electrical current is credited to Cremer in 1907. Furthering this pioneering work, Mann (1937) performed the first non-invasive impedance measurements using contact skin electrodes. At this point, an interest was created in the measurement and documentation of biological impedance, and many investigators began researching this field in earnest. In particular, these early studies explored the relationships among bio-impedance measurements and water content of the body (Barnett, 1940; Löfgren, 1951), and to physiological variables such as thyroid function, basal metabolic rate, and blood flow (Barnett & Bagno, 1936; Barnett, 1937a, 1937b, 1937c, 1937d; Burger & van Milaan, 1943; Schwan, 1955; Schwan & Kay, 1956a ,1956b). As often happens with pioneering research, these studies were limited by the available technology, reference methods, and sample size (Chumlea & Baumgartner, 1990).

It is believed that the turning point in the perceived success and support of applied bio-electrical impedance came about as a result of extensive work performed by Thomasset in 1962 and 1963 (van Loan, 1990). These two studies essentially elucidated the basic principles of bio-electrical impedance measurement.

Thomasset's work in the early 1960's inspired a renewed interest in the 1970's regarding the clinical application of bio-impedance, and resulted in numerous studies being published on body composition attributes. These studies included work on total body water volume (Hoffer *et. al.*, 1969, 1970), the intracellular and

fat-free mass (Bolot *et. al.*, 1977).

Present scientific interest in bio-electric impedance measurement is largely a result of the work performed in the 1970's. Perhaps the most eminent of the investigations of this period was that of Nyboer (Nyboer, 1970, 1972, 1981; Nyboer *et. al.*, 1976). Nyboer and co-workers reported numerous studies supporting the belief that changes in electrical impedance were due to changes in body composition, in particular the blood and fluid volume of the body. Nyboer's work is arguably the most significant work that subsequently led to the extended contemporary interest in, and application of bio-electrical impedance measurement (Chumlea & Baumgartner, 1990).

1.3.2. The Physical Basis of Bio-Electrical Impedance Measurement

Essentially, the impedance of a biological body is measured by passing a small alternating current (I) through the body in question, and measuring the voltage (V) developed across the body by this current. The magnitude of the impedance (Z) is then taken to be the ratio of the magnitudes of V to I, using Ohm's Law:

$$Z = \frac{V}{I} \qquad \text{.....(1.4)}$$

The impedance of the body to current is because biological tissues possess a dielectric potential produced by their net polarity. This polarity characteristic is a function of the atomic, electronic, and ionic components of the constituent molecules (Pethig, 1979).

The body is commonly separated into conducting and non-conducting portions. Due to the hydrous nature of the body environment and the cells present in this fluid environment, the content of the total body water is considered to form the bulk of the conductive compartment (Cooley & Lehr, 1972; Khaled *et. al.*, 1988). The cells of the body are essentially a semi-organised fluid surrounded by a thin membrane that separates them from a similar fluid that lies outside. Intracellular and extracellular fluids consist of water in which a number of ionised molecules are dissolved. These molecules include sodium chloride, potassium chloride, carbon dioxide and large proteins. As a result of the ionisation process, these molecules exist in solution as various species of ions, each having a small electric charge. Because they possess a charge, ions will migrate in the presence of an electric field. If the field is produced by two polar electrodes, this migration of charged particles produces a flow of current through the solution between the electrodes, which will continue as long as there are ions in directed motion. The presence of selectively permeable membranes and large organic, uncharged particles will impede the movement and thus the current flow of the mobile ions.

The electrical field produced is often in the form of an introduced alternating current (AC). Theoretically, if an AC, which oscillates at a characteristic frequency, is applied to a biological structure, a rapid reversing of the ions is produced. If the AC frequency is sufficiently great, then the ionic motion is periodically reversed sufficiently quickly that cellular membranes do not have time to collect ions on their surfaces. This ion collection functions as an electronic capacitance within the biological structure. From this, it is postulated that at low frequencies, the injected current only travels, and therefore represents, the extracellu-

lar fluid. At higher frequencies, this current theoretically has the ability to pass through the cell membrane, thereby representing the total body water. Impedance is therefore the frequency-dependent opposition by a conductor to the flow of an alternating current (Nyboer, 1959).

An electrical property of impedance is that it is composed of two vectors - resistance (R) and reactance (X_c). The relationship between the two impedance vectors, R and X_c, and impedance is described by the equation:

$$\boxed{Z = \sqrt{R^2 + X_c^2}} \quad \text{.....(1.5)}$$

Resistance is the pure physical opposition of the conductor to the current flow and is the reciprocal of conductance, or the ability of an object to convey an electric current. In a biological conductor, the current is due to the mobile ions, and the conductivity, or the amount of electricity that can be conducted, is proportional to the number of available ions per unit volume, and is strongly temperature dependent (Khaled *et. al.*, 1988). *Reactance* is the dielectric component that results from the additional opposition to the flow of an alternating current from the presence of capacitance, or the storage of voltage by a condenser (cell membranes, tissue interfaces, and non-ionic tissue) for a brief moment (Schwan & Li, 1953; Ackmann & Seitz, 1984). The nature of electrical capacitance, in this case cellular capacitance, theoretically causes the current to lag behind the voltage, creating a phase shift that is quantified geometrically as the arctangent of the ratio of reactance to resistance, or the phase angle (φ), i.e.,

$$\boxed{\tan \varphi = \frac{X_c}{R}} \quad \text{.....(1.6)}$$

All the relationships between Z, R, X_c and φ, with the influence of frequency, are illustrated in the impedance plot shown in Figure 1.5. This plot combines the relationships shown in equations 1.5 and 1.6 above. At low frequencies (f_{low}) the impedance of the cell membranes and tissue interfaces is too high for easy conduction of the current, and the measured impedance is purely resistive (R_0). As the frequency of the AC increases, the current has the ability to penetrate the cell membranes and X_c increases causing the phase angle (φ) to increase. Critical frequency (f_{crit}) is the frequency at which X_c and φ are maximal. Above f_{crit} the X_c decreases as the cell membranes and tissue interfaces lose their capacitive ability, and at very high frequencies of AC (f_{high}) impedance is again equivalent to pure resistance (R_∞). The reactance and the phase angle are considered to be small in most biological systems (Baumgartner *et. al.*, 1988; Chumlea & Baumgartner, 1990).

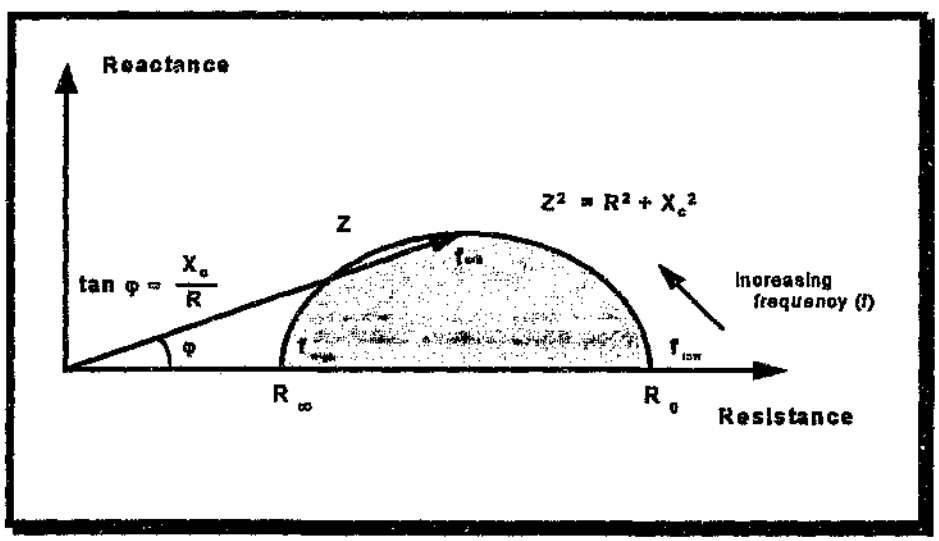


Figure 1.5: The relationships of impedance (Z), resistance (R), reactance (X_c) and current frequency (f).

In a uniformly isotropic solid-state conduction medium, the constant current will be conducted uniformly between injector electrodes, and the current density will be the same everywhere (Baker, 1989). Under these conditions, the measured impedance of the body is proportional to its length (L) and inversely proportional to its cross-sectional area (A) (Ackmann & Seitz, 1984), or:

$$Z = \frac{\rho L}{A} \quad \text{.....(1.7)}$$

Thus for a cylindrical conductor with a uniform cross-sectional area, the volume (V) of the conductor is proportional to its length squared (L^2), divided by its impedance (Z), or:

$$V = \frac{\rho L^2}{Z} \quad \text{.....(1.8)}$$

The coefficient, ρ, in the above equation (1.8) is called the specific resistivity. Specific resistivity is an electrical property of a particular material. For homogenous conductors, this coefficient is a constant physical property. The body, and its constituent tissues, is not homogenous, rather it exists as a heterogeneous electrical conductor, with each tissue having its characteristic resistivity (Burger & van Milaan, 1943; Schwan & Kay, 1956a, 1956b; Rush *et. al.*, 1963; Geddes & Baker, 1967).

Most current techniques of body composition assessment using electrical impedance measurements assume that:

- the specific resistivity of the body is constant for age and sex (Nyboer, 1972; Kushner & Schoeller, 1986),

- the conducting body acts as a homogenous, cylindrical conductor to electrical current, and
- the volume of the conducting body or compartment is related to the impedance and conductor length of the measured body or compartment using equation 1.8 shown above.

1.3.3. Developed and Current Measurement Practices[1]

The present use of bioelectrical impedance to estimate body composition is based upon the premise that the fat-free mass contains a greater ionised electrolyte content, and hence conductivity, than the adipose tissue (Settle *et. al.*, 1980; Kushner & Schoeller, 1986). These current methods may broadly be categorised as being techniques using either whole body or segmental impedance measurements in their predictions. Studies using whole body or segmental impedance measurements attempt to assess either the fat mass, the fat-free mass, the extra- and intra-cellular fluid compartments, or the total body water volume (Harrison & van Italie, 1982; Lukaski, 1987; Brodie 1988b).

[1] The historically different and unconventional methods of impedance measurement have resulted in numerous largely undefined and unstandardised techniques currently available today. In terms of definition, many studies use the term "bioelectrical impedance analysis" or BIA to describe their method of assessment. This term (BIA) is used in the context of these studies to exclusively refer to any technique based on the measurement of whole body impedance, and the inclusion of this impedance value into an conductor volume equation based on the relationship $V = (\rho L^2) / Z$. These volume equations are often developed as statistical regression equations. The terms "bioelectrical impedance measurement", "bio-impedance measurement", "body impedance", or "impedance" refer to the direct and simple measurement of impedance of the body or a part of the body.

a) *Whole-Body Impedance Methods*

Very few reported studies use an isolated measurement of whole body impedance to indicate aspects of body composition. Most studies combine the measurement of body impedance with other measurements, such as anthropometry, sex and age, in population-specific developed prediction equations (Katch & Katch, 1980). Whole body impedance measurements are defined as the impedance measurements of the body as measured between the wrist and the ipsilateral or contralateral ankle. Moreover, whole body impedance is largely related to the technique of bioelectrical impedance measurement known as BIA (for definition, see footnote 1). Essentially, BIA uses the relationship between conductor volume, and conductor impedance and length, i.e., $V = (\rho L^2) / Z$ (equation 1.8), to estimate the volume (V) of the measured compartment. In the BIA technique, the length of the conductor is taken to be the height, or stature, of the subject.

BIA studies have found that the statistical association between impedance measurements and body composition is improved with multivariate prediction equations. Theoretically, these equations include additional measures of the size and shape of the "conductor", and any other factors such as age, sex and even ethnicity that may correlate with body composition. The inclusion of further prediction variables causes the technique and the ultimate prediction equation to be very population specific (Deurenberg *et. al.*, 1991a). This addition results in the value of the impedance measurement being statistically obscured by other included variables.

The predictive strength of the developed equations using BIA is statistically assessed by comparison of the BIA estimate to a known standard measurement. These standard measurements are selected depending on the body composition aspect being considered.

i. Fat and Fat-Free Mass

In the assessment of the fat and fat-free masses, the calibration standard measurement may be hydrodensitometry (Lukaski *et. al.*, 1985; Segal *et. al.*, 1985; Guo *et. al.*, 1987), anthropometry (Jackson *et. al.*, 1988; Kushner & Haas, 1988; Little *et. al.*, 1989), total body potassium (Cordain *et. al.*, 1988) and dual photon absorptiometry (Wang *et. al.*, 1987).

ii. Intracellular and Extracellular Fluid

Attempts have also been made to assess intra- and extracellular fluid volumes using BIA. Perhaps not yielding as statistically significant results as the assessments of fat and fat-free mass, the impedance estimates of ICF and ECF have been almost entirely compared to values obtained in isotope dilution studies (Bolot *et. al.*, 1977; Gambini *et. al.*, 1980; Tedner *et. al.*, 1985; McDougall & Shizgal, 1986; Lukaski & Bolonchuk, 1988). Deurenberg and co-workers (1989a) found a significantly positive relationship between the ratio of the intra- and extracellular fluids, and impedance measurements. Scheltinga *et. al.* (1992) further showed a relationship between measured reactance (X_c) and ECF.

iii. Total Body Water

The assessment of total body water by BIA has yielded relative statistical success when compared to various isotope studies. The first investigators to show this relationship between BIA and total body water were Hoffer *et. al.* (1969). In many different validation studies, BIA has been compared to total body water measured by deuterium dilution, with correlation coefficients ranging from R = 0.78 to R = 0.99 (Gray, 1988; Fredrix *et. al.*, 1990; Kushner *et. al.*, 1990; Pullicino *et. al.*, 1990; Schols *et. al.*, 1991; Binnerts *et. al.*, 1992; Friedl *et. al.*, 1992). Heavy water dilution methods of measuring total body water have also been used in validation studies, yielding correlation coefficients between R = 0.68 to R = 0.98 (Davies *et. al.*, 1988, 1990; Gregory *et. al.*, 1991; Mayfield *et. al.*, 1991; Goran *et. al.*, 1993). A third, reasonably popular method of measuring total body water is tritiated water dilution. This method also yields significant and high correlations with BIA, with coefficients between R = 0.69 and R = 0.97 (Hoffer *et. al.*, 1969; Nyboer & Sedensky, 1974; Jenin *et. al.*, 1975; Segal *et. al.*, 1985; Hannan *et. al.*, 1990; Heitmann, 1990; Franssila-Kallunki, 1992).

With the same degree of success seen in the above reference methods, some investigations have considered the changing measurement of BIA during changing body hydration, which is indicated by acute weight change. Examples of weight change caused by shifting hydration are found in renal dialysis of nephropathic subjects (Kurtin *et. al.*, 1990; Petracca & Biasioli, 1990; de Lorenzo *et. al.*, 1991), in paracentesis of cirrhosis patients (Gugliemi *et. al.*, 1991), in protein-energy malnutrition (Vettorazzi *et. al.*, 1990), in post-surgical patient such as those having undergone gastrectomy (Miholic *et. al.*, 1990), and in patients undergoing

intrapleural fluid removal procedures (Spence *et. al.*, 1979). It must be noted that there is a statistical overlap between the weight changes (due to the examples given above), and the inclusion of weight in the BIA regression equations.

A further non-isotope dilution method of total body water measurement that BIA has been successfully compared with is the use of urea kinetics in haemodialysis patients (Pastan & Gassensmith, 1992). Changes is urea due to fluid shifts are regarded as an effective indicator of body hydration and total body water volume.

b) *Segmental Impedance Measurements*

Perceived problems associated with the inclusion of further predictive variables, the body existing as a homogenous cylinder, and the conductor length being the height of the measured subject have led to the recent consideration of segmental body impedance measurements (Settle *et. al.*, 1980; Smith, 1987). It is felt that segmental measurement of body impedance would reduce the effect of increased specialisation regarding the prediction equations (Baumgartner *et. al.*, 1989; Chumlea & Baumgartner, 1990). The segmental measurement of impedance takes into account that the specific resistivities of the body differ from tissue to tissue and between body segments. An attempt was made to standardise the segmental body impedance measurements as body composition indicators. It was theorised that the total conductive volume of the body can be derived from measures of the lengths of the segments being considered, and their biological impedance values. If the body is divided into five segments (excluding the head, they are the two arms,

the two legs, and the trunk), then the total conductive volume is theoretically represented as:

$$\text{Total Vol} = 2\left(\frac{\rho_1 \times L_{arm}^2}{Z}\right) + 2\left(\frac{\rho_2 \times L_{leg}^2}{Z}\right) + \left(\frac{\rho_3 \times L_{trunk}^2}{Z}\right) \quad \text{.....(1.9)}$$

It is naturally assumed that the specific resistivity of each segment (ρ_1, ρ_2, and ρ_3) is known. Using this summative method, segmental impedance measurements, with an indication of their conductor length, have been used to assess different aspects of the body composition. The total and segmental fat and fat-free masses (Chumlea *et. al.*, 1988a, 1988b; Fuller & Elia, 1989), and total body water (Fuller & Elia, 1989; Zillikens *et. al.*, 1992) have all been assessed by segmental impedance measurement. These assessments have proved to be fairly successful, although, they have consistently slightly underestimated body composition values.

Although by definition the measurement of blood impedance is not strictly segmental, it may be considered as a compartmental measure of body impedance. Deurenberg *et. al.* (1989a) performed bioelectrical impedance measurements on samples of venous blood, and found a significant relationship between the impedance value and the extracellular fluid.

1.3.4. Some Problems Associated with Electrical Impedance

Although the high degree of statistical correlation between the BIA method and body composition assessment has pointed to BIA as being a valid method of estimation, some doubts and technical problems have begun to appear (Smith, 1987; Grieve, 1993; Grieve & Henneberg, 1994). These problems can be summarised as being hypothetical, technical, statistical or biological, and are briefly discussed as such below.

a) *Problems Related to Hypotheses of the Impedance Method*

BIA is based on various assumptions about the composition of the human body. Regarding the conductive compartment, the relationship between the fat-free mass and total body water relies on the assumption that the fat-free mass is constantly hydrated at approximately 73%. The origin of the value and static nature of this figure is unclear. It seems that the value of 73% is a result of body composition analyses originally performed on the rabbit (Pace & Rathbun, 1945). Even if the water content of the fat-free mass is 73%, and varies little in the normally hydrated person, pathological conditions, such as fluid retention or dehydration, can cause considerable variation (Bengtsson *et. al.*, 1990). Furthermore, the normal process of ageing results in significant changes in the hydration status of the body (Schoeller, 1989).

An assumption that the body is a homogenous, geometrically equal conductive mass is plainly erroneous. The segmental approach to assessing body composition

using impedance measurements does acknowledge this problem, and does attempt to address it. Unfortunately, the segmentation of the body into the two arms, two legs and the trunk only partially addresses the inconsistent geometric problem, and does not consider the complex tissue heterogeneity in any way.

b) *Technical Problems*

Technical problems with the BIA method are beginning to cause doubts about the results obtained in BIA studies (Cohn, 1985; Deurenberg *et. al.*, 1991a; Grieve & Henneberg, 1994). One such problem is the nature and placement of electrodes (Gartner *et. al.*, 1991). There does not seem to be a standard, accepted placement position for the body electrodes. Furthermore, commercially published electrode sites are often difficult, if not impossible, to effectively locate and apply in children and infants (Barillas-Mury *et. al.*, 1987).

Skin temperature and blood flow at the site of the electrode placement have been found to influence impedance recordings (Caton *et. al.*, 1988; Smith, 1992). From the literature, it is seen that very little standardisation of the ambient and biological temperature during impedance measurement exists. Coupled to this, there is no uniformity regarding the hydration and prandial status of the subject (Deurenberg *et. al.*, 1988; Rising *et. al.*, 1991; Tagliabue *et. al.*, 1992). The individual influences of hydration and prandial states, skin temperature, and electrode placement may not significantly affect impedance recordings, but may cause additive errors.

A final potential technical error that may influence impedance recordings is the actual recording instrument. There are numerous commercially available instruments available, which are released with no documentation regarding their design and construction. Furthermore, some instruments do not possess any form of effective calibration. Due to the investigative nature of the scientific community, the application of these instruments is not limited only to the manufacturer's instructions and prediction equations. Investigators rely on the biological impedance values they obtain to develop prediction equations. These published prediction equations are then often used by other investigators using different instruments. Disturbingly, there have been significant differences between instruments reported (Graves *et. al.*, 1989; Newby *et. al.*, 1990). An additional query is beginning to surface regarding the use of 50kHz as the optimal AC frequency in body composition studies (Cohn, 1985; Piccoli *et. al.*, 1991).

c) *Statistical Difficulties*

The very specific nature of the populations being studied is resulting in many population-specific prediction equations. To name but a few, body composition studies based on BIA consider different races, ages, sexes, and numerous pathological states as being distinct research groups (Schutte *et. al.*, 1984; Cohn, 1985; Katch *et. al.*, 1986; Deurenberg *et. al.*, 1990).

A further problem related to the statistical basis of BIA as a body composition technique is the inclusion of anthropometric and other 'additional' prediction variables in regression equations to estimate body composition values. The inclusion

of anthropometric variables such as height and weight obscure the real or imagined value of impedance measurements in the estimation of the body composition value (Hergenroeder & Klish, 1990; Mazess, 1991a, 1991b; Forbes *et. al.*, 1992; Grieve, 1993; Grieve & Henneberg, 1994).

d) *Biological Influences*

Normal and abnormal biologic[illegible]riation possibly accounts for some technical errors in the BIA technique. Physiological factors such as the stage of the menstrual cycle (Gleichauf & Roe, 1989), hydration status (Khaled *et. al.*, 1988; Deurenberg *et. al.*, 1989a; Thompson *et. al.*, 1991), blood pressure (Siervogel *et. al.*, 1987), and respiration (Baumg[illegible] *et. al.*, 1988) have been reported to influence impedance measurements.

Furthermore, the current injected into the body may not travel in a direct linear path. In a homogenous system of electrolytes in solution, the electrical current will travel linearly between electrodes The electrical properties of the body may be such that the current will follow the path of least resistance. This path in the heterogeneous biological system is in all likelihood not linear, and may be extensively complex, favouting certain tissue types over others. Simply, the body may not behave as a linear conductor.

Biological extremes in body composition, such as obesity and malnutrition, may also cause problems regarding the method of BIA (Gray *et. al.*, 19[illegible] 1989).

1.3.5. The Unfolding Questions and a Need for Further Investigation

Considering the problems summarised above regarding the BIA method of body composition analysis, the technique has come under scrutiny amongst investigators. Of particular interest is the issue of the homogeneous-heterogeneous nature of the conductive body. Can the impedance of the body truly be related to the conductor length and the conductor volume? Does the body conduct electricity uniformly and in a linear fashion, or does the molecular, cellular and tissue heterogeneity prevent this? From the data available, it seems that these questions cannot wholly be answered.

Of even more concern is the manner that the inclusion of anthropometry in the BIA technique obscures the value of impedance measurements, which claims to be the essence of the technique. It is reasonable to presume that anthropometric variables such as height and weight are strong indicators of body composition in themselves. Rather simply, the height of a body is related to the fat-free mass, as height is a function of bone length and the fat-free mass includes the skeletal tissue. Body weight indicates the size of the body relative to the height, and can be considered a function of the fat mass of the body. Also, height and weight are directly related to each other.

The simplistic relationship between height and weight, and the fat-free mass can be extended to the total body water and the water compartments of the body. Hume & Weyers (1971) showed a highly significant correlation between height and weight, and total body water ($R = 0.96$). Does the inclusion of the measurement of body impedance add any value to body composition estimates assessed by anthro-

pometry alone? Recent tentative studies considering the individual effects of the variables involved in BIA prediction equations have concluded that measurements of whole body impedance do not significantly improve the statistical power of estimation of body composition values (Houtkooper *et. al.*, 1989; Hergenroeder & Klish, 1990; Deurenberg *et. al.*, 1991; Gregory *et. al.*, 1991; Mazess, 1991a, 1991b; Forbes *et. al.*, 1992). This lack of improvement in predictive ability holds true for all body composition values. Campos and co-workers (1989) conclude:

> "...results show that density, total body water, total body fat, and lean body mass in a population of healthy individuals under steady state conditions can be estimated by anthropometric-derived formulas with the same relative accuracy as that provided from estimates based upon BIA measurements."

1.4) Aims of the Present Study

From the problems of the BIA method, and the questions currently being asked by body composition investigators, the primary question remains: If it does not adequately indicate body composition, what does the measurement of body impedance indicate? If it indicates anything at all?

Electrical conduction of by the body, the volume of the conductive compartment, and the nature or concentration and distribution of the conductive electrolytes in a normal static subject are interrelated in some way (Thomasset, 1963; de Vries *et. al.*, 1989; Grieve, 1992). Considering this relationship, an important question is: what factor provides a change in body conduction? The volume of the conductive compartment or the composition of this compartment? If both factors are involved, what is the extent of each? In order for the measurement of the electrical impedance of the body to be a valuable and employable tool, this question needs to be answered, within both physiological and pathophysiological limits. Due to the ionic and electrolytic nature of the conductive path of the body, it is speculated that body impedance measurements may only indicate the nature or composition of the conduction medium, and that body impedance is not substantially affected by the volume of the conductive or non-conductice parts of the body.

The primary aim of the main part of the current study was to consider whether the electrical impedance measurements of the body indicate the nature, concentration or hydration of the ions in the total body water volume. These impedance measurements include whole body, segmental and blood impedance measurements.

The reference method of total body water volume measurement was tritiated water dilution. The ionic concentration of the body fluid, represented by blood, was measured for comparison with impedance values. To assess any possible influence of fluid and ionic movement on the impedance measurements, the study subjects were measured in a slightly dehydrated and then in a rehydrated state.

In order to provide a preliminary assessment of the hypothesis that body impedance is related to the nature of the conductive fluid compartment, it was felt the impedance measurements should be performed on a biological fluid, which would be indicative of the hydration status of the body. Sampled blood is a fluid without the complication of a vastly different tissue composition, it is relatively easy to obtain, and it is easily manipulated. The *in vitro* impedance measurement of blood (Chapter 2.1) would provide some indication of the direction the main study should progress. With significant results from this pilot investigation, the main study could reasonably progress with an attempt at *in vivo* measurements of body impedance during different hydration states.

Due to the indirect nature of the tritiated water dilution method, it would be necessary to validate certain aspects of the technique. Firstly, the equilibration time of isotope dilution in the body should be determined (Chapter 2.2). This is necessary as many conflicting time values exist in previously described work. Secondly, the technical aspects should be rehearsed in an animal model (Chapter 2.3). The purpose of the animal model would not be to validate the method's accuracy and reliability, but to assess its feasability. With successful completion of these two pilot investigations, tritium dilution would then be acceptable for use in the main study.

If the results of the main study showed that impedance measurements are related to the concentration of electrolytes in the body, then the issue of electrical current frequency should be considered. In order to determine the effect of frequency on body impedance measurements, a multiple frequency instrument should be constructed and validated (Chapter 4.1). The validation should include both electronic and biological validation. Preferably. the constructed instrument should be compared to other commercially available instruments (Chapter 4.2). The simplest way of assessing the involvement of frequency in impedance measurements would be to repeat the *in vitro* measurement of impadance in manipulated blood samples (Chapter 4.3).

If both the frequency and the ionic concentration exhibits an apparent effect on impedance measurements, then the degree and nature of this effect should be further considered. One biological application which provides a local, dynamic and acute ionic concentration flux is muscle contraction. In order to assess the degree and nature of the frequency and concentration effects, the local impedance changes during muscle contraction should be investigated.

With this interrelated body of information, it should be possible to draw some conclusions regarding the electrical conduction or impedance of the body to an applied current.

In summary,

- The question asked in this investigation is:

Regarding the composition of the body, what do electrical impedance measurements of the body indicate? If anything at all?

- And the proposed hypothesis is:

Impedance measurements of the body are dependent on the nature and concentration of the ions and electrolytes, which are found in solution in the total body water, and constitute the electrical conduction compartment of the body.

CHAPTER 2

PILOT INVESTIGATIONS

In order for the main thrust of this research project, that is, the electrical impedance assessment of body fluid volumes and electrolyte concentrations to progress efficiently, various pilot studies were performed. The pilot investigations were able to either provide answers that were needed for the main study, or they were able to assess the proficiency, efficiency and accuracy of instruments and methods used. Three such pilot investigations were performed.

1. *Electrical Impedance and Ionic Concentration of the Blood*

In the body, the conductor volume, the nature of the conductive compartment, and electrical conduction are interrelated. This relationship is true for both physiologically and pathophysiologically static states In order to understand the true essence of electrical impedance by a biological system, it is necessary to assess the factors which cause a change in the electrical conduction. Does a change in the volume or nature of the conductive compartment result in changing impedance measurement? It is hypotheised that the nature (ionic concentration) of the conductive compartment is a significant factor in impedance measurement. It is presumed that the fluid, and related, compartment, are involved in electrical conduction. In order to provide a preliminary indication of this relationship, impedance

measurements were performed *in* vitro in biological fluid samples. The ionic composition of blood plasma was assessed by means of electrical impedance measurements to determine whether the impedance measurement of a blood sample was an indicator of blood characteristics relating to the hydration status. Also, these ionic measurements were performed to develop a reliable and accurate method of assessing blood ionic characteristics. The instrumentation and measurement techniques were optimised in this pilot study, in preparation for the main study.

2. *The Equilibration Period of Tritiated Water in the Body*

Due to the relative insufficiency of methodological and technical information regarding the assessment of total body water by means of the tritiated water dilution method, a second pilot study was performed in order to:

(a) determine the optimal equilibration period of tritiated water in the human being for the purposes of accurate total body water measurement, and

(b) to serve as a trial run for the methods of venous blood sampling, sample management and dilution, and tritium concentration measurement, using a scintillation counter.

3. *TBW Measurement by Tritiated Water Dilution in an Animal Model*

The final pilot study was performed to assess the viability of the method selected for TBW determination. This determination was by tritiated water dilution, and was assessed in an animal model. Some of the proposed chemical analyses for

the main study were also assessed. Total body water measured by tritiated water dilution was compared to the direct measurement of total body water obtained from desiccation.

2.1) Electrical Impedance and Ionic Concentration of the Blood

The bio-electrical impedance method is based on the assumption that the electrical conductivity of the body is a function of the fat-free mass, and the fat mass impedes the flow of electrical current (Jenin *et al.*, 1975). Numerous studies have described relationships between different methods of bio-electrical impedance measurements and different body composition characteristics, including fat mass, fat-free mass and body fluid compartments (Lukaski, 1987).

Considering the fact that many published studies use regression analysis to predict body composition indices, some investigators have begun to question the validity of the bio-electrical impedance method (Cohn, 1985; Solomons, 1986; Grieve, 1993; Grieve and Henneberg, 1994). Unfortunately, the scientific basis of bio-electrical impedance analysis is often overlooked in routine consideration of the methods of body composition determination. In particular, the basic assumption that the electrical conductivity of the body relies on the availability of mobile electrolytes in the conducting fluid compartments is often erroneously ignored.

Without finding it necessary to use equations developed in regression analysis, other investigators have considered some of the electrical properties of the body, and have shown relationships between:

- impedance measurements of the blood and the intracellular and extracellular water spaces (Deurenberg *et al.*, 1989a),
- impedance measurements of the body and the histological structure of cells (Jenin *et al.*, 1975),

- the specific resistivity of blood and haematocrit (Spence *et al.*, 1979),
- body impedance and muscle anisotropy and excitation (Schwan and Kay, 1956a),
- and between body impedance and extracellular Na^+ concentration (Thomasset, 1962, 1963).

There are many studies describing the statistical relationships between various body compartments and anthropometric characteristics combined with bio-electrical impedance. However, very few studies consider the relationships between bio-electrical impedance measurements and the direct measurement of the fluid and electrolyte concentrations. Although some investigators have considered aspects related to body fluid and electrolyte concentrations, no comprehensive study of actual fluid and specific electrolyte measurements indicated by bio-electrical impedance measurements have been reported. Impedance of the body has been compared to measured volumes of the body fluid spaces, viz. intracellular and extracellular volumes and total body water (Cooley and Lehr, 1972; Jenin *et al.*, 1975; Settle *et al.*, 1980; Deurenberg *et al.*, 1989a; Scheltinga *et al.*, 1992). These assessments of the volume of body fluid compartments by bio-electrical impedance measurement are interesting in their apparent success, yet the mechanisms underlying bio-electrical impedance analysis and what specifically it is measuring are not adequately explained by the results.

Some investigators further considered what additional understanding would be gained by the electrical impedance measurement of blood. The blood acts as a representative medium of intracellular and extracellular fluids, and indicates the

distribution of water and electrolytes in the different compartments (Deurenberg *et al.*, 1989a; de Vries *et al.*, 1989). Unfortunately, these studies did not consider the nature and quantity of the electrolyte and water distribution in these 'conductive' compartments.

Almost ironically, the only investigations that have considered the issues of fluid and electrolyte concentrations, relative to electrical impedance measurements, were the pioneering studies performed by Thomasset (1962, 1963). These studies only describe, very briefly, the effects of extracellular Na^+ concentration on the electrical impedance measurement of the whole body. In desiring a comprehensive understanding of the electrical measurements of the body, Thomasset initiated a substantial, rapid, and perhaps premature move to application studies regarding general body composition.

In an attempt to test the theory that the electrical conductivity properties of the body depend largely on the amount of body water and dissolved electrolytes in the body fluids, impedance measurements were performed on venous blood samples with different ionic electrolyte and water concentrations. The methodological basis of this study was the report by Deurenberg *et al.* (1989a) which indicated that blood impedance is related to packed cell volume and the ratio of intracellular and extracellular fluid volumes. The packed cell volume relationship was also shown by the Spence *et al.* (1979) study, which considers body impedance and haematocrit.

For the purposes of this study, the actual ionic concentrations, including H_2O (measured as osmolarity), were considered in relation to the impedance measurements of a predetermined blood volume. Venous blood was selected as it is an

example of the body fluid compartment, having an extracellular component (the blood plasma) and an intracellular component (predominantly the content of the red blood cells). To this extent, donated venous blood samples were measured for various ionic concentrations (Na^+, K^+, Cl^-), H_2O concentration (osmolarity), and impedance to a constant 50kHz, 800μA alternating current. Furthermore, it was deemed necessary to manipulate some blood samples in order to produce a range of various concentration values against which impedance could be compared. This manipulation was achieved by adding either NaCl or H_2O to selected samples. The addition of NaCl was done to emulate a hyperosmotic or dehydrated state, and the addition of H_2O was done to emulate a hyposmotic or overhydrated state.

2.1.1. Materials and Methods

a) *Protocol*

During normal public blood donation at the South African Blood Transfusion Service, forty-four donors consented to the collection of an additional 10m*l* of their venous blood. This did not require further venous puncture, as the volunteers were already donating blood. The blood was collected by means of cubital fossa venipuncture, and was stored in heparinised collection tubes. The total collection of these venous blood samples was performed within 3 days.

Immediately on sampling, the impedance of the blood to a 800μA, 50kHz alternating current was measured. In an attempt to imitate an *in* vivo situation, the impedance measurements were performed at normal body temperature. This immediacy was in an attempt to control for blood temperature, measuring. The samples were then randomly divided into two groups. Each sample in both groups was then separated equally into two collection tubes to provide 88 x 5m*l* samples. A 1m*l* sodium-chloride (NaCl, 25μmol.ml^{-1}) solution was added to 22 of these samples, and 1 m*l* of distilled H_2O was added to another 22 of these samples (see Figure 2.1). After ensuring that the samples were all maintained at approximately 36° C by placing them in a heated H_2O bath, electrical impedance was then reassessed on all 88 samples. In summary, from the original 44 x 10m*l* samples, there were 44 x 5m*l* samples the same as the originals, 22 x 5m*l* samples with diluted NaCl solution added, and 22 x 5m*l* samples with distilled H_2O added.

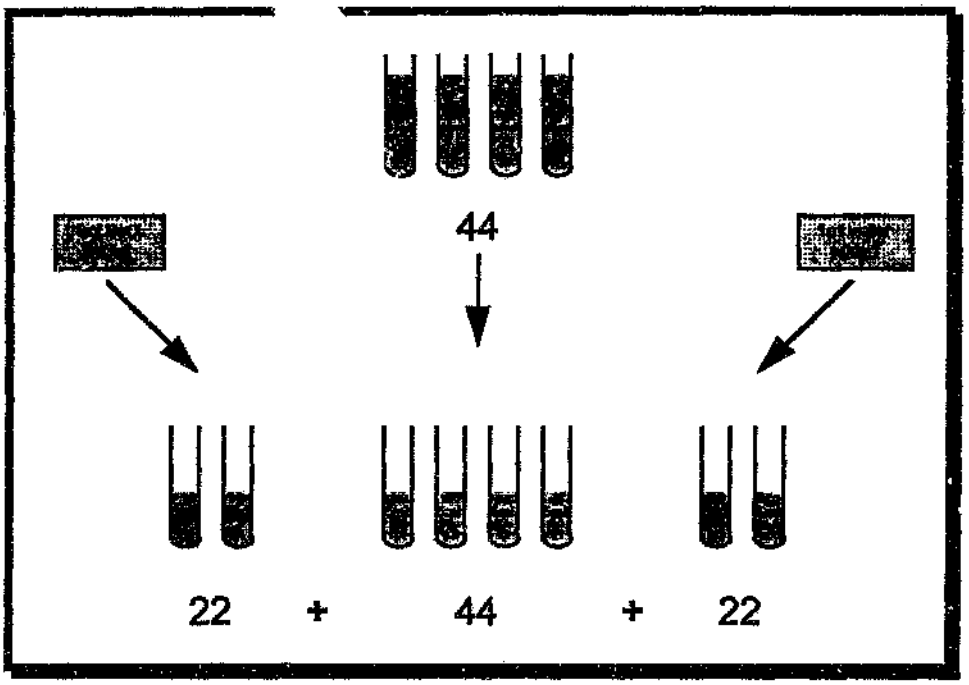


Figure 2.1: Diagrammatic representation of sample manipulation

Within 3-4 hours of the second blood impedance measurements, the samples were centrifuged for 10 minutes at 3000rpm (Technospin *R*, Sorval Instruments). The blood plasma was removed from the centrifugate by means of disposable pipettes, and placed in storage vials. The plasma was then frozen at approximately -20^0 C. Within one week of sampling, the plasma samples were unfrozen, and ionic concentrations of sodium, potassium and chloride, and plasma osmolarity were measured.

b) *Blood Impedance Measurement*

To measure the electrical impedance of the venous blood samples, a blood chamber was constructed. The blood chamber consisted of an open box constructed from clear perspex (see Figure 2.2). The dimensions of the chamber were 2.5cm x 2.5cm x 2.5cm. Two pairs of stainless steel electrodes were placed opposite each other in the sides of the box, and the level of 4m*l* of fluid in the container, which was well above the level of the electrodes, was marked.

The impedance of the constant volume (4m*l*) blood samples to a 800μA, 50kHz alternating current was measured by means of a commercially available bioelectrical impedance analyser (Holtain Body Composition Analyser; Holtain Ltd., UK). The pair of current injector electrodes and the pair of current detector electrodes were connected to the blood chamber, an injector and a detector lead attached on either side of the chamber (see Figure 2.2).

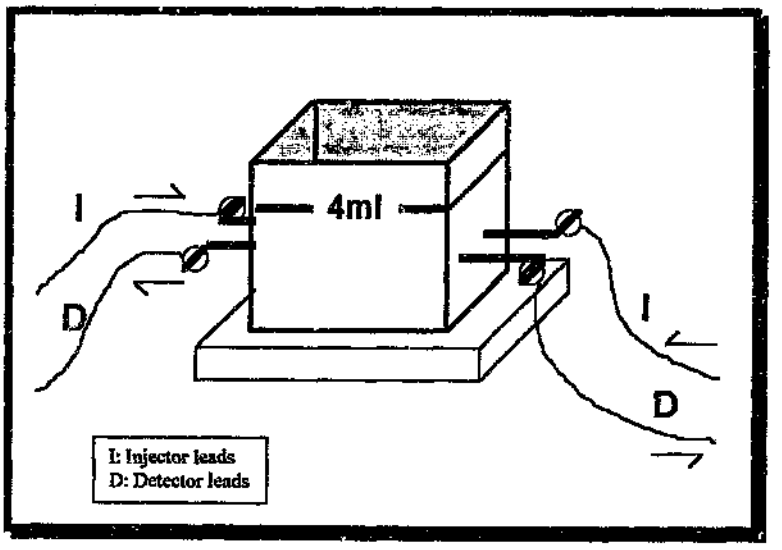


Figure 2.2: Blood impedance chamber

To perform the impedance measurements, the chamber was placed on a level mat of insulated material, and the blood sample was decanted into the chamber to the predetermined mark, indicating the correct volume of approximately 4m*l*. Before connecting the instrument to the blood chambers, the impedance analyser was calibrated, using the internal calibration resistance supplied by the manufacturers. The power supply was also checked. The instrument then was applied to the blood chamber, and the AC current was injected through the blood sample by way of the electrodes. The drop in voltage across the detector electrodes was measured by the instrument, and proportionately displayed as the impedance, in Ω, to the injected current. Each measurement was performed in duplicate.

c) *Sodium and Potassium Ion Concentrations*

Na^+ and K^+ concentrations were measured by means of a FLM3 flame photometer (Radiometer, Copenhagen). The procedure, which is suggested by the manufacturer, involved the following steps.

- STEP 1: Producing a blank or diluant solution

  7m*l* of a concentrated lithium solution (S3376) was added to 500m*l* of distilled H_2O. This diluant solution was used to calibrate the instrument to read zero values, and was used for all dilutions of serum flame-photometry standards and plasma samples.

- STEP 2: Producing a serum flame standard for calibration

  A 1:100 dilution of aspiration solution was produced by adding 0.1m*l* of concentrated aspiration solution (S3416) to 9.9m*l* of distilled H_2O. This as-

piration solution was again diluted in a ratio of 1:200 with the diluant, by an automatic fluid diluter (Radiometer A6241 diluter, Copenhagen), to give a serum flame-photometer standard. Using this standard, the FLM3 instrument was calibrated for Na^+ and K^+ concentration measurements of 140mmol.l^{-1} and 5mmol.l^{-1} respectively.

- STEP 3: Measurement of plasma samples

  In the same manner as the serum flame standard, the plasma samples were diluted in the ratio of 1:200 with the diluant, by means of the automatic diluter. This diluted sample was then assessed by the FM3 instrument for Na^+ and K^+ concentrations, measured in mmol.l^{-1}. All measurements were performed in duplicate.

d) *Chloride Ion Concentration*

Chloride ion (Cl^-) concentrations were measured using a CMT10 Chloride Titrator (Radiometer, Copenhagen). According to the manufacturer's instructions, a 0.4mol H_2SO_4 supporting electrolyte solution (S3224) was added to the titration vessel. The electrodes and the titration vessel were cleaned of any excess solution, and the instrument was switched on. The sample pipette volume was calibrated and set to 20μl. A blank sample was measured by the instrument, and the readout panel was zeroed. Using a clean pipette sheath for each sample, 20μl plasma samples were inserted into the instrument and were assessed for Cl^- concentration, measured in meq.l^{-1}. Each measurement was performed in duplicate.

e) *Plasma Osmolarity*

Plasma osmolarity was measured by means of a vapour pressure osmometer (Model 5100C, Wescor, Utah). Using the provided Wescor Osmolarity Reference Standards, a three-step calibration procedure was performed. Using the calibration controls on the instrument, the instrument was set for a standard value of 290mmol.kg^{-1}. Following this, the instrument was set for a standard value of 1000mmol.kg^{-1}. Finally, the 290mmol.kg^{-1} standard setting procedure was repeated to correct the offset. Zero-value compensation was then also performed.

Following the manufacturer's instructions, 8μl volumes of the plasma sample were added to the instrument, and assessed for osmolarity, measured in mmol.kg^{-1}. Each measurement was performed in duplicate.

f) *Statistical Analyses*

Using the statistical functions and other calculations provided in Microsoft Excel Software (Microsoft Corp., Ver 4.0), the following statistical analyses were performed on the data collected for this study (Appendix 2 describes in detail the statistical methods used):

1. From the *central limit theorem* of statistics (Hollander and Wolfe, 1973), and since the sample size was greater than n = 30, the sampling distribution of the mean, $\bar{x}$, could be approximated by a normal probability distribution. This normalcy was confirmed statistically. The mean, median and mode of each measured variable were considered for similarity - if the three indications of

central tendency were not obviously different, the sample measurements were considered to be relatively normally distributed.

2. Using a 5% ($\alpha \leq 0.05$) sampling error, the sample size (n) was assessed for adequacy, i.e., n = 44 for the non-manipulated samples, and n = 22 for the samples with NaCl and H_2O added.
3. The reliability of the measurements on the blood samples was assessed by means of two statistical estimates. The amount of error variance was assessed by calculating the standard error of measurement between two repeated measurements, and the proportion of error variance was obtained from a correlation analysis - indicating the variance (R^2) between the two measurements (see Appendix 2 for details on these two tests).
4. To assess the validity and repeatability of the method of measuring blood impedance, the impedance value measured for the 10ml blood sample was compared to the impedance value measured for the 5ml sample of unmanipulated blood. A two-sample *t*-test was used to compare the means and the standard deviations of the two samples. A correlation analysis was performed to assess the linear association between the two samples. Because only one investigator was responsible for the sampling and measurement of all data, it was not necessary to assess inter-observer reliability.
5. The predictive relationship between blood impedance values and the various blood ionic measurements was assessed by means of correlation analysis. The linear trend of this relationship was confirmed by means of simple regression analysis (Rawlings, 1988).

2.1.2. Results

a) *Data Description*

A summary of the data is presented in Tables 2.1 and 2.2. Values presented in Table 2.1 indicate the mean, standard deviation (STD), and coefficient of variation (ν) for the measurements of blood impedance and blood ionic composition for the two sample groups, i.e., the 10m*l* and 5m*l* samples, of unmanipulated blood. Table 2.2 presents a summary of the data from the two manipulated groups, i.e., the sample group with NaCl added, and the sample group with distilled H_2O added. Both summary tables include the values of the mean of the duplicate measurements.

Table 2.1: Summary of data of unmanipulated blood measurements

	10m*l* sample (n = 44)			5m*l* sample (n = 44)		
	MEAN*	STD†	ν (%)‡	MEAN	STD	ν (%)
Blood Z (Ω)	135.6	9.7	7.2	135.9	12.0	8.8
Na^+ (mmol.l^{-1})	141.8	5.5	3.9	142.7	5.8	4.1
K^+ (mmol.l^{-1})	4.9	0.6	12.2	5.0	0.8	16.0
Cl^- (meq.l^{-1})	108.8	5.6	5.2	110.0	5.6	5.1
Osmolarity (mmol.kg^{-1})	288.5	8.0	2.8	290.7	7.6	2.6

*: measurement mean
†: measurement standard deviation
‡: coefficient of variation

Table 2.2: Summary of data of manipulated blood measurements

	NaCl added (n = 22)			H_2O added (n = 22)		
	MEAN*	STD†	ν (%)‡	MEAN	STD	ν (%)
Blood Z (Ω)	113.8	9.5	8.4	147.9	12.1	8.2
Na^+ (mmol.l^{-1})	169.5	4.0	2.4	132.3	5.0	3.8
K^+ (mmol.l^{-1})	4.7	0.8	17.0	4.5	0.9	20.0
Cl^- (meq.l^{-1})	129.1	5.8	4.5	101.5	5.4	5.3
Osmolarity (mmol.kg^{-1})	338.5	8.9	2.6	269.9	10.6	3.9

*: measurement mean
†: measurement standard deviation
‡: coefficient of variation

b) *Type of Distribution*

Based on the Theorem of Central Limits one can often assume that a sample with more than 30 observations can be approximated by a normal probability distribution. In view of the parametric nature of the statistical procedures to be performed on the collected data, it was considered prudent to assess the degree to which the sample set approximated a normal distribution. To compare the mean, median and mode of each of the variables measured, each variable was plotted, giving the three measures of central tendency. The result of this plot is depicted in Figure 2.3. It is evident from this graphical representation that there is no marked difference between the mean, median or mode of any of the variables measured. This would qualitatively indicate that the data collected was reasonably approximated by a normal probability distribution.

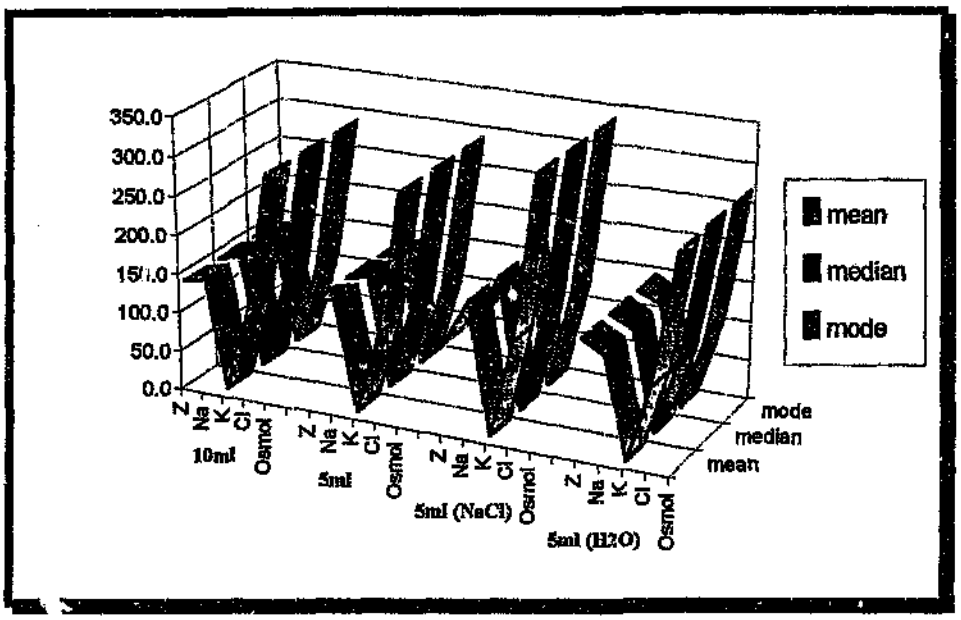


Figure 2.3: The means, medians and modes for the data set

c) *Sample Size Adequacy*

For the measurements performed on the blood samples, the sample size of n = 44, for both the 10m*l* and the 5m*l* samples, was within the limits needed for a probability level of $\alpha \leq 0.05$. Also, at this 5% probability level, the sample size of n = 22 for the 5m*l* samples was also adequate, except for the measurements of plasma K^+. At a 7.5% probability level, the sample size of n = 22 was adequate for all measurements, including the measurements of K^+. This increased sample size requirement of K^+ is largely due to the relatively high standard deviation of the measurement technique, with the coefficient of variation for this particular measurement being between 12% and 20% for the four sample measurements. Normal biological variation would account for this increased standard deviation.

d) *Reliability of Measurements*

All measurements obtained were performed in duplicate. This was done to assess the reliability and repeatability of the sampling techniques and measurement methods used in this study. To assess the amount of error variance, a calculation of the standard error of the measurements (SEM) was performed, and to assess the proportion of error variance in relation to true variance, a correlation analysis was performed, resulting in a variance (R^2) statistic. Table 2.3 indicates the SEM and R^2 values obtained for these two tests. The true variance was proportionately high in relation to error variance, ranging from $R^2 = 0.9950$ to $R^2 = 0.8169$ (i.e. 99.5% to 81.7%). This generally low proportion of error variance indicates that the measurements were reliable indications of the true values, and were repeatable.

Table 2.3: Reliability assessment of impedance and ionic measurements*

	Z	Na^+	K^+	Cl^-	Osmol.
10ml Sample - Plain					
R^2	0.9742	0.8901	0.9887	0.9919	0.9298
SEM	2.9396	4.0141	0.1394	1.2405	3.9345
5ml Sample - Plain					
R^2	0.9808	0.8903	0.9929	0.9883	0.9526
SEM	3.1630	3.9036	0.1357	1.3403	3.5043
5ml Sample - NaCl					
R^2	0.9745	0.9741	0.9942	0.9905	0.8169
SE	2.3176	3.4008	0.1288	1.5364	7.9412
5ml Sample - H_2O					
R^2	0.9773	0.8813	0.9950	0.9903	0.9081
SEM	3.0107	3.7721	0.1163	1.2497	5.4819

*: R^2 = proportion of true variance relative to total variance, SEM = standard error of measurement (indicates amount of error variance)

e) *Reliability and Validity of Blood Impedance Measurements*

The two unmanipulated samples (10m*l* and 5m*l*) were compared for significant differences to indicate whether two separate measurements of essentially the same sample are representative of each other. The means and standard deviations of the two sample groups were used to calculate the *t*-score for the difference between the two sample groups. Table 2.4 shows the calculated *t*-score for each of the variable pairs, the critical value for $| t |$ as well as the probability level of significance.

Table 2.4: Results of 2-sample *t*-test between blood variables

	t-Score	Critical Value*	Probability†
Impedance-Impedance	-0.1129	2.0167	0.9104
Na^+-Na^+	-0.7022	2.0167	0.4844
K^+-K^+	-0.5678	2.0167	0.5716
Cl^--Cl^-	-0.9297	2.0167	0.3551
Osmolarity-Osmolarity	-1.3101	2.0167	0.1936

*: critical value of $|t|$ for $\alpha = 0.05$
†: probability that sample group difference is explained by chance alone.

It is evident from the results presented in Table 2.4 that, for $\alpha = 0.05$, there exists no significant differences between the measurements of the 10m*l* plain sample and the 5m*l* plain sample.

f) *Data Analysis*

The associations between the blood impedance values, and the blood ionic composition indicators were investigated. The most obvious of these associations, the linear association, was considered first. To assess the linear associations between blood impedance and blood ions, a Pearson correlation calculation was performed on the 10m*l* data and the 5m*l* unmanipulated data, and the manipulated data. The values obtained in the correlation calculations were corrected using the results obtained for R^2 in the error analysis (see Appendix 2). The resultant correlation coefficients (R) were assessed for significance for $\alpha = 0.01$. At this level of significance, for n = 44, all R values greater or equal to 0.3845, and for n = 22, all R

values greater or equal to 0.423, are significantly different from R = 0. The method of assessing the correlation coefficient significance is described in detail in Appendix 2. Table 2.5 shows the correlation matrix obtained from the correlation calculations of the unmanipulated data, and Table 2.6 shows the correlation matrix obtained from the correlation calculations of the manipulated data.

Table 2.5: Correlation between unmanipulated blood impedance & ion concentration*

	10m*l* Samples					5m*l* Samples				
	Z	**Na^+**	**K^+**	**Cl^-**	**Osm**	**Z**	**Na^+**	**K^+**	**Cl^-**	**Osm**
Z (10m*l*)	1.00									
Na^+ (10m*l*)	-0.88	1.00								
K^+ (10m*l*)	-0.77	0.62	1.00							
Cl^- (10m*l*)	-0.79	0.80	0.53	1.00						
Osmol. (10m*l*)	-0.90	0.86	0.69	0.75	1.00					
Z (5m*l*)	0.98	-0.85	-0.77	-0.78	-0.90	1.00				
Na^+ (5m*l*)	-0.78	0.97	0.51	0.72	0.79	-0.73	1.00			
K^+ (5m*l*)	-0.74	0.66	0.96	0.55	0.70	-0.74	0.59	1.00		
Cl^- (5m*l*)	-0.84	0.85	0.57	0.98	0.79	-0.83	0.79	0.59	1.00	
Osmol. (5m*l*)	-0.88	0.90	0.67	0.73	0.98	-0.86	0.85	0.69	0.79	1.00

*: all corrected R values significant for $\alpha = 0.01$

Table 2.6: Correlation between manipulated blood impedance & ion concentration*

	NaCl added					H_2O added				
	Z	Na^+	K^+	Cl^-	Osm	Z	Na^+	K^+	Cl^-	Osm
Z (NaCl)	1.00									
Na^+ (NaCl)	-0.83	1.00								
K^+ (NaCl)	-0.92	0.78	1.00							
Cl^- (NaCl)	-0.82	0.82	0.79	1.00						
Osmol. (NaCl)	-0.95	0.86	0.86	0.79	1.00					
Z (H_2O)						1.00				
Na^+ (H_2O)						-0.80	1.00			
K^+ (H_2O)						-0.79	0.70	1.00		
Cl^- (H_2O)						-0.71	0.82	0.57	1.00	
Osmol. (H_2O)						-0.74	0.88	0.73	0.83	1.00

*: all corrected R values significant for $\alpha = 0.01$

In the above tables, Table 2.5 and Table 2.6, it can be clearly seen that there is a significant linear correlation between blood impedance and ionic composition measurements, with R values ranging from R = -0.95 (impedance and osmolarity of the 5m*l* NaCl sample) to R = -0.71 (impedance and Cl^- of the 5m*l* H_2O sample). It is also evident that the various ionic measurements are closely related to each other. Due to the ionic nature of blood, this is to be expected.

To assess the reliability of the correlation calculations, the two variables concerned were plotted against each other in a regression analysis. Figures 2.4 indicates a diagrammatic representation of a part of this linear regression analysis, where the impedance values of the 10m*l* unmanipulated samples (on the x-axis) are regressed against the blood ionic measurements of the same sample (y-axis). This graphical representation corroborates the linear association between the blood

impedance values and the blood ionic values, shown by the correlation results. The graphs for the 5m*l* unmanipulated and manipulated samples were very similar to those obtained in Figure 2.4. The significance of the regression statistics was confirmed by means of calculating the F-statistic for the regression. All the regression values were significant for $\alpha = 0.0$[illegible].

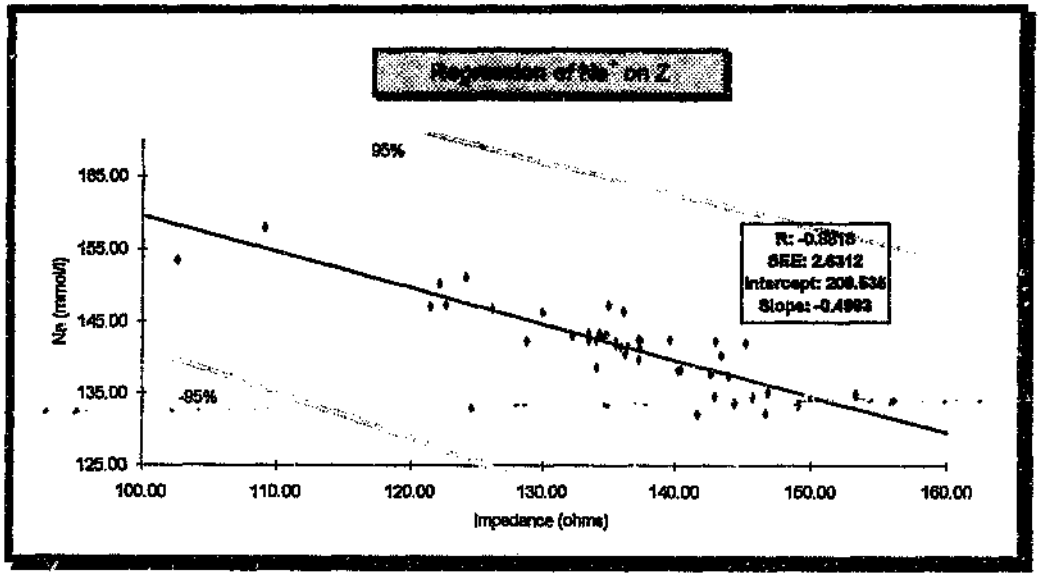
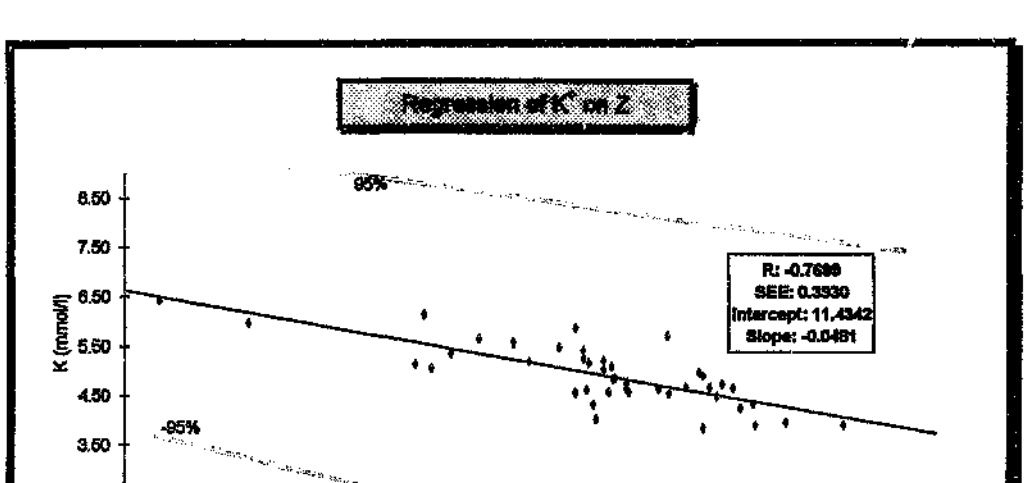
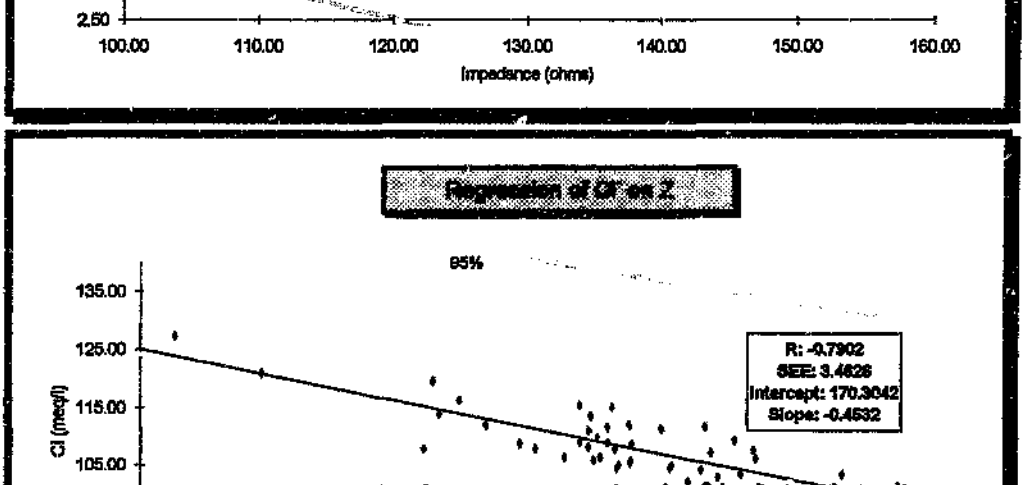


Figure 2.4: Regression analysis between blood impedance & ionic composition (10m*l* unmanipulated sample). continued overpage....


95%
8.50
7.50
6.50
5.50
4.50
3.50
2.50
K (mmol/l)
-95%
R: -0.7699
SEE: 0.3930
Intercept: 11.4342
Slope: -0.0481
100.00
110.00
120.00
130.00
140.00
150.00
160.00
Impedance (ohms)


95%
135.00
125.00
115.00
105.00
95.00
85.00
Cl (meq/l)
-95%
R: -0.7902
SEE: 3.4626
Intercept: 170.3042
Slope: -0.4532
100.00
110.00
120.00
130.00
140.00
150.00
160.00
Impedance (ohms)

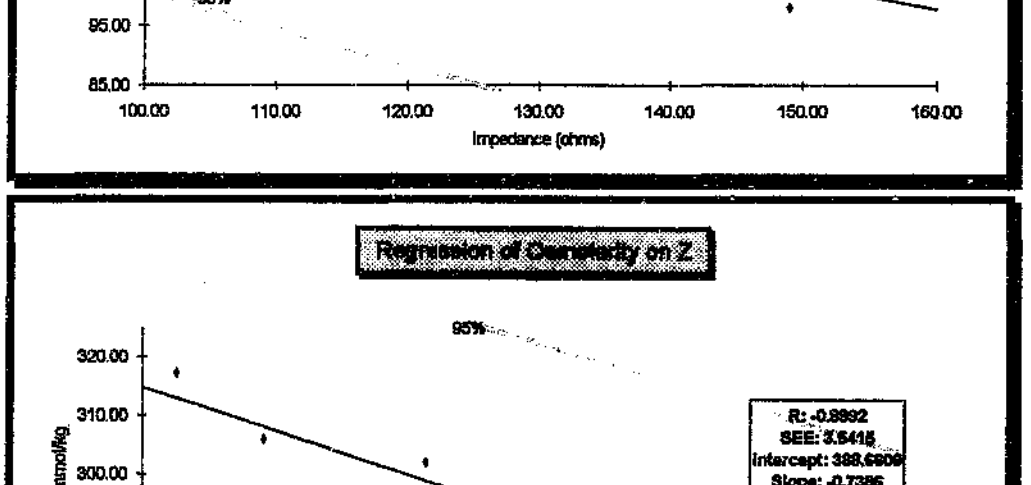

95%
320.00
310.00
300.00
290.00
280.00
270.00
Osmol (mmol/kg
-95%
R: -0.8992
SEE: 3.5415
Intercept: 388.6909
Slope: -0.7386
100.00
110.00
120.00
130.00
140.00
150.00
160.00
Impedance (ohms)

Table 2.7 shows the numerical values of intercept, slope, correlation coefficient, standard error of estimation, and F-value that were obtained in this regression analysis. The results obtained from the 10m*l* and 5m*l* unmanipulated samples were very similar. As the results of the regression analysis of the 10m*l* unmanipulated samples are shown in Figure 2.4, only the regression results for the 5m*l* unmanipulated samples are given in Table 2.7. It was not possible to combine the data for the regression equations for the three different groups, as either the slope, the intercept or both were significantly different (α=0.05) between the groups.

Table 2.7: Regression analysis of ionic measurements (y) on blood impedance (x) (y = mx + c)

	Intercept (b)	Slope (a)	R*	SEE†	F-value
Unmanipulated group‡					
Na^+	209.54	-0.499	-0.73	2.63	146.78
K^+	11.43	-0.048	-0.74	0.39	61.14
Cl^-	170.30	-0.453	-0.83	3.46	69.84
Osmol.	388.66	-0.728	-0.86	3.54	177.34
Manipulated group - NaCl§					
Na^+	209.49	-0.351	-0.83	2.28	45.42
K^+	13.55	-0.077	-0.92	0.32	111.37
Cl^-	186.09	-0.501	-0.82	3.39	41.21
Osmol.	439.57	-0.887	-0.95	2.77	194.24
Manipulated group - H_2O§					
Na^+	181.12	-0.330	-0.80	3.11	34.66
K^+	12.78	-0.055	-0.79	0.54	32.53
Cl^-	147.98	-0.314	-0.71	3.90	19.91
Osmol.	365.70	-0.648	-0.74	7.36	23.76

*: corrected correlation coefficient
†: SEE - standard error of measurement
‡: critical value for F = 4.08 (α = 0.05)
§: critical value for F = 4.35 (α = 0.05)

From the F-test values, it is shown that all regression equations are significant for $\alpha = 0.05$. It is evident from the significance of the regression results that they are valuable indications of the linear associations between the blood impedance and blood ionic measurements.

2.1.3. Discussion

The basis of bio-electrical impedance analysis is very often forgotten in the consideration of the electrical methods of body composition determination. In particular, the premise that the electrical conductivity of the body is largely dependent on the electrolyte concentrations of the conducting compartments is often erroneously ignored. This pilot study investigated the relationship between bio-electrical impedance measurements and the ionic nature and composition of the body.

The results obtained from the ionic concentration and osmolarity measurements of the unmanipulated samples (shown in Table 2.1) were all within normal ranges of healthy individuals (Ganong, 1989). The values of the ionic concentrations and osmolarity of the manipulated samples (shown in Table 2.2) were significantly different, for $\alpha = 0.05$, from the unmanipulated samples. All the data were normally distributed, and thus it was assumed that the use of parametric statistical methods would be valid. Furthermore, the sample size of the groups was seen to be adequate for significant statistical analyses.

The methods used for ionic and osmolarity measurements performed were assessed to be significantly reliable (see Table 2.3). This assessment was important in that, as a function of this particular pilot study, the instrumentation and measurement methods of ionic concentration and osmolarity were found to be reliable prior to the main study. The relatively unconventional assessment of blood impedance, using the custom-made perspex vial, was also found to be reliable and valid. The blood impedance values of the initial 10m*l* sample were not significantly different from the blood impedance values of the secondary 5m*l* sample group. This

agreement between the two separately measured samples indicates the reliability of the selected methods of sample handling and measurement. Differences in the procedure described by Deurenberg *et al.* (1989), regarding the maintenance of the blood samples at body temperature, evidently did not affect the validity of the impedance measurements, but rather served as an important standardisation method. It is uncertain whether the temperature of an electrolyte substance has an effect on the conduction or impedance of electricity. The individual measurements of ionic concentration for the 5m*l* and 10m*l* unmanipulated samples were highly correlated. Table 2.5 indicates the correlation between the measurements of each group, with R ranging from 0.96 to 0.98 (significant for $\alpha = 0.01$).

Table 2.5 also shows the linear relationships between the blood impedance values and the ionic concentration and osmolarity measurements for the unmanipulated samples. For both the 10m*l* and the 5m*l* samples, the impedance values show a negative correlation with the ionic and osmolarity measurements ($R = -0.74$ to $R = -0.90$). The correlation results shown in Table 2.6 for the manipulated samples also show similar significant negative correlations between the blood impedance values and the ionic and osmolarity measurements ($R = -0.71$ to $R = -0.95$). Interestingly, it is the osmolarity that correlates the best with blood impedance, but since osmolarity indicates the overall amount of electrolytes present in solution, this is to be expected. The linear regression and scatterplots of the relationships between electrical impedance and fluid and ion concentrations further support the high degree of correlation.

The effect of intracellular electrolytes, and the impact of the cell membrane were ignored in this study of blood impedance. It was assumed that the intra- and

extra-cellular ions would be in equilibrium as they were not electrically excited before the removal of the plasma. The cell membrane theoretically provides little impedance to current flow at the low frequency used. It was therefore possible to ignore the cell membrane and the intracellular ion effects. It is suggested that a detailed study be performed which should attempt to elucidate the effects of individual ions on electrical conduction.The results obtained in this pilot study indicate that there is possibly a relationship between the electrolyte concentrations in the body H_2O, and the measurement of bio-electrical impedance. There is a distinction between this conclusion and the idea that electrical conduction is determined by the conductor volume. The results of this study do not indicate any involvement of the fluid volume. They do, however, show a relationship between electrical conduction and composition of the conductive fluid. Although the measurement of impedance in blood does not automatically accurately reflect the entire body fluid compartments, it does provide a simplified direction for further research. Also, the sample handling, measurement methods and instruments were assessed to be reliable and valid. Further consideration of the bio-electrical impedance in a biological system was deemed worthwhile.

IN SUMMARY:

- The methods and measurements of blood impedance, ionic concentration and osmolarity were found to be valid and repeatable in this study.
- A good relationship was found to exist between bio-electrical impedance measurements and body electrolyte concentrations.

2.2) The Equilibration Period of Tritiated Water in the Body

Since the discovery of radioactive isotopes, the measurement of various body fluid compartments, in particular, total body water, has become more accurate and to a large degree far safer than previously used techniques involving chemical dyes. Although the technique and method of using isotopes to measure total body water was first described by Moore (1946) in his work on laboratory animals, the essential details of the method have never really been fully documented.

The interaction of isotopic water with body water is such that the hydrogen atoms of body water are fully exchangeable with isotopic water labelled by substituting hydrogen with either deuterium or tritium. This interaction forms the basis of the measurement of total body water (Culebras and Moore, 1977).

After ingestion or injection of a known concentration and volume of a tracer dose of tritiated or deuterated water, a period of equilibrium is allowed to pass. This equilibrium period is required for the maximal distribution of the isotope in the body spaces. Samples of blood or other body fluids are then taken, appropriately treated, and measured for final diluted isotope concentration. With the known values of ingested or injected isotope volume and concentration, and the final diluted isotope concentration, the diluted volume of the body compartment may be calculated.

Although both tritium and deuterium are similar in hydrogen exchange properties, tritium is relatively inexpensive and the measurement of tritium concentration easier to perform than that of deuterium (Liebman *et al.*, 1960). This often results in tritium being the isotope of choice.

The routes of isotope administration vary, with the most common being either intravenous injection or oral ingestion. Other routes include subcutaneous injection, intraperitoneal injection and intramuscular injection. The route of administration will ultimately affect the time needed for isotope equilibrium. The intravenous route involves the isotope being placed directly in the circulating body fluid. Although assumed to be faster than the oral ingestion route, the intravenous route does encounter some technical problems. Coleman *et al.* (1972) highlighted the fact that the rapid equilibration of intravenously injected isotope depends on the assumption that the peripheral circulation is homogenous rather than heterogeneous. This homogeneity is not the case, as the peripheral tissues are considered to have a variety of perfusion-to-volume ratios. The time of isotope equilibration is therefore dependent on the delivery of isotope to all compartments, both the poorly perfused and the well-perfused areas. The apparent speed of equilibration of the isotope administered in this fashion may indeed be an artefact of increased blood flow to the site of sampling. Although the perfusion-to-volume ratio problem is still evident in the oral route of administration, the time for the isotope to be absorbed and distributed by the gastro-intestinal tract will, to a degree, negate the problem of too short an equilibration time. Intravenous injection, and other routes of injection, also result in increased complexity of skills required by the researcher using human subjects, although in animal studies, the injected route is by far the more efficient.

Although the physical half lives of the isotopes are long enough (tritium = 11 years) not to cause problems regarding the equilibration period, the short period of the biological half life does influence equilibration. The biological half life of 7.5 ±

0.6 days of the isotope (Foy and Schneiden, 1960) is influenced by sensible and insensible water loss, i.e., respiratory, renal and sweat mechanisms (Coleman *et al.*, 1972; Hilson *et al.*, 1990). The most significant loss of isotope is renal loss, with normal urine production continuing during the equilibration period. Some researchers do correct for this isotope loss via the urine (Dyrbye and Kragelund, 1970). However, the bladder wall has been found to be selectively permeable to tritium in the urine stored in the bladder (Hilson *et al.*, 1990). This possible unknown reabsorbtion of tritium by the bladder would result in the lack of control of the quantities of tritium lost via the urine.

Directly related to the problem of water loss is the assumption that the body water volume and distribution remain constant during the measurement period. Nagy and Costa (1980) found that the error of total body water measurement approaches 15% in extreme situations over longer periods of time. In more typical circumstances, the error of distribution was found to be ± 3% in various species of animals.

Another source of error in the isotope dilution method is the exchange of the isotope with non-aqueous hydrogen. This exchange does occur, and results in an over-estimation of body water volume (Pinson, 1952). Sheng and Huggins (1989) reported that the longer the equilibration period of the isotope, the greater the exchange with non-aqueous hydrogen.

From these described problems, it is evident that the equilibration period is an essential part of the accuracy of the dilution principle. If the period is too short, the isotope will not distribute adequately and if the period is too long, the isotope may be lost, in part, by sensible and insensible water loss, and by non-aqueous

hydrogen exchange.

In practice, an equilibrium time of approximately 2 hours has been said to be satisfactory for isotope mixing and exchange with mammalian body water, although over 95% of the exchange occurs much more rapidly than that - within 1 hour (Culebras and Moore, 1977). Anbar and Lewitas (1958) confirmed this by finding that, in rabbits, the isotope reaches equilibrium within 60 minutes.

There does not seem to be any consensus regarding the equilibration time in human subjects, which tends to differ from study to study, ranging from less than 1 hour (Webster and Weathers, 1989) to 24 hours (Thylen *et al.*, 1991). To assess the optimum time for equilibrium, a study was performed to determine the shortest time needed for the tritium isotope to reach its maximum concentration in the blood. This time period was taken to be the optimum equilibration time, and the maximum concentration of tritium in the blood was taken to be the final diluted concentration of the isotope. These two variables, namely the optimum equilibration time and the maximum tritium concentration were subsequently used in the main study (Chapter 3). This pilot study was also performed in order to practice and assess the most efficient methods of venous blood sampling, blood sample management and dilution, and the measurement of tritium concentration by means of liquid scintillation counting.

2.2.1. Subjects, Materials and Methods

a) *Subjects*

Six male volunteers were assessed for tritiated water equilibration over a period of time. This pilot study was included in the ethical approval obtained from the University of the Witwatersrand Committee for Research on Human Subjects - Medical (Protocol No. 16/7/91 - see Appendix 4). All six volunteers read and signed an informed consent document before participating in the study. The volunteers were all young apparently healthy adult males, between the ages of 20 and 24 years. They were of similar weights (72.3kg ± 11.5kg) and heights (177cm ± 16.7cm).

b) *Study Protocol*

The subjects were all measured at the same time of day. After an overnight fast, an indwelling 21 gauge venous catheter (Venisystems™ Butterfly®-21, Abbott Ireland Ltd.) was inserted into a cutaneous vein on the dorsal surface of either the right or the left hand. A sterile technique of blood sampling and venipuncture was followed meticulously. On insertion of the catheter, a 5m*l* venous blood sample was withdrawn and placed in a heparinised blood collection tube. The dead space of the catheter (0.7m*l*) was then filled with anticoagulant fluid - 1000 units.ml^{-1} heparin sodium (Pularin®, Glaxo SA), and the catheter was sealed. This first blood sample served as the baseline blood radioactivity sample of the study.

Each subject then ingested a 150m*l* sample of tritiated water (Amersham Int., England) - for tritium concentrations and dilution techniques, see below in "Analysis of Tritium Concentration". The cup used by the subjects was rinsed with a small amount of tap water. The time of ingestion was recorded as T_o. From this time onwards, nine further 5m*l* blood samples were collected from each subject. The times of sampling were 10, 20, 30, 45, 60, 90, 120, 180 and 240 minutes after T_o. For each sample, the heparinised dead space of the catheter was withdrawn into a sterile 1m*l* syringe. A second 10m*l* syringe was then used to withdraw the 5m*l* sample, and the sterile heparin was replaced. Each sample of blood was placed in a heparinised collection tube, and stored for analysis of tritium concentration.

c) *Analysis of Tritium Concentration*

A 5mCi.ml^{-1} stock solution of tritium (Amersham Int., England) was diluted to give a 10μCi.ml^{-1} sample solution of tritiated water. This sample solution was stored refrigerated in a sealed, isolated radio-isotope room until required.

On the morning of the experiment, the necessary samples of tritiated water were made up by diluting 10m*l* of the tritiated water sample solution with 150m*l* of distilled water. To assess the initial radioactivity count (concentration C_1), a 10m*l* aliquot was withdrawn from the 160m*l* total sample solution, and prepared for radioassay. The total remaining sample (150m*l*) was then ingested by each volunteer. Therefore, each volunteer ingested a total radioactive dose of less than 100μ Ci (10m*l* x 10μCi.ml^{-1}) in the 150m*l* ingestion sample.

The concentration of the tritiated water was assessed by means of radioisotope liquid scintillation counting, using a scintillation counter with quench correction (Packard SC2660, USA). All blood plasma samples and initial ingestion samples were prepared in the same manner as described by Olsson (1970).

The blood samples were centrifuged for 10 minutes at 3000 rpm, and the supernatant plasma was removed. A 0.2m*l* sample of this spun plasma supernatant was diluted with 12m*l* of scintillation fluid (Aquagel I, New England Nuclear, USA) in polyethylene vials (UTP, Illinois). In a similar manner, 0.2m*l* of the initial centrifuged ingestion samples were also added to 12m*l* of scintillation fluid, and placed in polyethylene vials.

These prepared tritium samples were then inserted in the scintillation counter and assessed for radioactivity measured in disintegrations per minute (DPM) for a 5 minute period. Measurements (in DPM) provide an accurate assessment of concentration per standard volume of the tritiated water samples (Vaughan and Boling, 1961). All measurements were performed in duplicate.

The radioactivity count was then plotted over the period of time from T_0 in order to determine the optimal equilibration period of the tritiated water in the blood.

To assess the impact the equilibration period has on the measurement of total body water (TBW), the values of TBW were calculated from the isotopic study results. The method of calculation is described in detail later (Chapter 3.2.3).

2.2.2. Results

a) *Data Description*

A summary of the data is presented in Table 2.8. The values presented indicate the scintillation counter measurements of radioactive disintegrations (DPM) of each sample measured, as well as the mean and standard deviation (STD) for the measurements of each volunteer at t = 0, 10, 20, 30, 45, 60, 90, 120, 180 and 240 minutes. Each of these values is a mean of the duplicated measurements for each sample. The duplicate measurements were used in a test-retest reliability study, described below.

Table 2.8: Summary of data of tritium measurements (in DPM).

Subject	1	2	3	4	5	6	MEAN	STD
t=0	31.2	36.7	37.1	40.0	42.0	33.4	36.7	4.0
t=10	321.3	389.5	386.9	395.8	421.3	334.6	374.9	38.6
t=20	435.2	588.2	584.2	597.7	636.2	505.2	566.1	58.3
t=30	579.5	705.1	708 4	712.0	777.7	604.7	681.2	74.5
t=45	645.6	785.7	787.5	778.0	860.8	650.4	751.3	85.5
t=60	653.3	785.0	760.4	792.1	837.1	673.7	750.3	71.9
t=90	656.1	781.7	776.2	785.0	855.3	674.2	754.8	75.4
t=120	653.7	786.5	769.0	781.0	853.4	670.3	752.3	76.1
t=180	655.6	784.2	771.7	777.5	854.8	660.7	750.8	77.8
t=240	645.1	776.3	772.5	789.5	853.5	665.2	750.3	79.6

b) *Repeatability and Validity of Tritium Concentration Measurements*

All measurements obtained for the different time periods were performed in duplicate. This was done in order to assess the reliability and repeatability of the sampling techniques, theoretical bases, and measurement methods used in this study. Because only one investigator was responsible for the sampling and measurement of all the collected data, it was not necessary to assess inter-observer reliability. To assess the amount of error variance, a calculation of the standard error of the measurements (SEM) was performed, and to assess the proportion of error variance in relation to true variance, correlations coefficients were calculated, resulting in a variance (R^2) statistic. Table 2.9 indicates the SEM and R^2 values obtained for these two tests. The low proportion of error variance to true variance, and the low values for the standard error of measurement indicate that the measurements were reliable indications of the true values, and were repeatable.

Table 2.9: Reliability assessment of tritium concentration measurements*

	R^2	SEM	%†
Sample	0.9865	3580.79	1.54
t=0	0.9928	0.5986	1.63
t=10	0.9912	6.0492	1.61
t=20	0.9912	9.1343	1.61
t=30	0.9920	10.9806	1.61
t=45	0.9801	15.5625	2.07
t=60	0.9841	16.6058	2.21
t=90	0.9704	18.0299	2.38
t=120	0.9838	15.4322	2.05
t=180	0.9900	15.0955	2.01
t=240	0.9849	16.6344	2.21

*: all R^2 values are significant for $\alpha = 0.05$
†: % indicates the proportion of SEM to mean measurements

c) *Tritium Concentration Equilibration Time*

To indicate the equilibration period, the mean tritium radioactivity concentration in the blood, both prior to tritiated water ingestion and at the different periods of time after ingestion, were plotted against time. Figure 2.5 shows the results of this plot. Included in this figure are the 95% confidence limits of the data collected.

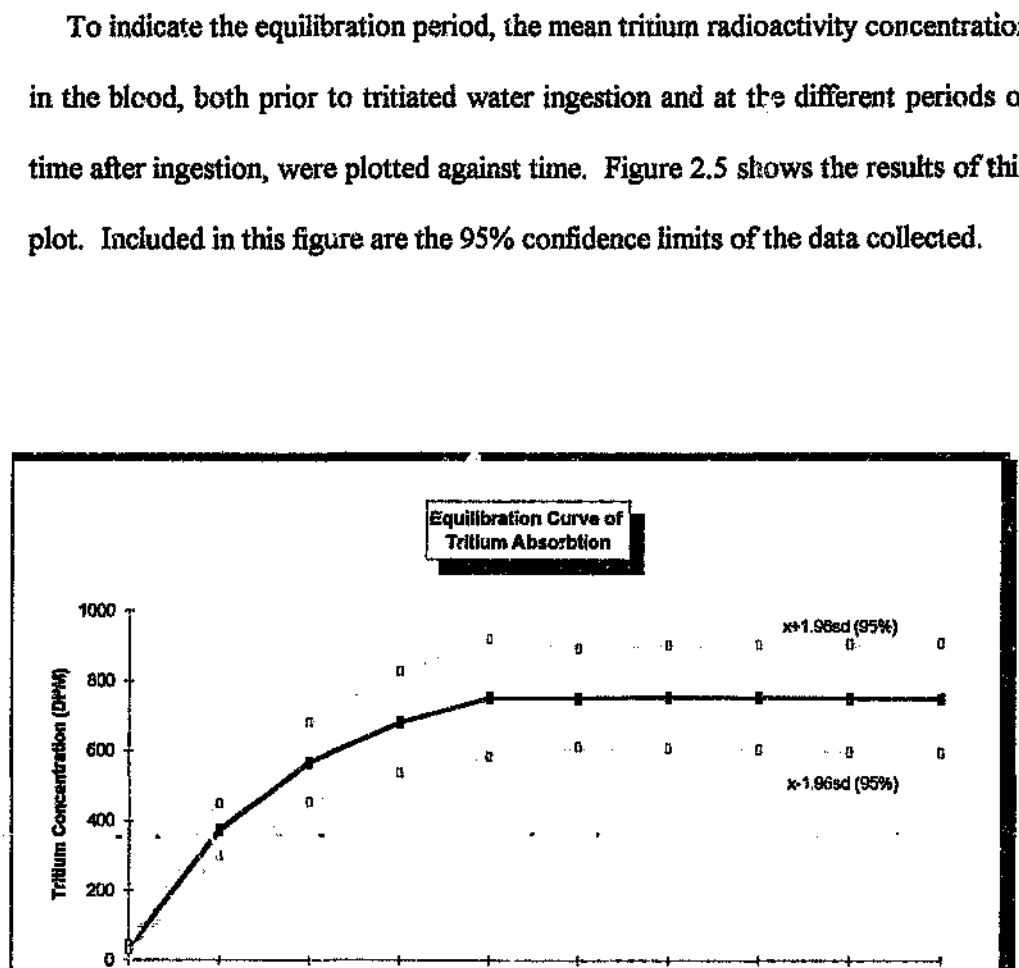


Figure 2.5: Equilibration plot of tritium concentration against time

d) *Further Data Analysis*

It is evident from the graph that the tritium equilibrates in the blood within 60 minutes. From the initial results, it was deemed necessary to perform two further statistical analyses. The first was to assess whether there is any significant difference between the 30 minute and the 45 minute measured tritium concentration. The second was to see if there is a difference between any of the tritium concentration measurements after equilibrium is reached.

A two-sample two-tail *t*-test was performed on the data for the 30 minute and 45 minute measured concentration samples. Because the sample variance of the two samples was combined, an F-test was performed on the two variance values to test if they could be combined. Table 2.10 gives the results of the *t*-test and the F-test. Although the combination of the variances was valid, the *t*-test showed that there is a significant difference between the 30 minute and the 45 minute samples.

Table 2.10: Two-sample *t*-test on 30 and 45 minute samples

TEST	VALUE	CRITICAL VALUE*
t	12.2201	2.5706
F	1.3158	3.4529

*: for $\alpha = 0.05$

To assess whether there were any differences between the 45 minute to 240 minute equilibration concentrations, a single-factor ANOVA was performed. Table 2.11 indicates the results obtained from this test. It appears that there is no signifi-

cant difference between any of the tritium concentrations measured from 45 minutes onwards. Observing the data (Table 2.8), a small but not statistically significant decline is observed after 2 hours (120 minutes). This is to be expected due to isotope elimination from the body, amongst other factors.

Table 2.11: ANOVA for tritium concentrations after 30 minutes

	$\Sigma\sigma^2$	σ^2	d.f.		
BG*	88.04	17.61	5	F = 0.0029	CV= 2.5336‡
WG†	181692.8	6056.4	30	P = 0.9998	
Total	181780.8		35		

*: between group variance
†: within group variance
‡: critical value

e) *Implications of Results on TBW Assessment*

The values for total body water for each subject, at each time interval, were calculated from the results obtained in the tritium concentration measurements. In this particular study it was more interesting to assess the effect of variation in tritium concentration measurements on the calculation of total body water. The values of the total body water calculation for t = 10 minutes to t = 240 minutes for the study group were as follows (Table 2.12):

Table 2.12: Calculated total body water (*l*) measurements for the study group

	MEAN (*l*)	STD (*l*)
t=10	98.57	4.54
t=20	62.97	2.90
t=30	51.72	2.23
t=45	46.64	1.93
t=60	46.71	2.31
t=90	46.42	2.20
t=120	46.58	2.18
t=180	46.68	2.13
t=240	46.71	2.08

The time periods of particular interest were the blood samples taken at time 45 minutes, 60 minutes and 90 minutes. The mean difference in total body water calculations between t = 45 and t = 60 minutes was $\Delta x = 0.58l \pm 0.29l$, and the difference between t = 60 and t = 90 minutes was $\Delta x = 0.61l \pm 0.28l$. Both of these differences account for less than 1% of the total body water measurement (0.68% for $\Delta x = 0.61l$), which is well within the estimated measurement error of the method of 0.5% to 2.0% (Sheng and Huggins, 1979).

2.2.3. Discussion

Due to the ease of obtaining tritium, and the relative ease and inexpense of measuring the concentration of the isotope in tritiated water, it was decided that for the purposes of this study, the isotope of choice for the measurement of total body water should be tritium. This pilot study successfully attempted to ascertain the optimum period of equilibration needed for the isotope to maximally distribute in the body fluids.

The results of the tritium concentration measurements in the blood after various periods of time, and the equilibration plot of the isotope concentration against time indicate that the equilibration of tritium occurs in this sample at approximately 60 minutes.

The assessment of the repeatability and reliability of the method used (Table 2.9), using the duplicate measurements of tritium concentration, shows a low amount of error variance - with the standard error of measurement ranging from 1.54% to 2.38% of the measured tritium concentration. The proportion of the error variance in relation to the true variance is also low, indicated by the high values obtained in the correlation analysis between the two duplicate measurements ($R^2 = 0.9704$ to $R^2 = 0.9928$). From these results, it may be concluded that the measurement of tritiated water by the water dilution method is both repeatable and valid.

The plot of mean blood tritium concentrations (values given in Table 2.8) against the time of sampling (Figure 2.5) gives an excellent indication of an equilibration curve for a substance in the body. This shape of the equilibration curve

obtained in this study is very similar to the curve obtained by Nagy and Costa (1980). Further analysis of the data obtained in this study showed that there are significant differences (for $\alpha = 0.05$) in tritium concentration measurement from time t = 0 to time t = 45 minutes. The concentrations measured at these times are significantly lower than the concentrations measured at time t = 60 minutes. Furthermore, there is no significant difference between the concentrations measured after t = 60 minutes. Although not significant, there does seem to be a small decrease in the concentration values from t = 60 minutes onwards.

The results of the total body water calculation from the tritium dilution study show the effects of the equilibration period and the small decrease in tritium after equilibration (Table 2.12). The values of total body water (46.7*l* at t = 60 mins) were found to be a good indication of the assumed normal hydration state of the volunteers. The calculated value of TBW shows that the mean hydration status of the sample was 64.6%, which is similar to the hydration status values of normal subjects reported by others (Dyrbye and Kragelund, 1970; Herath *et al.*, 1982; Heitmann, 1990; Deurenberg and Schouten, 1992).

Both the calculated values of total body water and the actual concentration measurements of tritium indicate that the optimum equilibration time for normal human subjects after oral ingestion is approximately 60 minutes. It is at this time of equilibration that the diluted tritium reaches maximal distribution in the body.

The methods of tritium preparation and administration, venous blood sampling, blood sample management and dilution, and the measurements of tritium concentration by means of liquid scintillation counting were shown to be workable and valid in this pilot study.

IN SUMMARY:

- The methods and measurements of tritium dilution used to assess total body water were found to be valid and repeatable in this study.
- The optimal equilibration period was taken to be from 60 minutes onwards. The time from 60 minutes was restricted to a limit of no more than 75 minutes after ingestion.

2.3) Measurement of TBW in an Animal Model

Many difficulties and errors involved in the isotope dilution technique have been reported (Lifson and McClintock, 1966; Nagy and Costa, 1980). To assess their effectiveness, reliability and accuracy, some investigators have compared dilution methods with direct methods of measuring body fluids in various animals, both in the field and in the laboratory. The direct method of total body water measurement is obviously not possible in humans as such measurements would normally involve the exsanguination of the organism being measured, and complete dehydration of the carcass (Bradbury, 1973). It is assumed that if the comparison between direct and indirect methods, using an animal model, show a low error of estimation, or there is a significant relationship between the two methods, then the indirect method may be applied to the human model.

The various animal studies mentioned below have reported errors in estimated total body water volume by tritium dilution ranging from 0.1% to 6.5% (Nagy and Costa, 1980). The magnitude of these errors appears to be within acceptable levels for an indirect technique of body fluid volume measurement.

Field work on experimental animals has recently included gray seals (Reilly and Fedak, 1990), poultry (Johnson and Farrell, 1988), and ruminants (Hix *et al.*, 1959). Laboratory investigations have included mongrel dogs (Coleman *et al.*, 1972), rabbits (Anbar and Lewitas, 1958; Olsson and Plantin, 1970) and rats. Due to the relative ease and economy of working with rats in the laboratory, the majority of validation studies have been performed with these animals.

Various aspects of the dilution method have been considered in relation to desiccation studies in rats. Pioneering work by Foy and Schneiden (1960) estimated the total body water as the diluted tritium space in not only the rat, but also the cat, rabbit, and guinea-pig. Other validation investigations of total body water dilution analysis have also been performed (Holleman and Dieterich, 1975; Culebras *et al.*, 1977; Sheng and Huggins, 1989). Furthermore, Gray *et al.* (1988) considered the implications of rapid and repeated weight loss and gain on the body composition and fluid compartments of rats, and Yasumura *et al.* (1990) considered the ratio distribution of body water by means of tritiated water dilution and desiccation studies.

The dilution method, using tritiated water, was applied to controlled laboratory rats, and compared to a direct assessment of total body water measured by exsanguination and desiccation. For the purposes of ensuring a valid and reliable method of measuring total body water by means of tritiated water dilution analysis, the techniques and methods that were to be used in the main study were assessed in a pilot study using laboratory rats.

2.3.1. Materials and Methods

a) *Animals Used*

12 Sprague-Dawley rats, 7 male and 5 female (mass = 267.12g ± 28.87g), were used in this study. The study was approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (AESC No: 92/117/2b - see Appendix 4).

b) *Protocol*

The total body water (TBW) of each of the animals was measured by two methods. An indirect assessment of TBW was made by means of tritium dilution, and a direct measurement of TBW was made by means of heat-desiccation.

The animals, which were allowed free access to food and water prior to the study, were measured early in the morning. They were first weighed, using an animal scale balance (Sauter SM2000), and then anaesthetised. The anaesthetic, 40mg/kg sodium pentobarbitone (Sagatal, Maybaker SA), was injected into the peritoneal cavity. After allowing 15 minutes for the anaesthetic to act, the animals were re-weighed to account for the weight of the anaesthetic. The animals were then placed on a dissection table, over a heating pad. All fluid and tissue from the animal was retained.

The left and right external jugular veins were carefully exposed by blunt dissection to minimise blood loss, and cannulated using narrow gauge plastic tubing (Portex, SA). The plastic tubing and the syringes used were heparinised prior to

being used.

On cannulation, 1.5m*l* of 10μCi.m*l*$^{-1}$ tritiated water was injected into the vascular system of the animal. The animal was then observed for approximately 45 minutes while the tritiated water equilibrated in the total body water space.

After this equilibration period, cardiac puncture was performed, either through the thoracic wall or through the diaphragm, by way of the abdomen. The animal was then exsanguinated by withdrawing the maximum amount of blood possible (between 5m*l* and 10m*l*) from the left ventricle of the heart. The volume of blood removed was weighed before it was assessed for tritium concentration.

Most of the viscera were then exposed and opened to enable effective desiccation, and the animal was re-weighed. The exposed carcass was then placed in an oven at 70^0 C for the purposes of desiccation. The weight of the desiccating carcass was taken daily, and the animal's carcass was removed from the oven when the carcass had reached a constant weight. This period was approximately two weeks. Following this desiccation period, the animal's carcass was carefully re-weighed.

c) *TBW Measurement - Tritium Dilution*

Total body water (TBW) was measured by means of the tritiated water dilution method (Vaughan and Boling, 1961). The tritium concentration was determined by radioisotope scintillation counting, using a scintillation counter (Packard SC2660, USA). Details of the tritium sample preparation and scintillation counting are furnished in Chapter 2.2.1. From these tritium concentration results, the total

body water was calculated. The calculation is described in detail in Chapter 3.2.3.

d) *TBW Measurement - Direct Analysis*

The direct total body water assessment was taken to be the overall loss of weight of the animal after the desiccation process. The hydrated weight of the animal was taken to be the weight measured after the tritium dilution study had been performed. This weight was measured after the removal of the animal's blood on exsanguination. Most of the viscera had been exposed and opened at the time of weighing. Corrections were made for the accurately measured volume of blood removed, the volume of tritiated water injected, and the volume of the anaesthetic injected.

After the animal's carcass had been desiccated in a drying oven for two weeks at 70^0 C, the carcass was removed and weighed. This weight was assumed to be the animal's desiccated weight. The use of this method to desiccate an animal was not expected to fully remove all fluids from the body, as the process of ashing does, but only to provide a good indication of easily removable total body water.

The desiccated weight was subtracted from the corrected hydrated weight, and the difference was interpreted to be the body fluids lost during desiccation, which approximates total body water. For the purposes of this study, it was assumed that the density of the animal's body fluids is approximately $1.00 g.dm^{-3}$ - hence 1g of water lost = 1m*l* of body fluid. This approximation is not entirely accurate, and does result in small, but insignificant errors.

2.3.2. Results

Table 2.13 shows the results obtained from the desiccation and tritiated water studies for the individual animals.

Table 2.13: Results of TBW measurement in rats

ANIMAL	WETWT (g)	DRYWT (g)	TBW_1* (ml)	$\%BM_1$†	TBW_2‡ (ml)	$\%BM_2$§
1	224.95	95.28	129.67	0.5764	154.84	0.6883
2	303.94	138.45	165.48	0.5445	191.39	0.6297
3	258.07	125.00	133.07	0.5156	150.53	0.5833
4	249.64	118.74	130.91	0.5244	150.22	0.6018
5	226.54	104.79	121.75	0.5374	137.89	0.6087
6	298.53	145.53	153.00	0.5125	160.01	0.5360
7	284.20	114.12	170.08	0.5985	193.33	0.6802
8	308.31	124.85	133.46	0.5950	216.67	0.7028
9	278.49	131.32	147.17	0.5285	171.85	0.6171
10	237.77	107.19	130.59	0.5492	149.76	0.6298
11	263.49	131.17	132.32	0.5022	150.09	0.5695
12	271.51	132.24	139.27	0.5129	160.93	0.5927
Mean	267.12	122.39	144.73	0.5414	165.63	0.6200
STD	28.87	14.83	19.34	0.0327	23.30	0.0500

*: TBW_1 = measured by desiccation (Wetwt-Drywt)
†: $\%BM_1$ = TBW as % of body mass (TBW_1/Wetwt)
‡: TBW_2 = measured by tritium dilution
§: $\%BM_2$ = TBW as % of body mass (TBW_2/Wetwt)

There is a significant difference between total body water measured by desiccation and total body water measured by tritium dilution. The desiccation method underestimates the amount of total body water. This underestimation was expected due to the inability to entirely remove all body fluids. To assess the degree

of association between the two methods, a correlation coefficient was calculated, and was found to be high: $R = 0.9499$ ($P \leq 0.05$). A scatter plot of this correlation is shown in Figure 2.6.

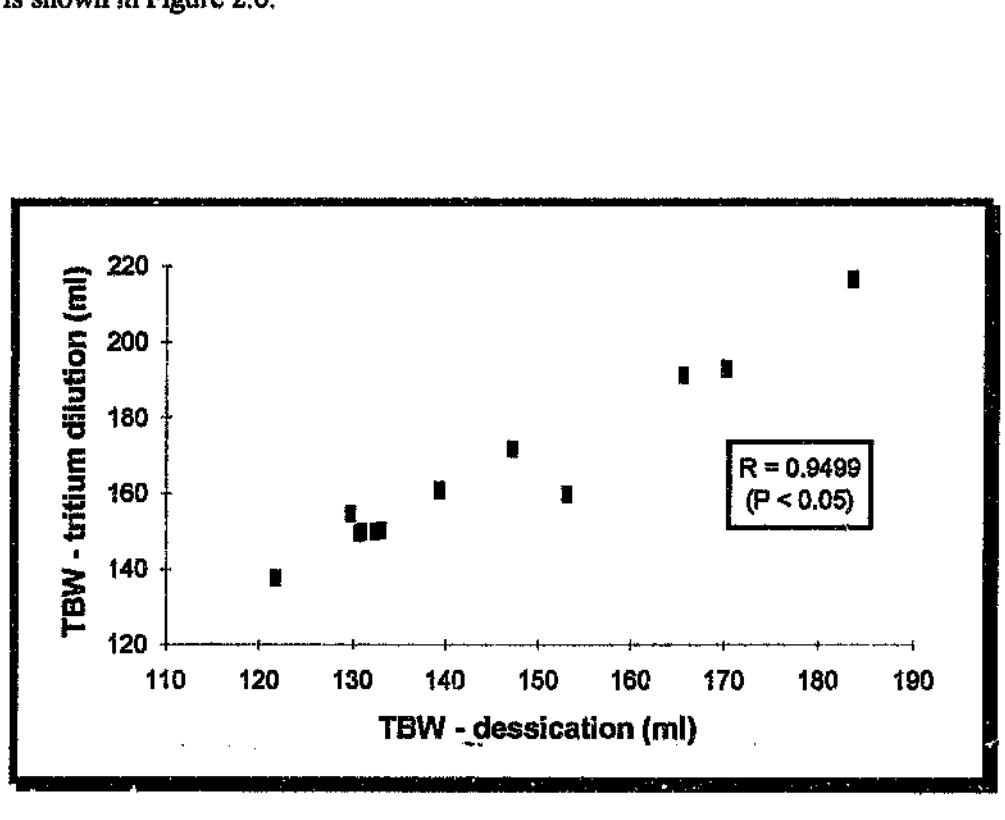


Figure 2.6: Scatterplot of two measurements of TBW

the purposes of this pilot study is the relationship between the two types of measurement - one direct and one indirect. The correlation between the results of the total body water measurements obtained by desiccation and isotope dilution is strong and ($R = 0.9499$, $P \leq 0.05$). Assuming that the direct method of measuring total body water - the desiccation method - is a valid, but not necessarily accurate, representation of the actual total body water, the significant correlation between the desiccation method and the tritium dilution method suggests that the tritium dilution method is valid and reliable in assessing total body water of an organism. This strong correlation is reinforced by the scatterplot of the results shown in Figure 2.6.

IN SUMMARY:

- The methods and theoretical bases of the tritium dilution principle, used to assess total body water in an animal model, were found to be valid and reliable when compared to a direct measurement of total body water.

CHAPTER 3

MAIN STUDY

3.1) Introduction

In Chapter 1, various methods were described to indirectly assess the different compartments of the body. These methods are generally accepted to provide valid body composition estimates (Lukaski, 1987; Roche, 1987; Elia & Jebb, 1990). Recently, yet another dimension to the existing methodological battery of body composition assessment was introduced - the technique of bioelectrical impedance analysis, or BIA (Malina, 1987). The electrical principles on which BIA was based were established by Thomasset (1962, 1963), and were given clinical prominence by the landmark publications of Nyboer (1970, 1972). Both pioneering authors detailed the relationship between the electrical impedance of the body and the nature of the blood and body fluids that constituted a representative conductive volume of the body. Since their work, the use of BIA has been expanded to provide other estimations of body composition, including total body water, fat and fat-free masses, extracellular water and segmental body composition (van Loan, 1990).

Studies of the estimation of total body water by means of BIA began with the work of Hoffer in 1969. In this work, Hoffer described an electrical impedance

measurement using a tetrapolar electrode configuration, and related the impedance value to the total body water measured by tritium dilution. Present day techniques of performing BIA are essentially based on this described method, with a few modifications. Customarily, the measured impedance value is included in a regression equation based on the electro-physical relationship described by:

$$V = \frac{\rho L^2}{Z} \quad \text{.....(3.1)}$$

In this relationship, the volume (V) of the measured component of body composition is directly related to the square of the length of the conductor (L), and inversely related to electrical impedance (Z). Published studies commonly use height or stature as the conductor length and may also include further variables, i.e., anthropometric measurements, age and sex, in the regression equation (Campos *et. al.*, 1989; Kushner *et. al.*, 1992). For the purposes of this study, the technique of BIA is defined as the inclusion of whole body impedance, with other variables, into a regression equation, which predicts body composition.

Relying on various assumptions, the general field of body composition assessment has spread its interests and applications to measurements of the fat and fat-free compartments of the body (Segal *et. al.*, 1985; Cunningham, 1987).

Recently, however, some researchers have begun to question the value of the method of BIA in indicating body composition characteristics (Cohn, 1985; Solomons, 1986; Deurenberg *et. al.*, 1988; Forbes *et. al.*, 1992). These queries have arisen from various documented issues, which are discussed in detail in Chapter 1. A key issue arising from these queries is the question of what, in terms of body composition, the measurements of body impedance really indicate (Deurenberg *et.*

al., 1991; Mazess, 1991a, 1991b; Grieve, 1993). This question may be specifically considered in the following manner:

- Does the technique of BIA adequately predict body composition?
- What role in this prediction, if any, does the inclusion of body impedance measurement play in body composition measurement?

It has been postulated that the impedance of the body is specifically reliant on the conduction compartment of the body (Thomasset, 1962, 1963; Nyboer, 1970; Grieve, 1992). Due to its electrolyte content, this compartment is the fluid compartment, or total body water. However, it is suggested that the conductance (or lack of, in the case of bioelectrical impedance) depends on the relative amount or concentration of the body's fluids and electrolytes rather than the volume of the fluid compartment (Khaled *et. al.*, 1988; Grieve, 1993; Grieve & Henneberg, 1994). This premise is based on the electro-physical concept that it is a fluid's electrolytes that provide the property of current flow, and not the fluid itself. If this is the case, the answer to the question of what impedance of the body indicates would be essentially provided.

The pattern of most kinds of validation studies begins with the consideration of a normal physiological population. The following stage is the application of a validated method in a pathological population. To date, most bioelectrical impedance validation studies have been in physiologically normal samples, although some studies have considered pathophysiological states (de Lorenzo *et. al.*, 1991; Binnerts *et. al.*, 1992; Cc nlisk *et. al.*, 1992; McDonald *et. al.*, 1993). Although the results from the pathophysiological studies have shown some promise for future

use, it is considered dangerous to extend the applicative value before the mechanisms of the basic method are fully understood.

Furthermore, recent application and validation of multiple frequency impedance measurements have been reported. As with the application of the single frequency results, the multiple frequency data reported do show some promise of future utilisation. However, the value of the use of more complicated multiple frequency technology is debatable, if the basic principles are poorly understood.

For the sole purpose of attempting to determine the basic mechanisms and value of body impedance measurements, it was deemed necessary to first consider the measurements within physiological limits. Moreover, measurements with a simple, single-frequency current input were selected in order to elucidate a functional understanding of body impedance.

This study assessed the value of BIA and body impedance measurements in predicting total body water. In this regard, an attempt to find out what bio-impedance measurements truly indicate was made. Total body water was measured by tritiated isotope dilution, and was compared to the prediction of total body water by the BIA technique. Impedance measurements were compared to total body water measurements and the measurement of body fluid and ionic concentrations. The impedance measurements performed were those of whole body, segmental, and blood.

Body fluid and ionic concentrations were assessed by the measurement of blood osmolarity and the blood electrolytes measured were Na^+ K^+ and Cl^-, and provided an indication of the cellular hydration of the body (Hill, 1990).

To assess the hydration state of the body, the isotopically measured total body water was related to body mass to indicate the percentage hydration of the body. This total body water volume to body mass ratio was performed to standardise for subject mass and size (Schoeller, 1989).

An indication of the influence of the content of the body fluid was obtained by performing the study on individuals with different hydration states. This variation did, however, need to be controlled. To observe the effects of hydration on impedance measurements, the measurements indicating the hydration of the subjects, i.e., total body water, haematocrit, and blood osmolarity and ionic concentrations, were performed with the subjects in a 'dehydrated' state, and a 'rehydrated' state. For ethical purposes, the simulated 'dehydrated' state of measurement was not a clinical dehydration (Gershan *et. al.*, 1990). Therefore, dehydrated and rehydrated states were created to produce a greater physiological range of ionic and water concentrations, against which impedance could be compared.

The purpose of the design of this study was therefore to consider the influence of the changing water and electrolyte concentrations of the body on bio-impedance measurements.

3.2) Subjects, Materials and Methods

3.2.1. Subjects

This study was approved by the University of the Witwatersrand Committee for Research on Human Subjects - Medical (Protocol No. 16/7/91). All subject volunteers read and signed an informed consent document before participating in the study (see Appendix 4).

Seventy-four apparently healthy adults, between the ages of 17.3 years and 33.5 years, volunteered for the study. This subject group, of 39 males and 35 females, was drawn mainly from various undergraduate degree courses at the University of the Witwatersrand, the remainder from the greater Johannesburg area. The so-called black, coloured, Indian and white groups were all represented in this sample. Table 3.1 details descriptive statistics for the study sample.

3.2.2. Protocol

The subjects were measured in two states of hydration (see Figure 3.1 for a diagrammatic representation of the study protocol). The subjects were measured after an overnight deprivation of fluid, and this deprivation was considered to provide a suitable state of mild dehydration of up to 1*l* of body fluid (Girandola *et al.*, 1977; Rising *et al.*, 1991). Rehydration was provided by simply allowing the subjects access to tap water and encouraging them to drink as much as possible. It was not expected that the total amount of water imbibed would be absorbed entirely nor

immediately by the body, but it was felt that allowing a small surplus would enable the best possible fluid absorption within the constraints of the study (Andersson, 1977; Bradbury, 1973). At each of these two states of hydration, total body water, body impedance, blood impedance, anthropometry, blood haematocrit, and blood ionic indices were made.

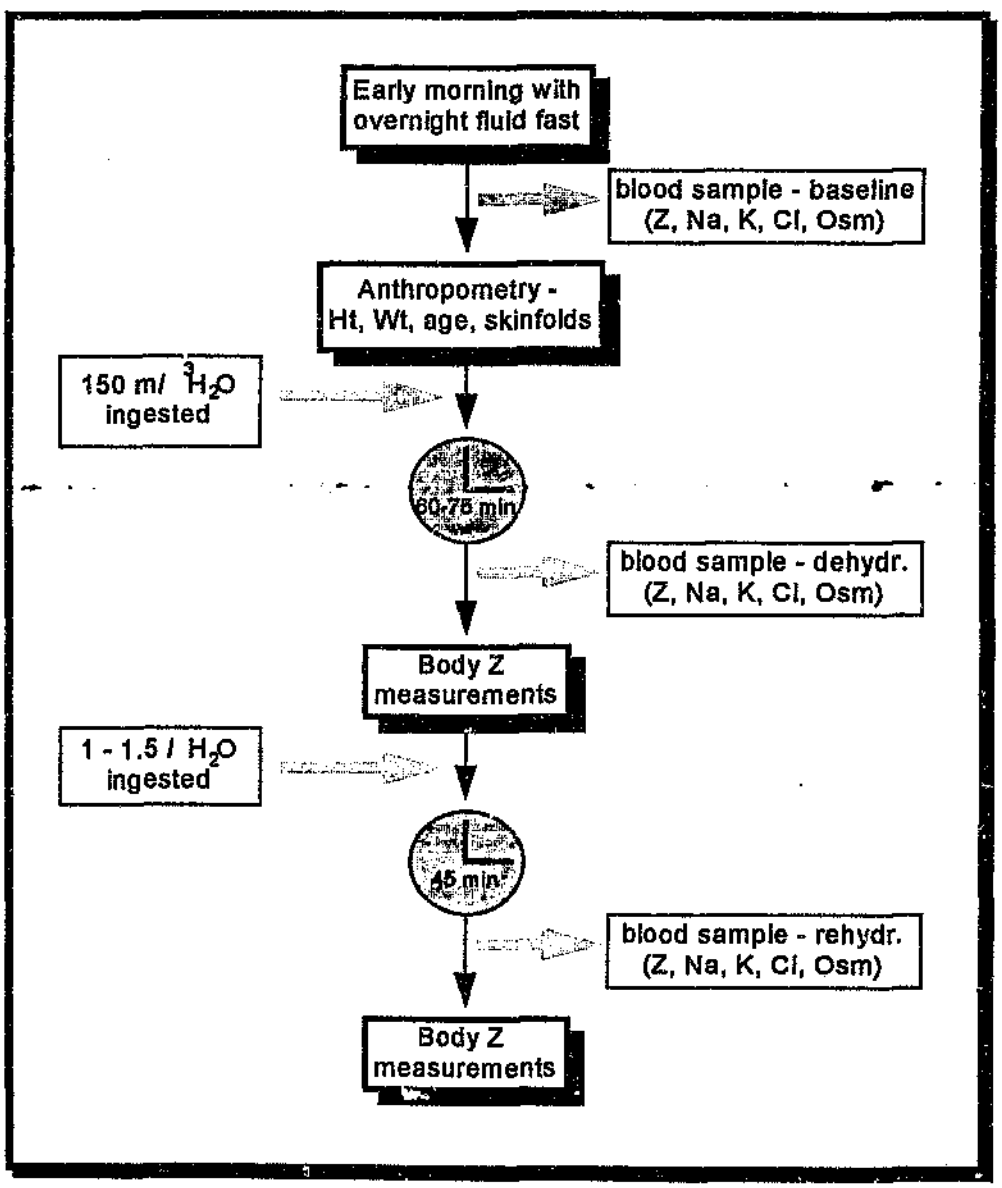


Figure 3.1: Diagrammatic summary of the study protocol.

Subjects presented themselves at the laboratory early in the morning of the day of study. An initial venous blood sample was drawn for measurement of baseline levels of blood radioactivity, ionic concentrations and impedance. Subject height, weight, age and skinfold measurements were then recorded. A 150m*l* sample of tritiated water was then ingested by each volunteer. The subjects then waited approximately 60-75 minutes for the total ingestion, absorption and distribution of the tritiated water. During this equilibration period, various body impedance measurements to a 50kHz, 800μA alternating current were recorded. Impedance of the blood sample to this current was also recorded.

After the equilibration period, a second venous blood sample was obtained for assessment of the dehydrated state. Further blood and body impedance measurements were performed, and the subject was then required to ingest approximately 1 - 1.5 *l* (15-20m*l*.kg^{-1} body weight) of fluid for rehydration purposes. The subjects then waited for approximately 45 minutes for adequate absorption and distribution of this fluid.

Following the rehydration of the subjects, a third venous blood sample was drawn. Impedance of the blood and the body for the rehydrated state was then recorded.

The three blood samples were analysed for haematocrit and then centrifuged. The blood plasma was removed and frozen at -20^{0} C for later ionic analysis. Na^{+}, K^{+}, and Cl^{-} concentrations, osmolarity, and blood haematocrit (Hct) were recorded within a week of sampling.

3.2.3. Procedures

a) *Blood Sampling*

Venous blood samples were obtained by venipuncture of either the median cubital vein, basilic vein, or the cephalic vein of both the left and right arms. After applying a blood pressure cuff to the arm, and measuring the subject's blood pressure[1], the cubital fossa area was thoroughly swabbed with alcohol-prepared swabs.

Using a sterile technique, blood was drawn using gauge 22 needles. The needle was inserted into the selected vein. After the vacuum container began filling, the blood pressure cuff was deflated, and the 10ml vacuum blood collection containers were filled in duplicate. The vacuum containers used were pre-heparinised glass tubes (Vac-u-Test, purple tops). The needle was withdrawn, and a cotton swab with antiseptic solution was placed over the puncture site.

Three blood samples were obtained, each from a different venous site, alternating between the left and right arms. For the comfort of the subject, no vein was used more than twice.

The resulting six sample tubes were initially labelled with the subject's coded study number and either blank-1, blank-2, dehydrated-1, dehydrated-2, rehydrated-1 or rehydrated-2, for the duplicate base-line, dehydrated, and rehydrated samples respectively.

[1] If the blood pressure recorded was significantly low, the subject was excluded from the study.

b) *Anthropometry*

After removing shoes and heavy overclothing, each subject was measured for height and weight. Standing **height** was measured, to the nearest millimetre, using a Harpenden stadiometer (Holtain, UK). The subject was asked to stand with his/her heels against a wall, in a fully upright position at full inspiration, with the head oriented in the Frankfurt position. The stadiometer was positioned vertically next to the subject, the blade of the stadiometer was lowered onto the vertex of the skull, and the height measurement was recorded (Cameron, 1984). Following the first measurement, the subject was told to relax, was then repositioned, and the measurement was duplicated.

Weight was measured, to the nearest 0.1kg, using a digital scale (Hanson Electronics). This measurement was also taken in duplicate.

Using the method described by Durnin and Rahaman (1967), a Harpenden skinfold calliper (British Indicators Ltd.) was used to measure **skinfold thickness**, at the following anatomical sites:

- TRICEPS: a reference point midway between the acromion and olecranon processes of the left arm was marked with ink. The fold was taken along the longitudinal axis of the arm, on the posterior aspect.
- BICEPS: a reference point at the same level as the triceps fold, on the anterior aspect of the left arm was measured.
- SUBSCAPULAR: a reference point inferior to the inferior angle of the left scapula, parallel to the axillary border, and vertically orientated, was measured.

- SUPRAILIAC: a point one centimetre superior and two centimetres medial to the left anterior superior iliac spine, and horizontally orientated, was measured.

The skinfold thickness measurements were obtained in duplicate, and were measured to the nearest 0.1mm.

c) *Total Body Water Measurement*

Total body water (TBW) was measured by means of the tritiated water dilution method (Vaughan and Boling, 1961), which is based in the relationship between the volume and the concentration of two fluid masses in equilibrium, shown as:

$$C_1V_1 = C_2V_2$$

Essentially, a sample of known volume (C_1) and known tritium concentration (V_1) was ingested by each volunteer. After the period of equilibration, a body fluid sample (venous blood) was assessed for final tritium concentration (C_2), thus allowing the determination of V_2, or the overall body fluid volume (TBW).

A 5mCi.ml^{-1} stock solution of tritium (Amersham Int., England) was diluted with distilled water to give a 10μCi.ml^{-1} sample solution of tritiated water. This sample solution was stored refrigerated in a sealed, isolated radio-isotope room until required.

On the morning of the experiment, the necessary samples of tritiated water were made up by diluting 10m*l* of the tritiated water sample solution with 150m*l* of distilled water. To assess the initial radioactivity count (concentration C_1), a 10m*l*

aliquot was withdrawn from the 160m*l* total sample solution, and prepared for radioassay. The total remaining sample (150m*l*) was then ingested by each volunteer. Therefore, each volunteer ingested a total radioactive dose of less than 100μCi (10m*l* x 10μCi.m*l*$^{-1}$) in the 150m*l* ingestion sample. Correction for non-aqueous dilution was not considered necessary.

i. Tritium Concentration Assessment

The concentration of the tritiated water was assessed by means of radioisotope scintillation counting, using a scintiilation counter with quench correction (Packard SC2660, USA). Both blood plasma samples, and initial 'cocktail' samples were prepared in the same manner as those in the pilot investigations. The details of the tritium concentration measurement, which were performed in duplicate, are described in Chapter 2.2.1.

d) *Body Impedance Measurements*

The impedance measurements performed were of the whole body, of different body segments, and of blood. The measurements of whole body impedance were performed according to the method described by Hoffer (1969). As well as being used in the direct impedance : total body water comparison, this measurement was also used in the conventional BIA technique of total body water measurement.

Although segmental body impedance measurements have been previously described (Baumgartner *et al.*, 1989; Chumlea *et al.*, 1987; Fuller & Elia, 1989; Zillikens *et al.*, 1992), the sites of measurement were not adequate for the investi-

gative nature of this study. There was a need for sites with specific natures and compositions, hence the selection of a unique set of segmental impedance measurement sites. The segmental body impedance measurements were performed across specific anatomical sites of set distances using an extended and modified method described by Scheltinga *et al.* (1992). The different sites were selected to represent controllable areas of differing fat patterning and water distribution (Katch *et al.*, 1974; Scheltinga *et al.* 1992). The specificity of the sites and the set length of the measurement was done in order to standardise for anatomical variation and conductor length. The blood impedance measurements were performed using a modification of the method described by Deurenberg *et al.* (1989a).

The body impedance across various sites, of constant conductor length, was measured by means of a Body Composition Analyser (Holtain, UK). Before measurements were made, the instrumental power supply was checked, and the instrument was calibrated using an internal set resistance of 400Ω.

The subjects were placed in a supine position, with the limbs away from the trunk. Silver silver-chloride disposable ECG skin electrodes (Promed, Protea Electro-Medical, South Africa) were applied to various sites on the body. Before applying the electrodes, the skin surface was swabbed clean with alcohol, and allowed to dry. The electrodes were not removed from the initial site for the duration of the electrical measurements. Although the skin under the electrode may have sustained a small degree of dehydration, this was considered to be negligable. Between the impedance measurements, which were performed in the supine position, the subjects were allowed to walk around freely. After placing the electrodes on predetermined sites (Figure 3.3), the pair of injector leads and the pair of detec-

tor leads of the bioelectrical impedance instrument were placed on their respective electrodes. A constant 800μA, 50kHz alternating current was applied to the site via the injector electrodes. The detector electrodes measured the impedance of the conductor as a drop in the voltage of the applied current, across the measured length. This voltage drop was converted into a representation of impedance, measured in ohms (Ω). This impedance was reflected on a 0-1000Ω scale. Due to the relatively low frequency of the applied alternating current, no attempt was made to separate the different components of impedance, viz. resistance and reactance. Each impedance measurement was recorded in duplicate, for test-retest assessment.. The various sites of the body for measuring different body impedance are shown in Figure 3.3, and are described below.

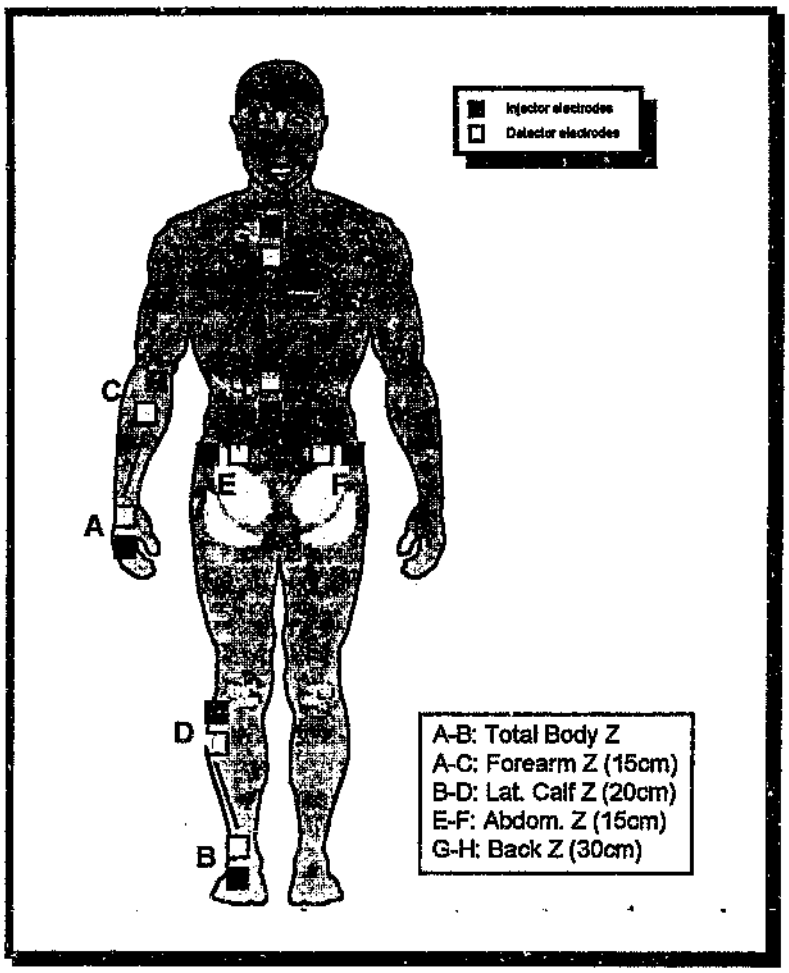


Figure 3.2: Diagrammatic representation of the sites of electrode placement for body impedance measurement

i. Total Body Impedance

According to the manufacturer's specifications, a pair of current injector electrodes were placed on the dorsal surfaces of the right hand and foot over the distal third part of the metacarpal and metatarsal respectively. A pair of detector electrodes were placed at the right pisiform prominence of the wrist and between the medial and lateral malleoli of the right ankle. Thus, a pathway from the distal part

of the right arm to the distal part of the right leg, and vice versa, was established, giving a total body impedance measurement.

ii. Forearm Impedance

An injector electrode was placed on the anterior aspect of the wrist of the right hand, immediately proximal to the middle wrist crease. A detector electrode was placed 5cm proximal to the injector electrode of the wrist. The other pair of detector\injector electrodes were placed on the longitudinal axis of the forearm, 5cm apart, with the detector electrode distal to the injector electrode. This detector electrode was placed 15cm proximal to the detector electrode of the wrist. This allowed a measurement of the impedance of a section of the right forearm, with a set distance between electrodes of 15cm.

iii. Lateral Calf Impedance

A pair of electrodes, one injector and one detector, were placed on the lateral aspect of the right ankle. The detector electrode was placed directly on the lateral malleolus, with the injector electrode 5cm distal to this, on the lateral aspect of the right foot. The second detector electrode was placed on the lateral aspect of the calf, 20cm proximal to the distal detector electrode, and the second injector electrode was placed 5cm proximal to this. This enabled a measurement of the impedance of a 20cm section of the right lateral calf.

iv. Abdominal Impedance

Four electrodes were placed on a horizontal line at the level of the umbilicus on the abdomen. The two detector electrodes were placed 15cm apart, one 7.5cm to the left of the umbilicus, and one 7.5cm to the right. The two injector electrodes were each placed 5cm lateral to its respective detector electrode. With this electrode arrangement, a measurement of abdominal impedance across 15cm could be recorded.

v. Back Impedance

A 30cm vertical length of the back was measured by placing the set of injector\detector electrodes in the following positions. A detector electrode was placed at the level of the iliac crest 5cm to the right of the mid-line of the back. An injector electrode was placed 5cm caudal to the detector electrode, in the same vertical plane. The other detector electrode was placed 30cm cranial to the first detector electrode, also 5cm right of the mid-line, with an injector electrode 5cm cranial to this detector electrode.

e) *Blood Impedance Measurement*

A volume of 5m*l* of blood was removed from the collection tubes immediately on venous sampling. The electrical impedance of the blood volume was then measured, in duplicate and at constant temperature, using the same blood-impedance chamber and method described in Chapter 2.1.1.

f) *Haematocrit Measurement*

Samples from each blood specimen were removed from the collection vials, placed in capillary tubes and centrifuged for 5 minutes at 3000rpm in a bench centrifuge. The capillary tubes were then removed from the bench centrifuge, and a haematocrit assessment was performed. The haematocrit was measured as a ratio of the red blood cells to the total blood volume, and expressed as a fraction, e.g., "0.52" - indicating 52% red blood cells relative to the total blood volume. Each haematocrit assessment was performed in duplicate.

g) *Blood Plasma Ionic Measurements*

After the blood impedance and haematocrit measurements were performed on all the blood samples, the collection vials, with their contents, were centrifuged for 10 minutes at 5000rpm (Technospin R, Sorval Instruments). The blood plasma was removed from the centrifugate by means of disposable pipettes, and placed in storage vials. The plasma was then frozen at approximately -20^0 C. Within one week of sampling, the plasma samples were unfrozen, and were assessed for ionic concentrations of Na^+, K^+ and Cl^-, as well as plasma osmolarity. The methods of ionic concentration and osmolarity determination are identical to those described in Chapter 2.1.1.

3.2.4. Calculations and Equations

For the purposes of the description of the study sample, the body mass index (BMI), body density (BD), the fat mass (FM), and the fat-free mass (FFM) of each individual was calculated.

Further, equations developed for the use in bioelectrical impedance analysis, using whole body impedance measurements, were used to predict total body water in both the dehydrated and the rehydrated stages.

The equations used were selected from the available published literature according to the study size and the character of the sample measured (van Loan, 1990). The equations selected were:

a) *Anthropometry*

$$BMI = \frac{Wt}{Ht^2}$$

$$BD = 1.1423 - 0.0632 \ln (\sum S.F.) \qquad \text{(Durnin \& Wolmersley, 1974)}$$

$$\%FM = \frac{4.95}{BD} - 4.5 \qquad \text{(Siri, 1961)}$$

$$FFM = Wt - \%FM$$

b) *Bioelectrical Impedance Analysis*

1. $$TBW = 2.03 + \frac{0.63 \times Ht^2}{Z}$$

2. $$TBW = 4.96 + \frac{0.42 \times Ht^2}{Z} + 0.13 \times Wt + 3.34 \times (sex^a)$$

3. $$TBW = 9.9868 + 0.000723 \times Ht^2 + 0.2822 \times Wt - 0.0153 \times Z - 2.3313 \times (sex^b) - 0.1319 \times (age)$$

4. $$TBW = 4.65 + \frac{0.377 \times Ht^2}{Z} + 0.14 \times Wt - 0.08 \times (age) + 2.9 \times (sex^a)$$

---

where Ht = height (cm), Wt = weight (kg), Z = impedance (Ω), age in years, S.F. = skin folds
and sex is dummy coded - a: males = 1, females = 0
b: males = 0, females = 1

references: 1. Lukaski, 1985
2. Kushner *et al*, 1986
3. van Loan *et al*, 1987a
4. Lukaski *et al*, 1988

c) *Total Body Water Calculation*

The total body water was calculated, using the accepted technique of tritium dilution (Pinson, 1952; Prentice *et al.*, 1952; Liebman *et al.*, 1960; Culebras *et al.*, 1977). The calculations included the values obtained in the tritiated water concentration measurements, and total body water was calculated by means of the following equations:

from ... $$C_1V_1 = C_2V_2$$

$$V_2 = \frac{C_1V_1}{C_2}$$

where ... C_1 = concentration of the sample tritium (in dpm.0.2ml^{-1})
V_1 = volume of the ingested sample (=150m*l*)
C_2 = concentration of equilibrated[2] plasma tritium (in dpm.0.2ml^{-1})
and V_2 = 'unknown' volume of total body water (in m*l*)

Example:
baseline plasma level = 18.4 dpm
equilibrated plasma level = 417.8 dpm
ingested sample level = 133451 dpm
ingested volume = 150 ml
measured volumes = 0.2 ml (in 12 ml scintillation fluid)

$$V_2 = \frac{C_1V_1}{C_2}$$

$$= \frac{133\,451 \text{ dpm.0.2m}l^{-1} \times 150\text{m}l}{(417.8 - 18.4) \text{ dpm.0.2m}l^{-1}}$$

$$= 50\,119.3 \text{ m}l$$

[2] the equilibrated plasma tritium is equivalent to the difference between measured plasma levels of tritium and the baseline plasma levels of tritium, i.e., equilibrated plasma level - baseline plasma level.

3.2.5. Data Capture and Analysis

The data were captured and analysed using an IBM compatible 80386DX personal computer, and software applications running under the Microsoft Windows environment.

a) *Data Capture*

An automatic-prompting software application was developed using MS Word-Basic v 2.0 (Microsoft Corp.) to automatically capture, format, and store all data variables collected. This data set was modified and converted into other software-compatible formats. The data were transferred into a spreadsheet package (MS Excel v 4.0, Microsoft Corp.), for purposes of data manipulation, various calculations, and data file exporting. The statistical analyses needed were also performed by MS Excel.

b) *Statistical Analyses*

The following statistical techniques were performed on the data collected:

1. From the *central limit theorem* of statistics (Hollander and Wolfe, 1973), and since the sample size was greater than n = 30, the sampling distribution of the mean can be approximated by a normal probability distribution. This normalcy was confirmed statistically. The mean, median and mode of each measured variable were considered for similarity - if the three indications of central tendency were not obviously different, the sample measurement was

considered to be relatively normally distributed.

2. Using a 5% ($\alpha \leq 0.05$) sampling error, the sample size ($n = 74$) was assessed for adequacy.
3. The reliability of the measurements on the blood samples was assessed by means of two statistical estimates. The amount of error variance was assessed by calculating the standard error of measurement between two repeated measurements, and the proportion of error variance was obtained from a correlation analysis - indicating the variance (R^2) between the two measurements (see Appendix 2 for details on these two tests).
4. In the final analysis of the data, Z-tests were used to compare the means of two measurements. The Z-test was selected over the *t*-test due to the sample size being greater than $n = 30$ - therefore, it was not necessary to correct for a small sample size (Rawlings, 1988). Partial correlations and Pearson product-moment correlations were used to consider the amount of association between data sets. Finally, stepwise linear regression was used to determine the predictive ability of specific variables.

The results of these statistical techniques presented in graphical and tabular form. Unless otherwise stated, all statistical results are considered significant for $\alpha = 0.05$.

3.3) Results

3.3.1. Subject Description

Summary information of the volunteers who participated in this study is provided in Table 3.1. In total, the study measurements were performed on 74 subjects (35 female and 39 male), of which, 46 were white, 15 were black, 7 were so-called 'coloured', and 6 were Indian.

Table 3.1: Summary statistics of study volunteers (n=74)

	MEAN*	STD†	v (%)‡	min	max
age (years)	23.8	3.4	14.3	17.3	31.5
height (cm)	173.7	9.2	5.3	151.2	191.4
weight (kg)	71.6	13.3	18.6	52.5	94.0
BMI ($kg.m^{-2}$)§	23.6	2.7	11.4	16.4	28.8
body density ($g.cm^{-3}$)**	1.049	0.010	0.01	1.022	1.074
fat mass (kg)††	15.66	3.61	23.05	7.88	26.49
fat-free mass (kg)††	55.93	9.91	17.72	45.11	63.72

*: sample mean
†: sample standard deviation
‡: coefficient of variation
§: body mass index
**: from skinfold thickness measurements (see calculations - 3.2.4)
††: see 3.2.4 - calculations and equations

3.3.2. Data Description

A summary of the data collected is presented in Tables 3.2 and 3.3. Values presented in Table 3.2 indicate the mean, standard deviation (STD), and coefficient of variation (ν) for the measurements performed on the subjects in the slightly dehydrated state. Table 3.3 presents the same information of the data from the subjects in the rehydrated state. The value of each measurement presented is the mean of duplicate measurements.

Table 3.2: Summary of data of dehydrated measurements

	MEAN*	STD†	ν (%)‡
Tritium Analysis (DPM)			
[blood baseline]	36.50	10.30	28.22
[sample]	232477.00	1296.70	0.56
[blood final]	880.36	185.49	21.07
Calc. Total Body Water (*l*)	43.14	8.91	20.65
Blood Ionic Analysis			
Na^+	145.6	16.4	11.26
K^+	5.2	0.6	11.54
Cl^-	107.8	16.2	15.03
Osmol.	291.2	32.8	11.26
Hct	0.46	0.07	15.22
Z Measurements (Ω)			
blood Z	129	11	8.52
body Z	459	91	19.83
arm Z	114	25	21.93
leg Z	132	29	21.96
abdomen Z	56	9	16.07
back Z	66	13	19.69

*: measurement mean
†: measurement standard deviation
‡: coefficient of variation

Table 3.3: Summary of data of rehydrated measurements

	MEAN*	STD†	ν (%)‡
Tritium Analysis (DPM)			
[blood baseline]	36.50	10.30	28.22
[sample]	232477.00	1296.70	0.56
[blood final]	850.26	180.74	21.25
Calc. Total Body Water (*l*)	44.79	9.39	20.96
Blood Ionic Analysis			
Na^+	141.2	14.0	9.92
K^+	4.9	0.5	10.20
Cl^-	106.3	14.0	13.17
Osmol.	282.7	30.6	10.82
Hct	0.40	0.06	15.00
Z Measurements (Ω)			
blood Z	136	12	8.82
body Z	464	68	14.66
arm Z	125	22	17.60
leg Z	138	25	18.12
abdomen Z	59	10	16.95
back Z	65	16	24.61

*: measurement mean
†: measurement standard deviation
‡: coefficient of variation

3.3.3. Type of Distribution

Considering the Theorem of Central Limits, one can often assume that a sample with more than 30 observations can be approximated by a normal probability distribution. In view of the parametric nature of the statistical procedures performed on the collected data, it was considered prudent to assess the degree the sample set approximates a normal distribution. One such method of assessing normalcy is to

consider and compare the three measures of central tendency, i.e., the mean, the median and the mode. To compare the mean, median and mode of each of the variables measured, each variable was plotted giving these three measures of central tendency. The result of these plots are depicted in Figure 3.3 for the sample data, in Figure 3.4 for the measurements of dehydrated subjects, and in Figure 3.5 for the measurements of rehydrated subjects. It is evident from this graphical representation that there is no marked difference between the mean, median or mode of any of the variables measured. This would indicate that the data collected were reasonably approximated by a normal probability distribution.

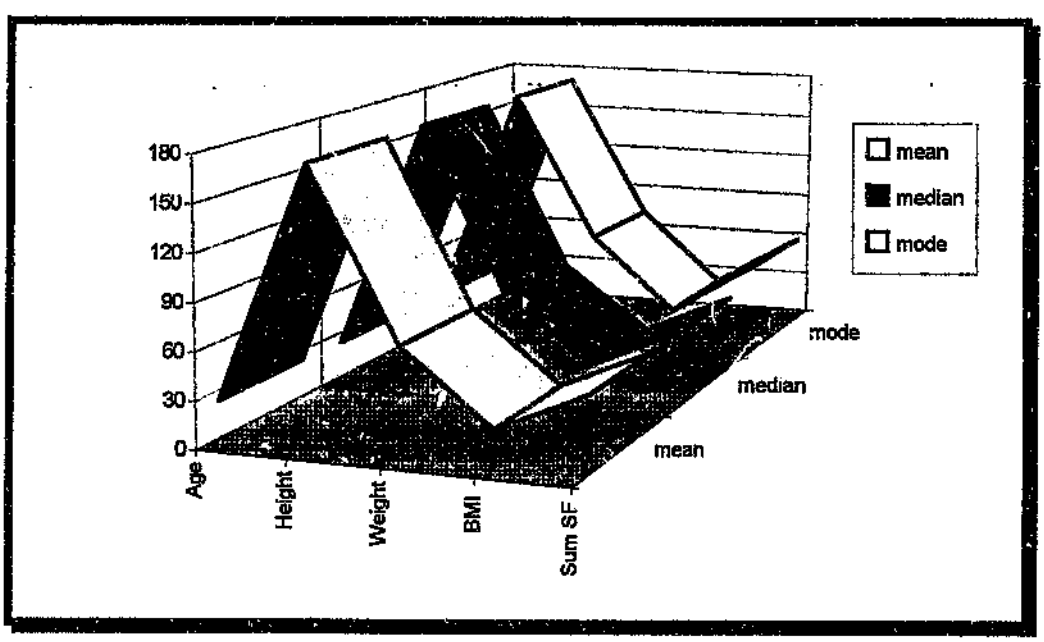


Figure 3.3: The mean, median and mode for the sample data

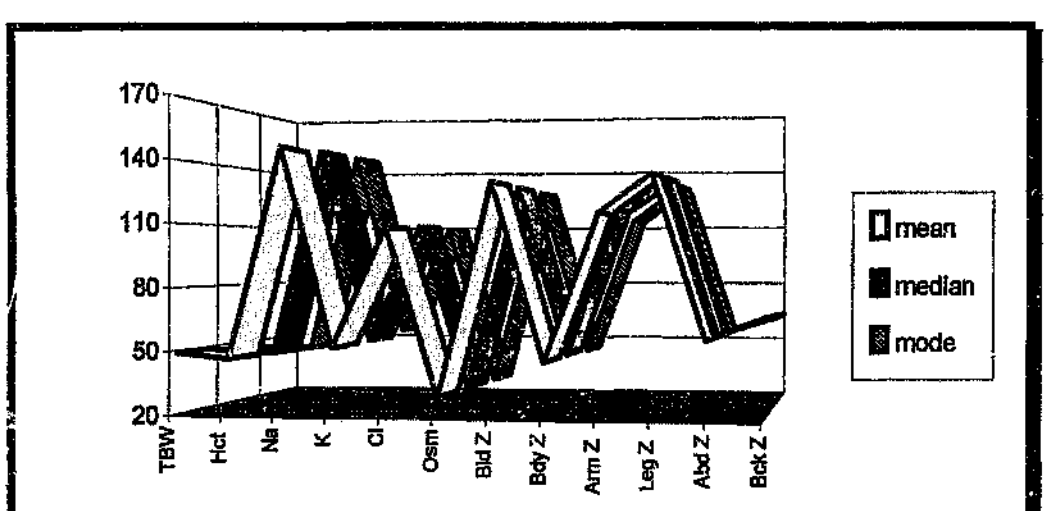


Figure 3.4: The mean, median and mode for the dehydrated state

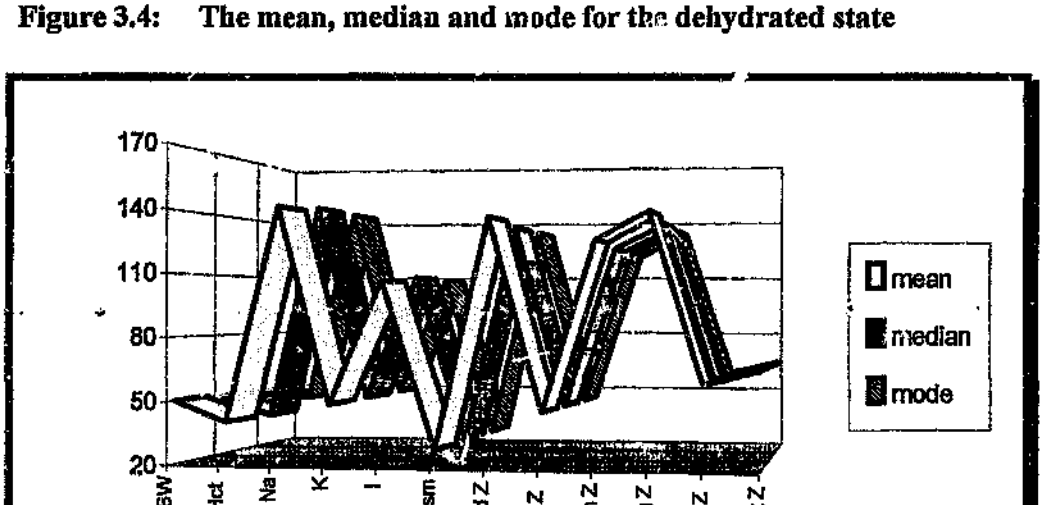


Figure 3.5: The mean, median and mode for the rehydrated state

From this convincing evidence, it was safely assumed that the data collected were normally distributed, and the parametric statistical analyses were performed on the data with confidence in their suitability and validity.

3.3.4. Sample Size Adequacy

For all the measurements performed on the volunteers, the sample size of n = 74 was within the limits needed for a probability level of $\alpha \leq 0.05$. The sample size significance was assessed by considering the variance, size of acceptable sampling error, and the Z value for $\alpha = 0.05$. See Appendix 2 for a detailed description of this assessment.

3.3.5. Reliability of Measurements

All measurements recorded were performed in duplicate. This was done to assess the reliability and repeatability of the sampling techniques and measurement methods used in this study. Because only one investigator was responsible for the sampling and measurement of all data, it was not necessary to assess inter-observer reliability. To assess the amount of error variance, a calculation of the standard error of the measurements (SEM) was performed, and to assess the proportion of error variance in relation to true variance, a correlation analysis was performed, resulting in a variance (R^2) statistic. See Appendix 2 for details on the statistical methods. Table 3.4 indicates the SEM and R^2 values obtained for these two tests. The true variance was proportionately high in relation to error variance, ranging from $R^2 = 0.9990$ to $R^2 = 0.9005$ (i.e. 99.95% to 90.05%). This low proportion of error variance indicates that the measurements were reliable indications of the true values, and were repeatable.

Table 3.4: Reliability assessment of impedance and ionic measurements

	R^2	SEM		R^2	SEM
height	0.9984	0.1907	**^3H_2O blank**	0.9987	0.3561
weight	0.9990	0.1301	**^3H_2O sample**	0.9890	4.609E3
triceps s.f.	0.9812	0.0898	**^3H_2O dehydr**	0.9911	8.9322
biceps s.f.	0.9900	0.0302	**^3H_2O rehydr**	0.9876	12.2451
subscap. s.f.	0.9763	0.8742	**Hct dehydr.**	0.9756	0.0115
suprail. s.f.	0.9490	1.1623	**Hct rehydr.**	0.9808	0.0080
Dehydrated Measurements			**Rehydrated Measurements**		
Na^+	0.9322	3.8433	**Na^+**	0.9005	3.3921
K^+	0.9788	0.1211	**K^+**	0.9796	0.1105
Cl^-	0.9803	1.0334	**Cl^-**	0.9829	1.1693
Osmolality	0.9193	4.0990	**Osmolality**	0.9169	3.8685
Blood Z	0.9730	3.0302	**Blood Z**	0.9752	3.1441
Body Z	0.9743	14.6325	**Body Z**	0.9664	15.7764
Arm Z	0.9898	2.2822	**Arm Z**	0.9856	2.5603
Leg Z	0.9862	2.6432	**Leg Z**	0.9844	2.7030
Abdomen Z	0.9645	2.2409	**Abdomen Z**	0.9585	2.8653
Back Z	0.9790	1.4542	**Back Z**	0.9802	1.3205

3.3.6. Data Analysis

The objectives of these data analyses were to consider:

- whether the study design provided a significant change in the hydration status of each individual.
- whether total body water is indicated by bio-electrical impedance measurements
- the associations between various impedance measurements and hydration status, and impedance measurements and body fluid electrolyte concentrations. The hydration status was considered as the total body water per body mass.
- the ability of impedance measurements to predict changes in total body water and electrolyte concentrations in individual measurements.

a) *Hydration Status Change*

Table 3.5 indicates the results of a two-sample Z-test to consider the significance of the difference in hydration status between dehydration measurements and rehydration measurements of body fluids and electrolytes. The hydration status was taken to be the ionic concentration of the blood, and the proportion of TBW per body mass.

Table 3.5: Significant differences between dehydrated & rehydrated states

		MEAN	STD	|Z|*	P ($\|Z\| \leq Z_{crit}$)
TBW	dehydr.	43.14	8.91	1.0948	0.1367
	rehydr.	44.79	9.39		
TBW.kg^{-1}	dehydr.	0.6005	0.0012	4.6187	1.93E-06
	rehydr.	0.6226	0.0005		
Hct	dehydr.	0.46	0.07	5.9704	1.19E-09
	rehydr.	0.40	0.06		
Na^+	dehydr.	145.58	16.4	1.7356	0.0413
	rehydr.	141.23	14.0		
K^+	dehydr.	5.21	0.6	3.1044	9.53E-4
	rehydr.	4.92	0.5		
Cl^-	dehydr.	107.83	16.2	0.5958	0.2756
	rehydr.	106.34	14.0		
Osmol.	dehydr.	291.15	32.8	1.6800	0.0526
	rehydr.	282.71	30.6		

*: $Z_{crit} = 1.6449$, for $\alpha = 0.05$

From the results shown above, it is evident that, although the change in TBW is not significant for $\alpha = 0.05$, there is a significant change in the amount of body fluid per unit body mass, as well as most of the ionic concentrations of the blood. The other exception is Cl^- that, although not significantly for $\alpha = 0.05$, does change in the same direction as the other ions. To assess the degree of relationship between the ionic changes and the TBW changes, without the influence of individual body size, a partial correlation analysis was performed considering the change in TBW and each of the ionic measurements, viz. Na^+, K^+, Cl^-, and osmolality, with height and weight held constant. Change in haematocrit was also partially correlated with change in TBW, with height and weight constant. Table 3.6 indicates

the results of this partial correlation analysis (all correlation coefficients are significant for $\alpha = 0.05$).

Table 3.6: Partial correlations between Δ TBW and ionic indicators.

Relationship	$R_{partial}$*
Δ TBW - Δ Na^+	0.8594
Δ TBW - Δ K^+	0.7601
Δ TBW - Δ Cl^-	0.6868
Δ TBW - Δ Osmol	0.9106
Δ TBW - Δ Hct	0.9498

*: with height and weight held constant

b) *BIA and Total Body Water*

To determine whether the described technique of bioelectrical impedance analysis (BIA) indicates total body water measurements, various published equations were used to predict total body water from whole body impedance measurements. These whole body Z measurements were included with other variables in regression equations for the prediction of total body water (see Chapter 3.4.2 - Calculations and Equations).

To assess the degree of agreement between the tritium dilution method of total body water measurement and the various whole body BIA methods, a correlation coefficient was calculated. Table 3.7 shows the results obtained for the correlation calculation, comparing calculations of total body water using the tritiated water

method (Prentice *et. al.*, 1952; Olsson, 1970; Szeluga *et. al.*, 1984) and whole body BIA (Lukaski *et. al.*, 1985; Kushner *et. al.*, 1986; van Loan & Mayclin, 1987a; Lukaski *et. al.*, 1988). In Table 3.7 Pearson product-moment correlation coefficients are shown. Standard error of estimation (SEE) values are also included in this table. The correlation coefficients were all significant for $\alpha = 0.05$.

Table 3.7: Correlation between TBW (*l*) measured by tritium dilution and whole body impedance, using various BIA regression equations.

BIA Equation	Hydration	R*	SEE†
Kushner *et al*, 1986	Dehydrated	0.96	1.31
	Rehydrated	0.94	1.42
Lukaski *et. al.*, 1985	Dehydrated	0.94	2.21
	Rehydrated	0.93	2.24
Lukaski *et. al.*, 1988	Dehydrated	0.95	1.42
	Rehydrated	0.91	1.49
van Loan, 1987a	Dehydrated	0.90	3.39
	Rehydrated	0.88	3.04

*: all coefficients significant for $\alpha = 0.05$
†: SEE: standard error of estimation

Although there exists a high correlation between the results of the TBW assessments and the BIA predictions of TBW, the BIA results of TBW did not significantly differ from each other for the dehydrated and the rehydrated states of measurement (for $\alpha = 0.05$).

The BIA regression equations used to indicate total body water include various anthropometric and other measurements. To assess the inherent value of the dif-

ferent individual measurements, a multiple regression analysis was performed, considering the prediction of total body water (measured by tritium dilution) by whole body BIA techniques. The pertinent information gained from this analysis is presented in Table 3.8, showing the partial correlation coefficients and the F-values of inclusion into the regression equation for each of the relevant variables.

Table 3.8: Stepwise linear regression analysis of the predictive value of BIA variables in assessing total body water

Variable	Dehydration Values		Rehydration Values	
	$\|P_{corr}\|$*	F_{enter}†,‡	$\|P_{corr}\|$	F_{enter}
Weight	0.7402	29.67	0.7596	29.98
Height	0.5949	18.23	0.5850	17.43
Height^2	0.6406	22.49	0.6537	23.02
Sex	0.4751	12.76	0.4759	12.84
Body Z	0.4047	7.40	0.3929	7.08
Age	0.1565	0.12	0.1602	0.15

*: partial correlation coefficient (all significant for $\alpha = 0.5$)
†: F-value for entry into regression equation
‡: d.f. = 73

c) *Impedance Measurements and Hydration Status*

Having considered the degree of change exhibited between the dehydrated and rehydrated measurements and that impedance may or may not indicate body composition other than the total body water volume, the next step was to consider the degree of association between the various impedance measurements and the hydration status. To consider the hydration status of each individual, the following

indicators were used:

1. the measured TBW volume per body mass ($l.kg^{-1}$), and
2. the blood electrolyte concentrations.

Table 3.9 summarises the results of a correlation analysis between the Z values, and the hydration status for the dehydration measurements. Table 3.10 shows the same analysis, for the rehydration measurements. All Pearson correlation coefficients (R) shown are significant for $\alpha = 0.05$.

Table 3.9: Hydration status correlated with Z measurements - dehydration

	Hydration Status				
Impedance	**TBW.kg^{-1}**	**Na^+**	**K^+**	**Cl^-**	**Osmol.**
blood Z	-0.9715	-0.9014	-0.8208	-0.8885	-0.9014
body Z	-0.5237	-0.4334	-0.4477	-0.4662	-0.4334
arm Z	-0.8911	-0.7988	-0.6908	-0.7733	-0.7988
leg Z	-0.9232	-0.8236	-0.7190	-0.8032	-0.8236
abdom. Z	-0.6704	-0.5221	-0.4317	-0.5381	-0.5221
back Z	-0.6864	-0.5396	-0.4226	-0.5339	-0.5396

Table 3.10: Hydration status correlated with Z measurements - rehydration

	Hydration Status				
Impedance	TBW.kg^{-1}	Na^+	K^+	Cl^-	Osmol.
blood Z	-0.9600	-0.8898	-0.8513	-0.9202	-0.9201
body Z	-0.4851	-0.6221	-0.5976	-0.6400	-0.6399
arm Z	-0.8485	-0.8109	-0.7746	-0.8371	-0.8371
leg Z	-0.9000	-0.8375	-0.8038	-0.8647	-0.8646
abdom. Z	-0.5904	-0.5746	-0.5735	-0.5764	-0.5765
back Z	-0.6303	-0.6075	-0.6026	-0.6107	-0.6107

Figure 3.6 shows the results of a scatter-plot between the body fluid index ($l.kg^{-1}$) and the six impedance measurements (Ω) for the dehydrated and the rehydrated states. The ionic measurements yielded very similar scatter plots when considered with the impedance measurements.

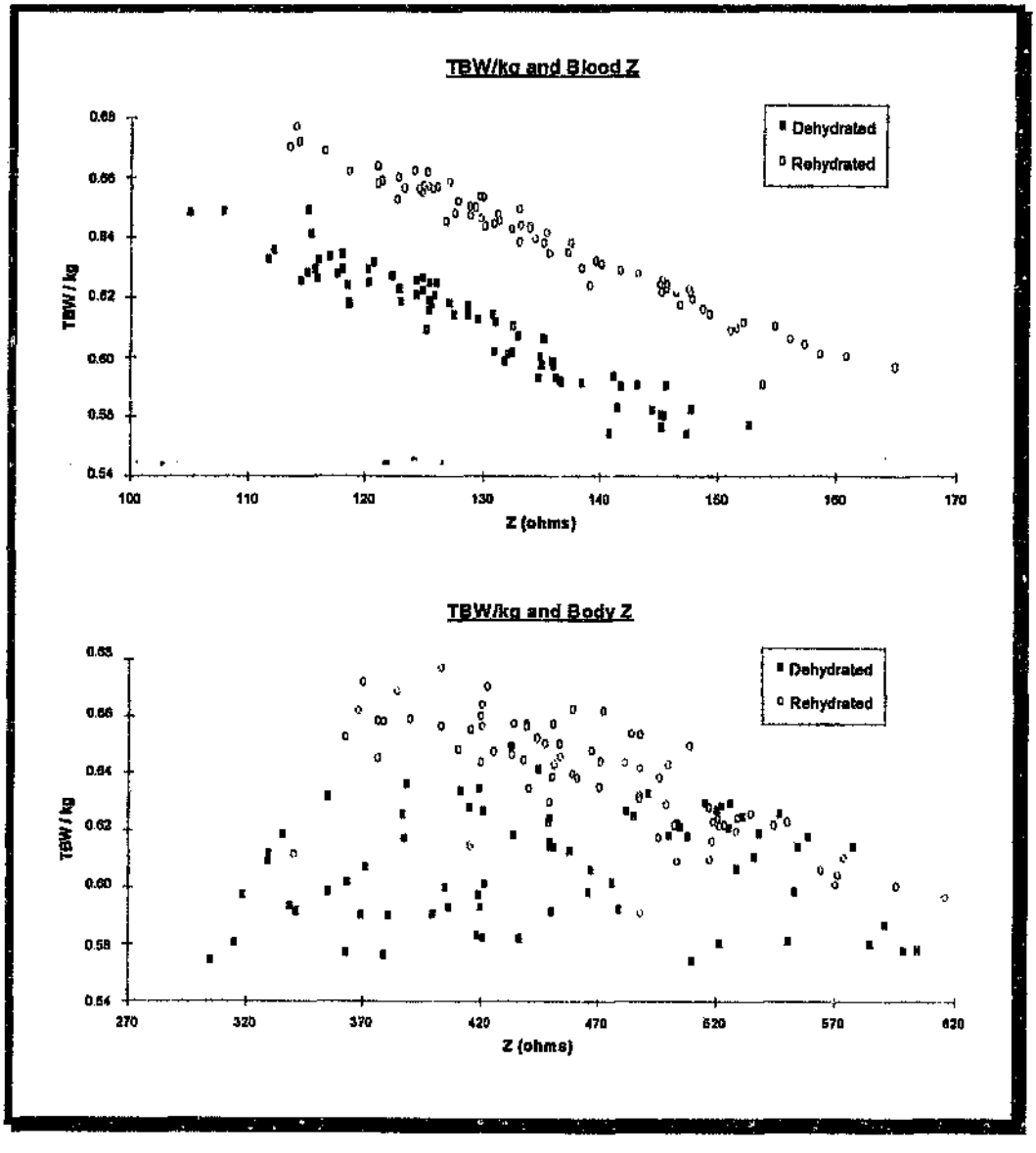


Figure 3.6: Body fluid index vs. body and blood Z (cont. overpage)

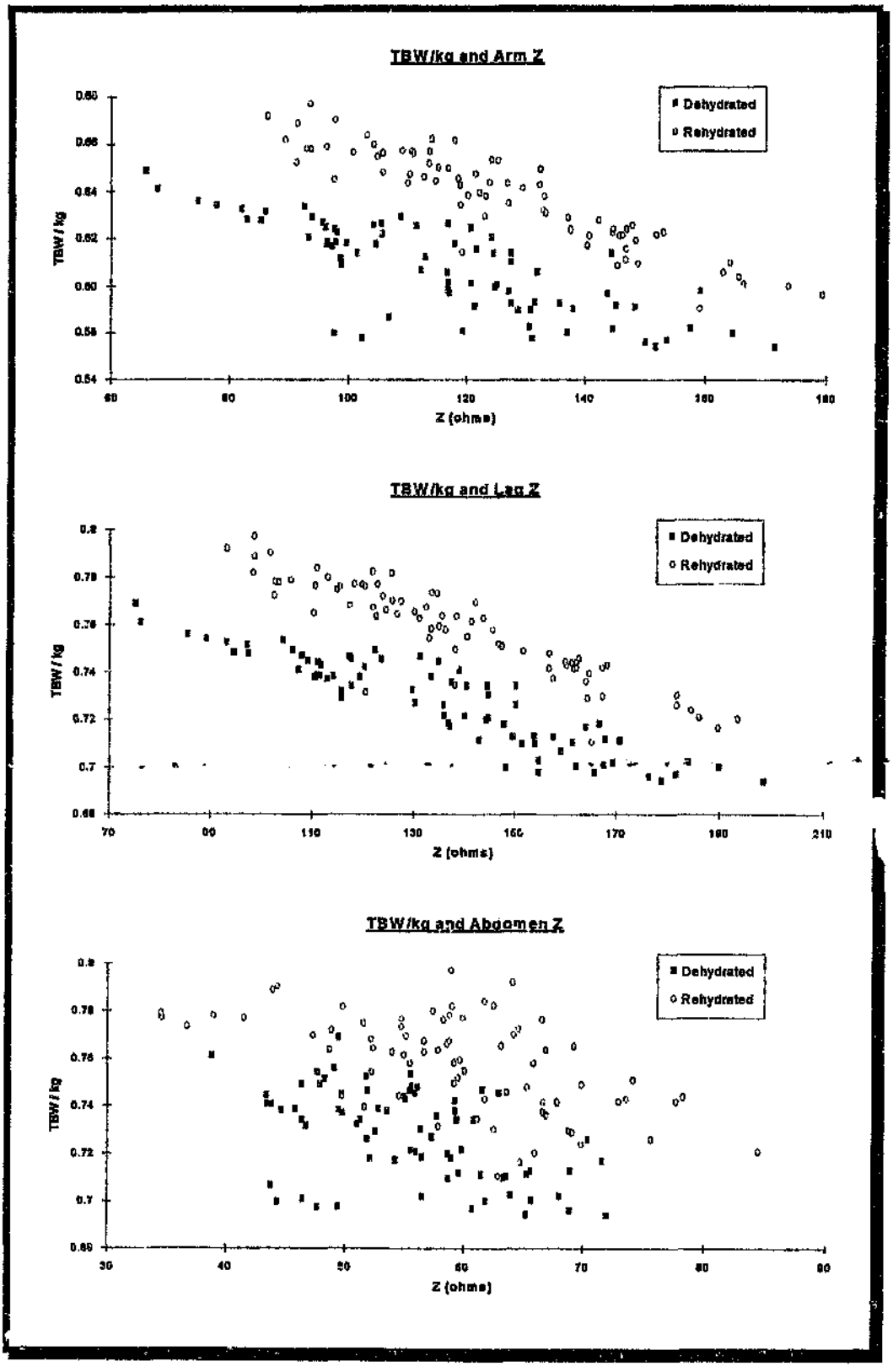


Figure 3.6: Body fluid index vs. body and blood Z (cont. overpage)

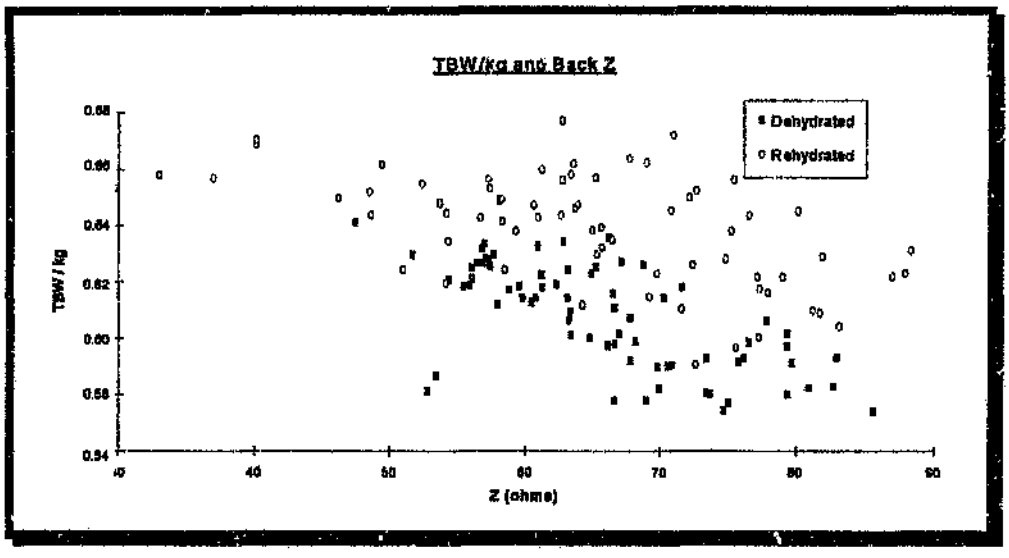


Figure 3.6: Body fluid index vs. body and blood Z

From the results of the correlation calculations and the above graphical representations, it is evident that there is a reasonable association between the hydration indicators and the impedance measurements, particularly impedance of the blood, arm and leg. To assess how much each of the individual impedance measurements indicate the hydration status of the body, a multiple regression analysis was performed considering the ability of the various impedance measurements to predict the body fluid index (TBW/weight). Table 3.11 presents the results of this regression analysis, showing the values of the partial correlation analysis and the F-values for the entry of the impedance measurements into the regression equation for the prediction of the body fluid index.

Table 3.11: Stepwise linear regression of fluid index and impedance

	Dehydration Values		Rehydration Values	
	P_{corr}*	F_{enter}†,‡	P_{corr}	F_{enter}
Blood Z	-0.9715	1207.69	-0.9600	845.74
Leg Z	-0.9232	415.57	-0.9000	306.97
Arm Z	-0.8911	277.71	-0.8485	185.13
Back Z	-0.6864	64.14	-0.6303	47.45
Abdom Z	-0.6704	58.79	-0.5904	38.52
Body Z	-0.5237	27.21	-0.4851	22.16

*: partial correlation coefficient (significant for $\alpha = 0.05$)
†: F-value for entry into regression equation
‡: d.f. = 73

d) *Impedance Measurements and Changing Hydration Status*

To assess whether the various impedance measurements are indicators of the change in body hydration status, Pearson product-moment correlation coefficients were calculated considering the change in the TBW and ionic status and the change in the various impedance measurements, viz. blood Z, body Z, and segmental Z. Table 3.12 shows these results. All R values are significant for $\alpha = 0.05$.

Table 3.12: Δ hydration status correlated with Δ Z measurements*

	Hydration Status				
Impedance	Δ TBW	Δ Na^+	Δ K^+	Δ Cl^-	Δ Osmol.
Δ blood Z	0.8836	0.8804	0.8629	0.8253	0.8775
Δ body Z	0.5287	0.5120	0.4398	0.5541	0.5327
Δ arm Z	0.8034	0.8497	0.7871	0.7649	0.8060
Δ leg Z	0.8450	0.8665	0.8175	0.8083	0.8368
Δ abdom. Z	0.7477	0.7421	0.7169	0.7224	0.7419
Δ back Z	0.7691	0.7826	0.7279	0.7248	0.7653

*: Δ indicates change in measured variable

A stepwise multiple linear regression analysis was performed to consider the qualitative prediction of changes in TBW volume and ionic concentrations by the change in impedance values. The F-value for entry into the regression equation, as well as the partial correlation coefficients, for each of the impedance values was calculated. From this information, the regression model was established, using only the most predictive impedance values. Table 3.13 shows the F-values and partial correlation coefficients calculated.

Table 3.13: Stepwise regression selection of Δ Z for Δ hydration prediction†

		Impedance					
		Δblood Z	**Δbody Z**	**Δarm Z**	**Δleg Z**	**Δabd. Z**	**Δback Z**
Δ TBW	**F-enter**	256.47*	27.92	131.07*	179.84*	91.29	104.23*
	P_{corr}	0.8836	0.5287	0.8034	0.8450	0.7477	0.7691
Δ Na+	**F-enter**	248.17*	25.58	187.01*	216.98*	88.23	113.81*
	P_{corr}	0.8804	0.5120	0.8497	0.8665	0.7421	0.7826
Δ K+	**F-enter**	209.81*	17.27	117.24*	145.11*	76.12*	81.14*
	P_{corr}	0.8629	0.4398	0.7871	0.8175	0.7169	0.7279
Δ Cl-	**F-enter**	153.76*	31.90	101.51*	135.68*	78.57	79.70
	P_{corr}	0.8253	0.5541	0.7649	0.8083	0.7224	0.7248
Δ Osm.	**F-enter**	241.00*	28.52	133.54*	168.19*	88.14	101.76*
	P_{corr}	0.8775	0.5327	0.8060	0.8368	0.7419	0.7653

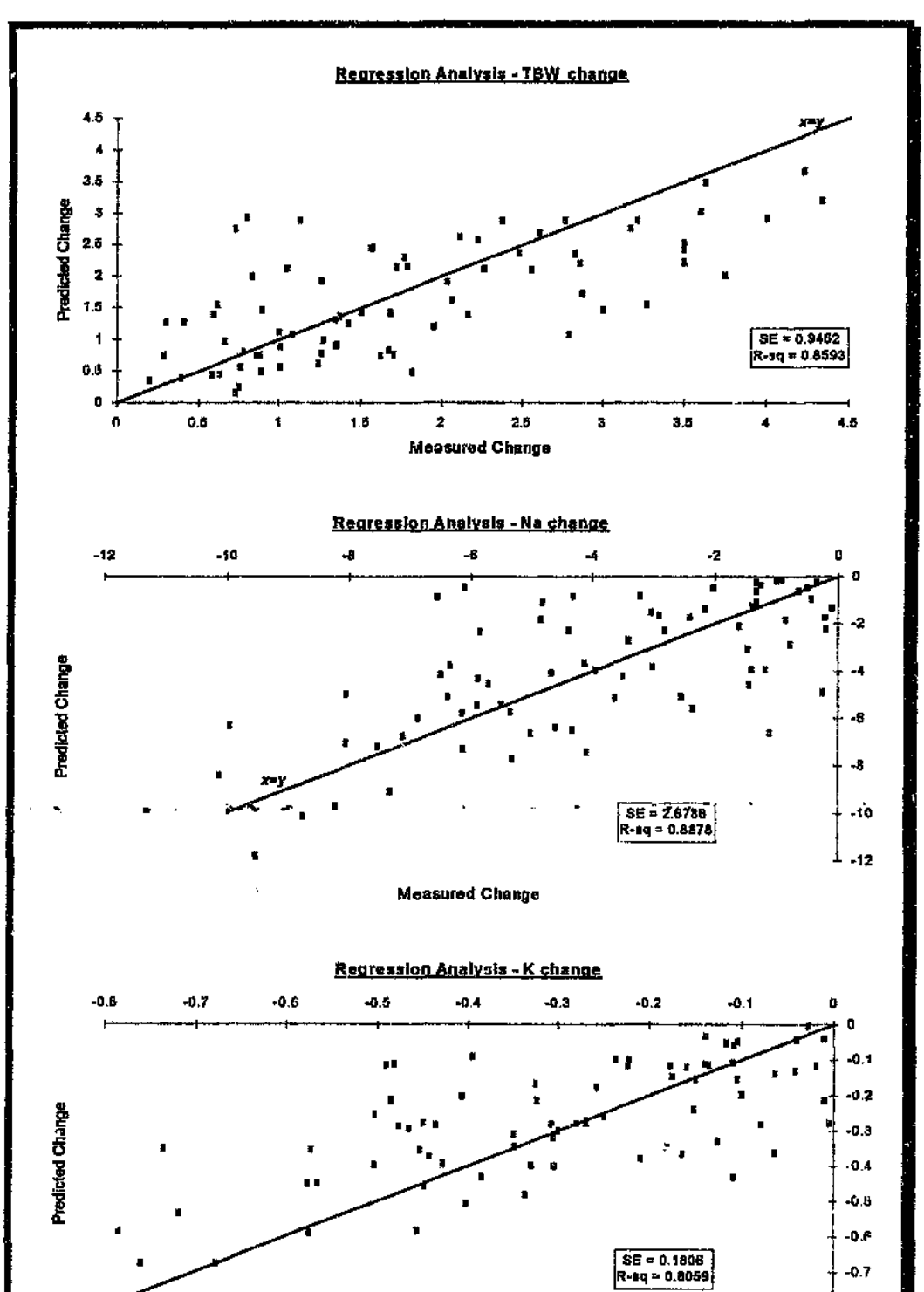
†: ***F-enter*** = F-value for entry into regression model, P_{corr} = partial correlation coefficient
*: variables included in multiple regression models

The six multiple regression models from the selected impedance measurements for the prediction of changing hydration indicators are:

Δ TBW:	$-2.7990 + 0.3056\alpha + 0.0477\beta + 0.3337\delta + 0.2459\varepsilon$	SE=0.9462	R^2=0.8593
ΔNa^+:	$-18.0996 + 0.7959\alpha + 0.3803\beta + 1.0559\delta + 0.7155\varepsilon$	SE=2.6786	R^2=0.8878
ΔK^+:	$-0.9823 + 0.0477\alpha + 0.0107\beta + 0.0403\delta + 0.0187\phi + 0.0289\varepsilon$	SE=0.1806	R^2=0.8059
ΔCl^-:	$-3.0625 + 0.1119\alpha + 0.0340\beta + 0.1703\delta$	SE=0.5008	R^2=0.7483
Δ Csm:	$-10.7051 + 0.1234\alpha + 0.0272\beta + 0.1244\delta + 0.1002\varepsilon$	SE=0.4054	R^2=0.8477

where: $\alpha = \Delta$ blood Z
$\beta = \Delta$ arm Z
$\delta = \Delta$ leg Z
$\phi = \Delta$ abdomen Z
$\varepsilon = \Delta$ back Z

Using the above regression models, the measured values of the changes in hydration indicators were plotted against the predicted values. The results of this plot are shown in Figure 3.7.


Regression Analysis - TBW change
Predicted Change
Measured Change
x=y
SE = 0.9462
R-sq = 0.8593
Regression Analysis - Na change
Predicted Change
Measured Change
x=y
SE = 2.6738
R-sq = 0.8878
Regression Analysis - K change
Predicted Change
Measured Change
x=y
SE = 0.1606
R-sq = 0.8059

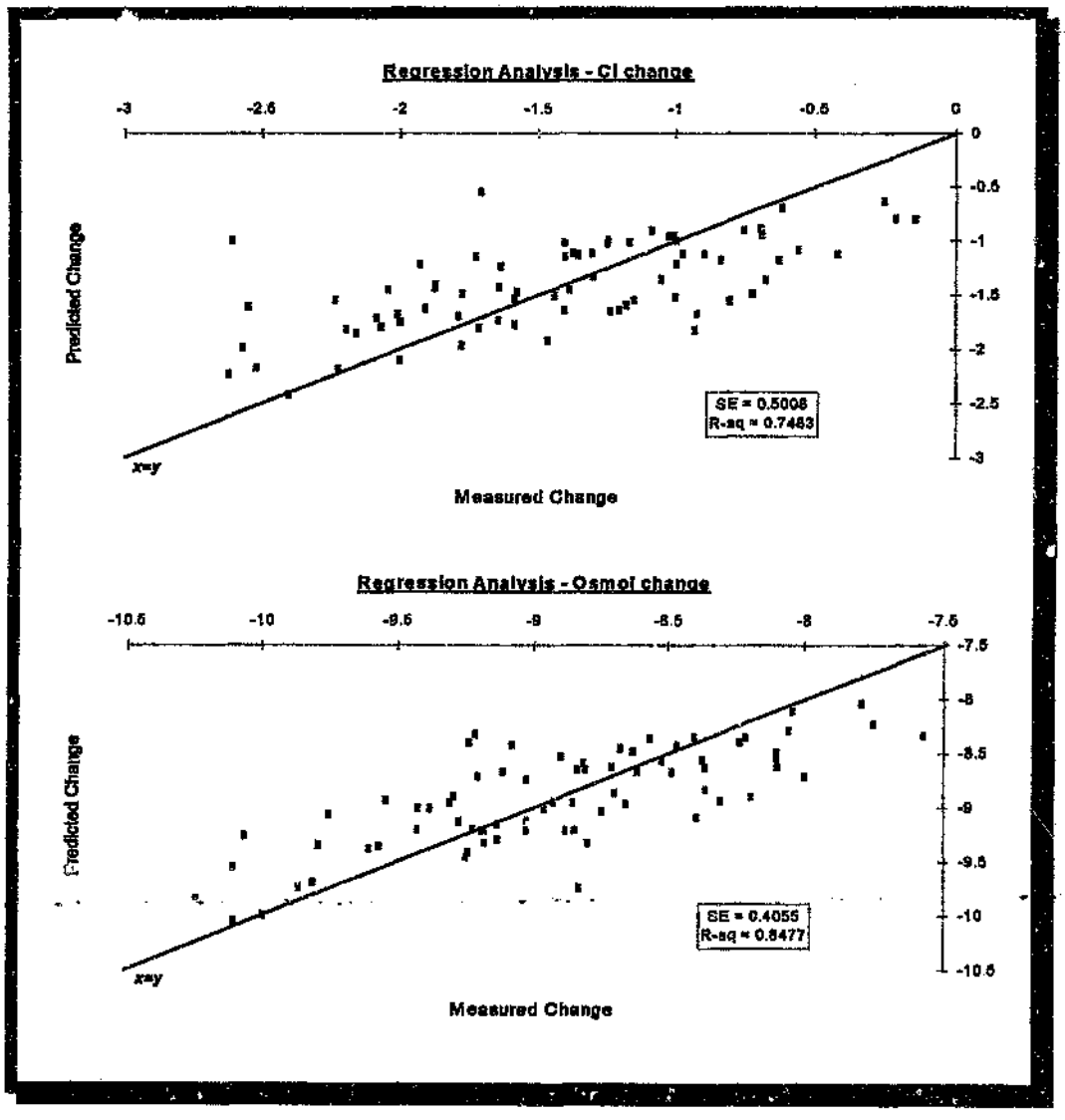


Figure 3.7: Graphical depiction of stepwise regression analysis

3.4) Discussion

3.4.1. The Study Sample

The results of this study were used in an attempt to elucidate an understanding of the nature of bio-electrical impedance measurements of the body. The sample size of n = 74 was estimated to be adequate for the statistical purposes of the study. Although the study sample selected was within reasonable normal limits regarding height and weight for the age of the sample (the body mass index was relatively normal for the mean age of the studied sample), there existed a certain degree of heterogeneity within the sample used. The study group was not divided into very specific categories according to age, sex, and race. This deliberate lack of sample separation was done to minimise the effects of one of the highlighted problems of the BIA technique, namely the extensive development of population-specific relationships (see Chapter 1 for further discussion). The ultimate goal of this study was not to develop further prediction equations for indirect assessment of hydration status of the body. The purpose was essentially to understand what, if any, body composition characteristics contributed to impedance measurements of the body.

3.4.2. Data Collection and Assessment

The study sample used fitted the description of a young, healthy adult sample of reasonably normal height and weight. Tables 3.1, 3.2 and 3.3 indicate the means and standard deviations of the measurements performed for the sample description, the dehydrated state and the rehydrated state respectively. The coefficients of variation (v) indicate reasonable standard deviations from the means in all the reported measurements. In particular, the calculations of body density (Table 3.1) from skinfold measurements show a low v, which is normally found in study samples with subjects of similar height and weight (Durnin & Wolmersley, 1974; Forbes, 1974; Jackson & Pollock, 1978; Norgan *et al.*, 1985). Coefficients of approximately 20% found in the fat and fat-free mass calculations are also similar to other reported studies for the described sample group (Durnin & Wolmersley, 1974; Katch *et al.*; 1979; Lohman, 1981; Baeke *et al.*, 1982; Gray & Bauer, 1991). The v values for the dehydrated and rehydrated states - shown in Tables 3.2 and 3.2 - also show standard deviations within acceptable limits. A measurement with a notably high v value is the tritium concentration measurements of the blood baseline level. This is probably due to the inherent inter-individual variation of baseline radioactivity of the subjects. Two v values which are relatively low in both the dehydrated and the rehydrated measurements - namely the tritium concentration of the ingested sample (0.56%) and the blood impedance measurement (8.52% - 8.82%) - are low because of the advanced control in the measurement technique. In the case of the sample concentration, all ingested samples were prepared in the same manner, and the low v indicates a concomitantly low tech-

nique error component of the measurement method.

In order for the selection of statistical techniques to provide significant results, it was important to assess whether the data collected was normally distributed or not. Two criteria were used to assess normalcy - sample size and the similarity between the measures of central tendency. The sample size of $n > 30$ should theoretically indicate a normal distribution. Furthermore, the mean, median and mode of the data were compared in Figures 3.5 and 3.6. It was shown that for all measurements, the three indications of central tendency were significantly similar, allowing the assumption to be made that the data was adequately normally distributed. Normal parametric statistical techniques were selected for further data manipulation and analysis.

The reliability of repeated measurements was considered by assessing the amount of error variance and the proportion of error variance relative to true variance (Table 3.4). The amount of variance due to measurement error, assessed by the calculation of standard error of measurement (SEM) was significantly low, with all the measurements having SEM values within 5% of the mean measurement value. The highest SEM was recorded for the rehydrated measurement of abdominal impedance (4.84%). The proportion component of this reliability assessment was indicated by calculating the variance (R^2) of the two measurements. For all the measurements, this value was low, ranging from 0.05% to 9.95%. It was assumed from this assessment that the measurements performed were reliable and valid.

3.4.3. Interpretation of the Statistical Analysis of the Data

Using selected parametric statistical procedures, the data was analysed in order to attempt to provide an answer to the original questions posed by this study. Specifically to show whether BIA indicates total body water or whether prediction of the latter is reliant on included anthropometric measurements. Furthermore, it was considered if impedance measurements of the body are largely determined by the fluid and electrolyte compartments of the body. The electrical impedance properties were considered over a hydration range. The limited methods of creating dehydration and allowing rehydration did provide statistically significant changes in hydration status measurements, i.e., total body water index, blood osmolarity and blood ion concentrations. Because of these significant physiological changes, and because weight is included in the TBW index, changes in body weight were not considered in isolation in the statistical analyses. Table 3.5 shows these significant changes. The only measurements not to change significantly were TBW and Cl^-. The lack of change of the latter measurement was probably due to the high degree of permeability of the cell membrane to Cl^-, allowing rapid equilibration to occur. The ionic measurements seemed to change due to the change in hydration status as evidenced in Table 3.6, showing significantly high partial correlation coefficients between the change in measured total body water, and the ion and water concentrations. As would be expected, the difference in osmolarity - which is an indication of all the ions present in the body fluid - has a very high partial correlation ($R = 0.91$) with the change in total body water, whereas Cl^- has the lowest partial correlation with changing total body water ($R = 0.67$). A further

indication the change of hydration status is a change in haematocrit (Gershan *et al.*, 1990). This relationship is significant, and is very highly correlated with the change in total body water. It can therefore be assumed that the attempt to physiologically manipulate the hydration status was successful, and further statistical analysis of the two sets of data - dehydrated and rehydrated - may be considered valid.

a) *Total Body Water and BIA*

Using the whole body impedance measurements in the techniques described to predict total body water from BIA (Lukaski, 1987; Brodie, 1988a, 1988b; van Loan, 1990), the value of the BIA technique was assessed in this study. The selected regression equations used in the BIA method were those published by Kushner *et al.* (1986), Lukaski *et al.* (1985, 1988), and van Loan & Mayclin (1987). The whole body impedance, measured in the dehydrated and rehydrated states, and other necessary variables were used to predict total body water measured by isotope dilution. The two techniques of assessing total body water were compared by means of a product-moment correlation. This statistical technique was selected because it was the technique used in the original studies. Table 3.7 indicates the correlation coefficients and the standard errors of estimation (SEE) for this analysis. The correlation coefficients, ranging from R = 0.88 to R = 0.96, and the SEE's, ranging from 1.31*l* to 3.39*l*, were comparable to the original published values (Lukaski *et al.*, 1985; Kushner *et al.*, 1986; van Loan & Mayclin, 1987;

Lukaski *et al.*, 1988; van Loan, 1990). There appeared to be a slight but neither consistent nor significant difference between the total body water predicted by BIA for the dehydrated and the rehydrated states, however it was seen that the dehydrated values provided slightly better correlation coefficients.

BIA based regression analysis does, from the results of this study, predict total body water with a high degree of reliability and a relatively low degree of technique error. One of the primary reasons for this study was to understand the mechanisms of the BIA technique. It has been reported recently that there is a direct and strong relationship between total body water and body size. Although this may appear obvious, Hume and Weyers (1971) quantified the prediction of total body water from height and weight. Their study showed a linear relationship between total body water and height and weight with significant correlation coefficients of R = 0.711 and R = 0.920 in males and R = 0.770 and R = 0.913 in females for height and weight respectively. Hoffer *et al.* (1969) also reported a strong correlation for the relationship between weight and total body water (R = 0.83) and between height and total body water (R = 0.58). Houtkooper *et al.* (1989) found a similar relationship between weight and total body water (R = 0.88), however, this study did not report the relationship between height and total body water. In a limited study of 40 obese and normal subjects, Kushner & Schoeller (1986) found strong correlations between weight and total body water (R = 0.86, SEE = 5.23) and a lesser correlation between height and total body water (R = 0.65, SEE = 7.93). In assessing body composition in adolescents and young adults, Conlisk *et al.* (1992) found that the inclusion of impedance added no significant value to simple anthropometry.

The method of BIA involves the inclusion of the square of height (supposedly as the conductor length), the whole body impedance measurement, and, as in three of the selected four equations used in this study, weight and other variables. This fact is causing concern regarding the issue of whether, in the BIA technique, weight and height, and not impedance, provide the predictive power for the assessment of total body water volume (Hergenroeder & Klish, 1990; Mazess, 1991a, 1991b; Grieve, 1993; Grieve & Henneberg, 1994). Furthermore, the added value of the BIA technique over simple anthropometry is also being questioned - Gregory *et al.* (1991) found no difference in either the predictive ability or the precision between the two methods. To determine the predictive ability of the many variables used in the BIA technique, a stepwise linear regression analysis was performed. This analysis provided the partial correlation coefficients of the relationship between the variables and total body water. Furthermore, the F-value of the variable inclusion in the regression equation was indicated. These values are reported in Table 3.8. It was interesting, yet hardly unexpected, that weight and height provide the majority of the predictive power with partial correlation coefficients of approximately $R = 0.75$ and $R = 0.60$ for weight and height respectively. The improvement of the predictive power of height by using the square of this variable was expected. Even sex accounted for a greater predictive capability than the variable under investigation - whole body impedance. Although impedance does not feature prominently in the prediction equation, it still has a small, yet significant, relationship with total body water or a concept related to total body water volume. The measurement of body impedance explains less than 16% of the total variance in the measured total body water (Table 3.8), and less than 25% - 30% of the total variance in the indi-

cation of hydration status (Table 3.9). The inclusion of age in the prediction equation was not a principal component, which was to be expected considering the relatively limited age range of the sample.

It is evident, within the confines of this study, that it is largely the strength of the relationships between height and weight, and total body water volume that provides the predictive power of the BIA method. It is, however, also evident that bio-electrical impedance measurements of the body do provide an indication of body composition related to total body water. It was postulated in the introduction to this study that measurements of the impedance of the body are related to the composition of the fluid in the body, i.e., the concentrations of the electrolytes and ions present in the body water compartment. These ionised electrolytes are often related to the hydration status of the body. In an attempt to determine whether body impedance indicates the composition of the conduction compartment, the present study further investigated the relationship between these named hydration status indicators and various pre-defined body impedance measurements.

b) *Hydration Status and Body Impedance*

The first step in investigating the relationship between bio-impedance measurements and hydration status indicators was to perform a product-moment correlation analysis for the various parameters. Tables 3.9 and 3.10 show the results of this analysis. All the coefficients were found to be significant. Further, a stepwise linear regression analysis was performed to ascertain the strength of impedance measurements in predicting the body fluid index (total body water : weight). This

index is a reasonable indicator of acute hydration status (Schoeller, 1989). The partial correlation coefficients and F-values for inclusion into the equation, are shown in Table 3.11.

The results of these analyses showed that the measurement of blood impedance had the greatest relationship with hydration status indicators, in particular with total body water per body mass. This relationship was very strong (R = -0.97 and R = -0.96 for the dehydrated and rehydrated states respectively). A strong (negative) correlation was expected as the measurement of the impedance of blood is the most direct body impedance measurement with respect to hydration status. Similarly, the ionic composition of the blood also correlated strongly with the blood impedance measurement. This would suggest the premise that bio-impedance measurements are dependent on the ionic nature of the fluid in the body.

Of the actual body impedance measurements, whole body impedance showed a relatively modest relationship with the hydration status indicators (approximately 25% of the total variance). This modesty is probably due to various compounding factors such as the complexity and heterogeneity of the tissues involved in electrical conduction across this large distance between electrodes (Löfgren, 1951), and the effect of skin blood flow and temperature (Caton *et al.*, 1988; Smith, 1992).

The remaining four segmental body impedance measurements, viz. arm, leg, abdomen and back, indicate an interesting trend. The segmental sites of impedance measurement that had a relatively high subcutaneous fat deposition, or had underlying viscera (the back and the abdomen) showed a less strong relationship with the hydration indicators. This is to be expected due to the relatively inert electrical

nature of the underlying tissue. On the other hand, the arm and the leg segmental sites of impedance measurement, which have a relatively high proportion of skeletal muscle and blood supply as underlying tissue, indicated a substantial relationship with the hydration indicators. This is perhaps due to the high content of fluid, and particularly, electrolytes normally found in skeletal muscle (Cooley & Lehr, 1972; Epstein & Foster, 1983; Pethig, 1987). Thus , it was seen that two groups of similar segmental impedance exist, the arm and the leg, and the abdomen and back. The abdomen-back group measurements were across a larger distance, but showed a decreased Z value when compared to the arm-leg group measurements. This may initially be interpreted to be due to a volume dependence of body impedance, i.e., an increased cross-sectional area results in a decreased Z. Another possible explanation to this phenomenon is the influence of tissue composition of the measured area. The back-abdomen area has a high fluid content and a low bone and connective tissue content. This could explain the decreased impedance. Furthermore, although the arm-leg areas have a decreased volume (smaller cross-sectional area), the tissue composition is of a higher density, with increased bone and connective tissue composition. Again, this could explain the increased impedance. Taken in context with the other findings of this study, of the two explanations, the latter is more probable.

The small and insignificant changes of relationship strength in the measurements of the dehydrated and the rehydrated states suggest that there is a compounding factor relative to impedance measurements. The manipulation of the hydration states used in this study would largely affect the extracellular fluid (ECF) more than the intracellular fluid (ICF) volume and concentration. It has been shown that

the ratio of the ICF and ECF distribution has some influence on the electrical impedance measurements of the body (Deurenberg *et al.*, 1989b; de Vries *et al.*, 1989). By changing the distribution of the fluid and electrolyte concentrations of the ECF and the ICF, the impedance of the blood and the body may have been affected - this would explain the small changes in the predictive capacity of specific impedance measurements for the two hydration states studied.

Again, these results suggest a dependence of the impedance measurements of the body on the fluid and electrolyte concentrations and patterns of distribution, rather than on pure volumes of body compartments.

The change in the extracellular and intracellular fluid and ionic distributions may affect the changes in measured body impedance in the dehydrated and the rehydrated states. To assess the degree with which impedance measurements predict the change in the hydration state was the next logical step.

c) *Prediction of Total Body Water Changes*

Again, the relationship between the changes in hydration status and the impedance measurements was assessed by straightforward product-moment correlation analysis (shown in Table 3.12). The results were very similar to the results of the direct impedance and fluid and electrolyte concentration measurements (see above). The association of blood, arm and leg impedance with hydration status indicators was high, with abdomen and back impedance slightly lower. Whole body impedance showed a reduced, yet significant association with hydration status change (Table 3.12).

Although it was not the purpose of this study to develop prediction equations for the hydration level of the body, an interesting exercise was performed, more to indicate the relationships between body impedance measurements and the changing status of body hydration. A stepwise linear regression analysis was performed using changing impedance measurements to indicate the changing hydration of the body (results shown in Table 3.13). Subsequently, six multiple regression models were developed using the more powerful predictive impedance values. These models provide an assessment of changing total body water, ionic concentrations and osmolarity. Figure 3.7 shows the graphical representation of these models. Although the volume of water ingested for rehydration was not controlled, the subjects generally drank 1-1.5*l*. The higher values indicated in Figure 3.7 may be either measurement artefact, or a result of bulk fluid shift into the ECF. The uncontrolled water ingested volume would also account for the relatively high SEE for the prediction equation of TBW change. Due to the limited nature of this study regarding the sample selected and the uncontrolled nature of the changing hydration status, it is neither assumed nor suggested that these models be used further. What can be seen from the results of this regression analysis is that the changes in total body water volume, Na^+ and K^+ concentration, and the osmolarity are the parameters best predicted by the different impedance measurements (R^2 = 0.86, 0.89, 0.81 and 0.85 respectively). Cl^-, again, is not as strongly predicted (R^2 = 0.75). This may be explained by the relatively small involvement of Cl^- in hydration indicators.

3.4.4. Conclusions

Although the statistical analyses of the results obtained in this study are somewhat extended beyond the confines of the original question posed, they all aid in the clearer understanding of what impedance of the body really indicates. Essentially, two main conclusions may be drawn from the results of this main study:

1. The measurement of impedance in the BIA technique is a very poor predictor of total body water. The success of BIA in predicting total body water relies heavily on the included anthropometric measurements. The emphasis on impedance measurements seems to be inappropriate.
2. The bio-impedance measurements are not indicators of body fluid volume, but are rather influenced by the fluid and electrolyte concentrations of the fluid compartment. It is the fluid and ionic concentrations that provide the electro-conductive faculty which is indicated by the measurement of biological impedance. It also seems that there is an influence of the ECF and ICF distributions of fluid and electrolytes on these impedance measurements.

CHAPTER 4

FURTHER INVESTIGATIONS

From the results of the main study, and from very recent literature regarding the impedance measurements of the body, further studies were contemplated and performed. The main study provided strong evidence that the impedance measurements of the body were highly reliant on the fluid and electrolyte concentrations of the body fluids. It is postulated that these fluids are the conductive compartment of the body, and manipulation of the ion concentrations in this compartment will influence the electrical conduction and impedance of an injected current. The frequency of the injected AC current has been said to influence the path of conduction (Ackmann & Seitz, 1984; Baker, 1989; Chumlea & Baumgartner, 1990). Theoretically, current of lower frequencies does not penetrate the cell membrane due to capacitance of the membrane and is conducted in the extracellular fluid space (Rush *et al.*, 1963). Higher frequency currents overcome the cell capacitance and penetrate the membrane, therefore being conducted in both the extracellular and the intracellular fluid spaces (Nyboer, 1959).

These issues led to certain questions being asked regarding the impedance measurement of the body. After showing that the less complicated single-frequency measurements performed in the main study provided little support for the currently accepted theories, and these measurements supported the proposed

hypothesis, further consideration was given to multiple frequency current input. As most of the questions leading to further investigation involved the measurement of impedance of the body to an injected AC current at varying frequencies, an instrument was developed to measure bio-impedance at a relatively low frequency (1kHz), the normally considered frequency (50kHz) and a relatively higher frequency (100kHz). This range of frequencies was selected as lower frequencies than 1kHz cause problems in electrode lead polarisation, and the higher frequencies, beside being potentially harmful to the measured subject, cause problems in constant current generation (Epstein & Foster, 1983; Pethig, 1987). The relatively narrow range does result in the imposition of some limitation for further study. Issues involved with high and low frequency measurements include the problems of increased skin impedance at low frequencies, and the 'shell-effect' seen at high frequencies. The 'triple-frequency' instrument was developed and calibrated against known impedances, and was then used in an attempt to provide some solution to these issues. Due to the limitations, the study was of a preliminary nature.

A point of concern was the repeatability of impedance recordings from different impedance instruments. As such, it was decided to consider the strength of relationship and association between impedance recordings from different commercial instruments and the 'triple-frequency' instrument.

The first question that was considered was the effect of the ionic and fluid concentrations in blood on the measured electrical impedance at different frequencies. Due to obvious similarities in the investigation aims, the study protocol of Chapter 2.1 was used for this investigation.

The second question posed was due to an unexplained quirk of measurement seen during the measurement of whole body impedance in the main study. The impedance recording fluctuated markedly when the subject, who was lying in a supine position, moved any of the limbs, underwent deep, forced respiration, or isometrically contracted muscles in the thorax, abdomen or neck. It was thought that these movements would produce some ionic change or movement during the excitation and/or contraction of skeletal muscle. Possibly, these ionic changes influence the measurement of impedance of the body.

4.1) Triple-Frequency Impedance Instrument Development

This section describes the design of an instrument to measure the bio-impedance of a body or substance to an injected constant AC current. The main purpose of the design and subsequent construction of such an instrument was to enable impedance measurements at varying frequencies of AC current injection. In essence, this instrument was designed to convert a DC current to constant AC at the required frequency. This current is then passed to a pair of biological surface electrode leads, injecting the current into the measured body between two electrodes. The impedance detection is, in essence, a measurement of the voltage change, via two further electrodes, between the points of injected current. Applying Ohm's Law,

$$V = IR$$

showing the relationship between electrical current (I), voltage (V) and resistance (R), it was assumed that at a constant current, the value of the measured voltage would indicate the value of the resistance. Furthermore, it was also assumed that the reactance of the measured body approximated zero, allowing the resistance value to indicate the impedance value. It was necessary to amplify the current passed between the two current-injection points, and to reconvert this AC into a direct current (DC) value. The voltage of this DC was measured, and then was calibrated to impedance and displayed.

The discussion below provides further technical details regarding the design of this instrument. A diagrammatic representation of the description is provided in the block diagram shown in Figure 4.1. A complete illustration of the instrument

design is provided in Appendix 3. Also provided in Appendix 3 is a list of the technical parts used in this design and a more technical description of the electronic circuitry.

a) *Current Production*

The DC source, provided by two 9V PP-3 batteries, was converted into an effective alternating current, or root mean square alternating current (RMS), by means of Weim Bridge oscillator technology (Schwan & Sittel, 1953). This oscillator design is commonly used in DC-RMS conversion. At this point in the instrument design, a switching mechanism was included to select the required frequencies. The AC frequencies produced by the oscillator were 1kHz, 50kHz and 100kHz.

The produced RMS current was then set to a constant value of 800μA for all three frequencies. This setting of a constant current enabled the instrument to be calibrated against five different standard resistors of known resistance, i.e., 100Ω, 220Ω, 330Ω, 470Ω and 562Ω

This set, triple-frequency current was passed to the current lead output. Essentially, the current was passed between two electrode leads connected to skin electrodes attached to the body or substance being measured. The returning current was earthed at the level of the instrument.

b) *Impedance Detection*

The injected RMS alternating current of 800μA, at three frequencies, was detected by a pair of detection leads, which were attached to the body being measured at the same level as the injection leads. This tetrapolar arrangement was used to reduce the effects of electrode polarisation impedance at the low frequencies used (Schwan & Ferris, 1968; Epstein & Foster, 1983). For measurement purposes, the detected AC was amplified by means of differential instrumentation amplification, and passed to a standard RMS to DC converter. The converted DC current was then passed through the frequency switching mechanism to standard set resistors.

Because the current and the resistance values remain constant, the voltage measured at this point indicates the impedance of the body. Thus, it is at this level that the measured voltage across the two electrode-leads is converted to a value of estimated impedance of the body being assessed. This converted value of impedance is sent to a LED display, and indicated in Ω. The LED display is driven by a separate 9V PP-3 battery.

One area that did cause some concern was that the detection-lead cabling was passing current through it at a relatively high frequency. There is a tendency for the cabling to exhibit capacitative reactance at higher frequencies, thus the electrode-lead frequencies were buffered at the level of input.

c) *Miscellaneous*

A further instrumentation design, not shown in Figure 4.1, but shown in Appendix 3, included a LED lamp to indicate when the DC power supply was below a certain level (<7.6V). The system was adequately driven at all three frequencies at this level of power supply, and the low-level indicator lamp provided sufficient warning of an impending deleteriously low power supply.

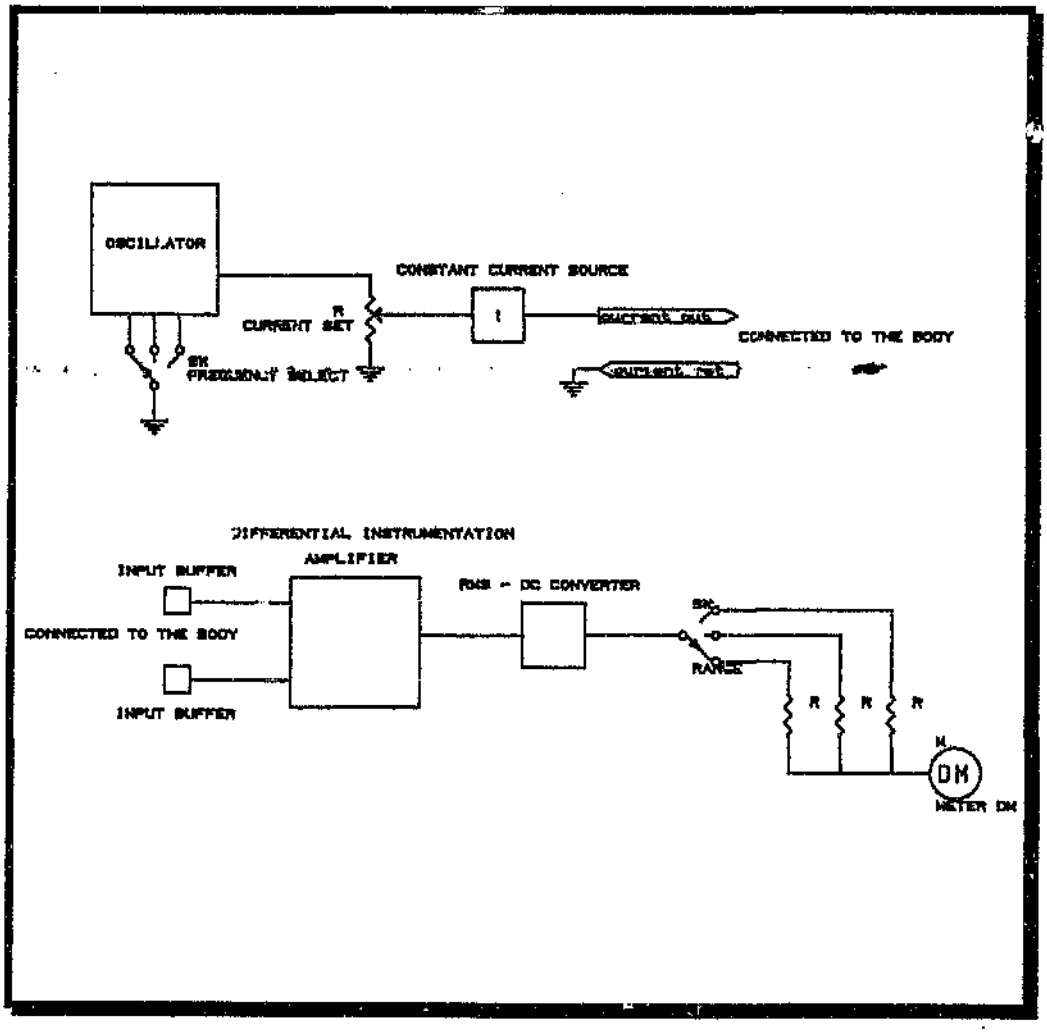


Figure 4.1: Block circuit diagram of impedance instrument

4.2) Triple-Frequency Impedance Instrument Validation

4.2.1. Introduction

Before any further investigation proceeded with the constructed impedance instrument, it was deemed necessary to directly validate and confirm the results obtained from this instrument. It was decided that the instrument should be validated by comparison with impedance results in both *in vitro* and *in vivo* situations. The primary purpose of this validation was not to assess whether the instrument could effectively and accurately predict body composition, but whether it could accurately measure electrical impedance. Therefore, as has been the general case in other published validation studies (Lukaski, 1987), no 'gold standard' was assumed, and the impedance values obtained were considered at face value.

The *in vitro* assessment was accomplished by comparing the impedance values obtained from the instrument with electronic resistors of known impedance. These resistors were arranged in such a fashion so as to provide a simple phantom model of the human body, with tetrapolar electrode placement.

The *in vivo* study involved a comparison of electrical impedance measurements performed by the constructed instrument and two other commercially available and accepted instruments. The impedance of the whole body was measured by the different instruments in a sample of 22 young, apparently healthy, adults, using described techniques of tetrapolar bio-electrical impedance measurement (Hoffer *et al.*, 1969). To reiterate, the commercial instruments were not considered as standards for validation assessment, rather as some basic reference point from which to

work.

There exist numerous commercially available impedance instruments. These instruments have all been used in studies that develop sample and population specific regression equations for the assessment of body composition by means of bioelectrical impedance analysis. There is certainly a lack of comparison between the results obtained from these different instruments, even though the results are considered compatible with all general regression equations. Graves *et al.* (1989) recently compared the results obtained from three different impedance analysers. They found a significant difference in the predicted body composition values when using the different instruments and suggested that caution be practised when attempting to generalise the impedance results from different instruments. Therefore, although not comprehensive, a comparison was performed to elucidate the differences, if any, in impedance values measured by the three instruments.

4.2.2. *In-vitro* Set Resistor Validation

In an attempt to determine the inherent accuracy and precision of the triple frequency instrument, the constructed instrument was tested against a range of resistors with known impedance values. Possible errors incurred from the measurement techniques were minimised by accurately measuring the resistors by means of oscilloscope instrumentation. This provided a reliable known impedance with which to compare the instrument's reported values of impedance.

a) *Validation Methods and Design*

After the accurate calibration of the triple-frequency instrument using the provided five set resistors of 100Ω, 220Ω, 330Ω, 470Ω and 562Ω, the accuracy of the instrument was assessed by comparing measured values of impedance, at different frequencies, with a range of established impedance values, provided by a constructed phantom model of the body. The construction of the phantom models. as shown in Figure 4.2, involves the electronic, *in vitro* emulation of a predetermined body impedance (Body Z). Furthermore, an attempt was made to account for the resistance to the injected current offered by the skin and skin electrodes. (Skin Z).

The placement and use of skin electrodes have been described as being the 'weak link' in biological electrical measurements (Gatzge, 1974). Although the use of a tetrapolar-electrode system does reduce the effects of contact impedance or skin-electrode impedance interactions (Hoffer *et al.*, 1969; Nyboer, 1970), the skin impedance does exert some influence on the impedance measurement (Gatzge,

1974). An electrical impedance to a current is exerted by the skin which is between each injector/detector pair of electrodes. This impedance is assumed to be a constant error in the measurement of bio-electrical impedance if

1. the distance between the injector and detector electrodes is constant, and
2. the specific resistivity of skin is assumed to be the same in all measured subjects.

The applications of commercial impedance instruments do recommend a constant distance of 5cm between the current injection and detection electrode placement. This distance, although constant, does present problems when it is necessary to place the electrodes in a small measurement area, e.g., in segmental body impedance measurement, and in the measurement of small children (Gartner *et al.*, 1991).

Most commercial instruments rely on the assumption that the skin possesses a constant specific resistivity. Although this is true within limits, a degree of normal biological variation does exist (Woodard & White, 1986; White *et al.*, 1991). Normal variation in skin resistivity results in a possible source of technique error. Furthermore, the effects that skin pathologies have on electrical impedance measurements have not been investigated.

To emulate the biological state, making the assumptions used in commercial instruments, a consistent design for the skin Z component of the model was used. In the construction of the phantom models, a set resistance, which approximates the skin resistance across 5cm, was placed between each injector/detector electrode pair. Furthermore, to reduce the effects of changing electrode and skin temperature, the validation measurements were all made at the same constant room tem-

perature (Smith, 1992).

Although the variable body resistors used in the phantom models are homogenous with regard to their electrical conduction properties, and do not exactly represent the heterogeneous nature of the biological system, the phantom models do provide some indication of the accuracy and precision of the instrument

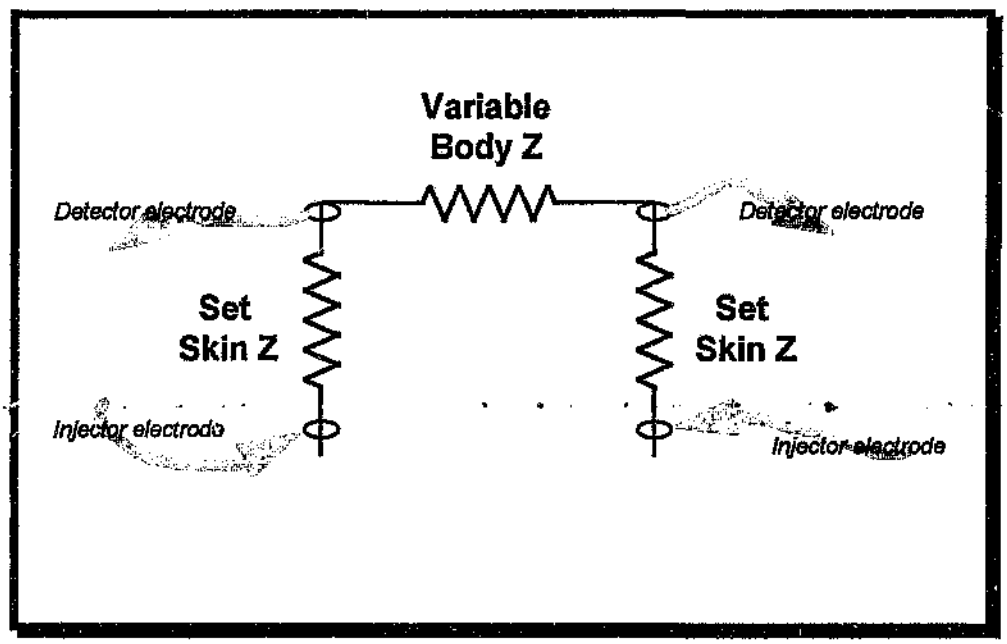


Figure 4.2: Graphic depiction of phantom impedance model of the body

b) *Validation Results*

Table 4.1 shows the known impedance values used in the phantom model, as well as the measured impedance (measured in duplicate, A and B) values obtained with the triple-frequency instrument, at all three frequencies. Assessed by means of a two-tailed *t*-test, no significant differences for $\alpha = 0.05$ were found between the duplicate measurements. Furthermore, for each of the impedance measurements performed, ANOVA showed no significant difference between the known impedance measurements of the phantom model and the recorded impedance measurements performed at the three frequencies of the instrument.

Table 4.1: Measured impedance of phantom model at three frequencies

	Z (1kHz)		Z (50kHz)		Z (100kHz)	
Phantom	**A***	**B***	**A**	**B**	**A**	**B**
40	40	40	40	40	39	40
80	80	80	80	80	79	79
120	119	120	119	120	120	119
160	160	159	160	160	160	159
200	200	200	200	200	199	199
240	239	240	239	240	239	239
280	280	279	280	280	279	280
320	320	320	320	320	319	320
360	360	360	360	359	359	359
400	400	399	400	399	400	399
440	440	439	440	440	440	440
480	479	479	480	479	479	479
520	519	519	520	519	519	520
560	559	559	560	560	560	559
600	599	600	599	599	599	600
640	639	640	639	639	640	640
680	680	679	680	680	679	679
720	720	719	719	720	719	719
760	759	760	759	759	759	759
800	799	799	800	799	800	799

*: measurements A and B are separate duplicate measurements performed on the phanton.

c) *Discussion and Conclusions*

It is evident from the data shown in Table 4.1 that the accuracy of the instrument assessed against known impedance values is very high for all frequencies used. At the higher impedance values, both the 1kHz (low) and the 100kHz (high) frequencies exhibited slight variations of impedance measurement from the real value, but these variations were not significant, being $\pm 0.25\%$.

A second valuable point noted from the data is the high degree of repeatability. The duplicated measurements do not differ from one another in any significant way. This would indicate the instrument to be of an accurate and precise design, theoretically measuring the intended values.

4.2.3. Commercial Instrument Validation: *In vivo* considerations

The next step in assessing the validity of the developed instrument was to compare the impedance values obtained in a biological setting. A comparison was made between two commercially available and accepted electrical impedance instruments, and the developed triple-frequency instrument.

a) *Instruments and Methods of Validation*

Standard whole body impedance measurements were performed in 22 male and female volunteers between the ages of 17 and 22, using three instruments.

1. Holtain Body Composition Analyser - this instrument was used in the main study (Chapter 3).
2. Bodystat 500 Body Composition Analyser
3. Developed triple-frequency instrument

The whole body impedance measurements were performed following the described method of BIA (see Chapter 3.2.3. for details), measuring the body impedance between the right hand and the right foot of the supine subject (Hoffer *et al.*, 1969). The electrodes were placed in the predetermined anatomical sites, and were not removed or changed between measurements, therefore providing consistency of electrode placement for the different impedance measurements.

b) *Results of Validation Study*

Before performing the impedance measurements, the Holtain instrument was calibrated against the internal 400Ω resistor (the Bodystat had no provided calibration means). The study instrument also was calibrated, against the five resistors of known impedance, and as a matter of comparison, the impedance value was recorded by each of the instruments used for each of the five known resistors. Table 4.2 shows the values obtained from these measurements. Although this is not in biological setting, it does provide interesting comparative results.

Table 4.2: Impedance measurements of 5 known resistors at 50kHz

Resistors	Holtain	Bodystat	iple-Frequency
100Ω	128	94	100
220Ω	29?	201	220
330Ω	443	294	330
470Ω	633	418	469
562Ω	761	497	560

The results of the whole body impedance values measured at 50kHz in the 22 volunteers are presented in Table 4.3. Also shown are the volunteers' height and weight. ANOVA and two tailed *t*-tests show significant differences (α=0.05) between the means of the measurements provided by each of the three instruments. It was thought that, although significant differences existed among the three measurements obtained by the different instruments, there may exist some relationship

between the obtained values. Ratios of the values obtained by the two commercial instruments against the values obtained by the constructed instrument are also presented in Table 4.3. Interestingly, although the Holtain measures impedance values significantly higher than the 3-frequency instrument, this difference appears to be constant (a mean of 134% of the values recorded by the 3-frequency instrument, with a small standard deviation of 0.01). Alternatively, the Bodystat shows a smaller overall difference in impedance measurements when compared to the measurements of the 3-frequency instrument. This difference is, however, not consistent (on average, the values are 83% of the 3-frequency values, with a larger standard deviation of 0.11). For confirmation of these results, correlation coefficients were calculated, and the correlation matrix is given below:

	Holtain	**Bodystat**	**3-Freq.**
Holtain	1		
Bodystat	0.8230	1	
3-Freq.	0.9878	0.8190	1

The correlation coefficients confirm that there is a strong relationship between the values obtained by the Holtain and the values obtained by the constructed 3-frequency instrument. A weaker relationship exists for the other commercial instrument and the 3-frequency instrument. Likewise, an obvious similar relationship exists between the two commercial instruments.

Table 4.3: Biological whole body impedance measurements at 50kHz

Subj.	Ht (cm)	Wt (kg)	Holtain	Bodystat	3-Freq.	H/3F*	B/3F†
1	186.1	62.6	738	500	550	1.34	0.91
2	164.5	48.7	773	520	571	1.35	0.91
3	180.0	64.4	720	438	537	1.34	0.82
4	184.6	66.5	668	448	498	1.34	0.90
5	167.0	62.0	609	413	453	1.34	0.91
6	179.6	68.8	708	456	532	1.33	0.86
7	172.6	66.7	671	398	498	1.35	0.80
8	163.6	52.2	648	369	479	1.35	0.77
9	181.0	67.5	594	259	439	1.35	0.59
10	173.0	77.4	520	292	382	1.36	0.76
11	175.6	77.0	603	360	454	1.33	0.79
12	166.0	57.9	694	452	521	1.33	0.87
13	168.5	53.6	661	434	492	1.34	0.88
14	174.4	59.7	714	475	534	1.34	0.89
15	194.9	68.8	659	258	497	1.33	0.52
16	185.6	71.1	620	331	462	1.34	0.72
17	173.2	64.8	650	438	481	1.35	0.91
18	174.7	57.5	704	466	527	1.34	0.88
19	175.0	64.1	590	414	445	1.33	0.93
20	175.0	63.9	614	364	452	1.36	0.81
21	179.2	61.3	677	456	500	1.35	0.91
22	178.3	79.4	632	439	471	1.34	0.93
MEAN	175.9	63.6	657.6‡	404.7‡	489.8‡	1.34	0.83
STD	7.89	7.28	57.9	72.8	43.9	0.01	0.11

*: ratio of Holtain measurement to 3 frequency instrument measurement
†: ratio of Bodystat measurement to 3 frequency instrument measurement
‡: significantly different from each other ($\alpha = 0.05$), shown by ANOVA and *t*-test.

c) *Discussion and Conclusions*

It is evident from the data presented in Tables 4.2 and 4.3 that there is a significant difference between the measured values of biological impedance obtained by the three instruments. The purpose of this comparison was not to develop a commercially viable instrument, but to assess the technical standards used in commercial instruments. This study has no direct impact on the consideration of the main study hypothesis, but due to the fact that a commercial instrument (Holtain) was used in the main study, it was deemed advantageous to provide an *in vivo* comparison. Furthermore, the impact of different instruments on developed generally-applied prediction equations is a source of concern with regard to the BIA technique.

From the positive results obtained on the phantom model in Chapter 4.2.2, it can be assumed that the triple-frequency instrument does indicate the real value of impedance relatively well. How this translates to the biological situation cannot be concluded definitively from this study. The results presented do, however, tend to indicate that the triple-frequency constructed instrument does have a reasonable degree of accuracy and precision. These results include the high degree of repeatability, the relationship of the instrument's recordings with electronically set standards, the reasonable standard deviations and the consistent calibration settings.

The two commercial instruments exhibit a departure from accuracy and precision. When compared to the constructed instrument, the Holtain appears to be a precise instrument, but it seems to lack in accuracy. Even though the Holtain was calibrated against an internal set resistor, its impedance readings were consistently greater (1.34x) than the developed instrument. An explanation for this may be that

either the instrument design does not account for problems related to the injector and detector cabling not being shielded, or the skin contacts are not adequately electrically driven. The internal resistor of the Holtain is immune from these associated problems. Whwn questioning the suitability of using the Holtain in the main study, the high degree of precision and lack of accuracy was not considered a serious dilemma as the purpose of the main study was to consider relationships and not to develop prediction equations. The opposite holds true for the Bodystat, which is relatively accurate, but fails to exhibit any reasonable degree of precision. Although the manufacturer instructs the user to return the instrument for repairs if it is faulty, until the instrument was independently compared to external resistors, it was not known that the instrument was faulty. This had no significance to results obtained in the current studies, and only serves as a warning about the danger of cross applicability of regressed prediction equations.

It would be nothing less than pretentious to finally conclude from this limited study that the commercial instruments were inaccurate, unreliable or deficient in relation to the instrument that was developed and constructed for further investigation. Nevertheless, the results obtained do allow for a certain degree of confidence that the developed instrument is a reasonable assessment of the impedance of biological material to alternating electrical current. The developed instrument was accepted for the purposes of further investigation, allowing for and recognising its limitations.

Of real concern is the disparity between the instruments recorded values. In particular, the disparities between the commercial instruments are disconcerting. Impedance values obtained in the indirect technique of BIA from the two instru-

ments would result in large and significant differences in body composition values calculated. It is accepted that each manufacturer develops its own regression equations, but as previously published studies have shown, the prediction equations of body composition parameters, using BIA, are highly population specific (Jackson & Pollock, 1978; Katch & Katch 1980; Deurenberg *et al.*, 1990; Deurenberg *et al.*, 1991b; Wilson *et al.*, 1992). The problem created by the difference in instrument measurement values urgently needs to be addressed and further investigated.

4.3) Triple-Frequency Impedance Assessment of Blood

The positive results obtained in Chapter 2.1, which considered the relationships between blood impedance measurements made at a single frequency of 50kHz and the blood ionic composition, led to the further investigation of this relationship. A compounding issue that needed to be addressed was whether this relationship depended in any way on the frequency of the injected current. The reported study that the work in Chapter 2.1 is similar to (Deurenberg *et al.*, 1989a) uses simple single frequency impedance measurements of the blood samples. Thomasset (1962, 1963) does report findings of blood impedance measured at different frequencies, but does not fully consider the ionic composition of the measured fluid. Furthermore, Thomasset's research involves a two-electrode method, which reportedly causes electrode polarisation problems (Schwan, 1959; Tedner, 1978; Tedner & Lins, 1984; de Vries *et al.*, 1989).

The current interest and speculation regarding the value of multiple frequencies in body composition-type assessments (Segal *et al.*, 1991; Deurenberg & Schouten, 1992; Cornish *et al.*, 1993) led to the further investigation of the blood ion concentration : measured impedance relationship using the developed instrument (Chapter 4.1) operating at three frequencies, namely 1kHz, 50kHz and 100kHz. Other than the frequency difference, the intended purpose, methods used and statistical analyses were identical to those of Chapter 2.1.

4.3.1. Materials and Methods

Forty 10m*l* venous blood samples were collected by the South African Blood Transfusion Blood Transfusion Service, Hillbrow. The blood was collected by means of cubital fossa venipuncture, and was stored in heparinised collection tubes. This collection was completed within 2 days.

a) *Protocol*

Immediately on sampling, the blood was assessed for electrical impedance to a 800μA, variable frequency, alternating current. The frequencies used were 1kHz, 50kHz and 100kHz. The samples were then randomly divided into two groups and processed in the same manner as described earlier (Chapter 2.1). In summary, from the original 40 x 10m*l* samples, there were 40 x 5m*l* samples the same as the originals, 20 x 5m*l* samples with diluted NaCl solution added, and 20 x 5m*l* samples with distilled water added. Electrical impedance at all three frequencies was then reassessed on all 80 samples. To control blood temperature, all the samples were maintained at 36° C prior to the impedance measurement. Within 3-4 hours of the second blood impedance measurement, the samples were centrifuged for 10 minutes at 3000 rpm (Technospin *R*, Sorval Instruments). The blood plasma was removed from the centrifugate by means of disposable pipettes, and placed in storage vials. The plasma was then frozen at approximately -20° C.

b) *Blood Impedance and Ionic Measurements*

Within one week of sampling, the plasma samples were unfrozen, and were assessed for ionic concentrations of sodium, potassium and chloride, as well as plasma osmolarity, as described earlier (Chapter 2.1.1).

The impedance of the constant volume ($\pm$ 4m*l*) blood samples to a 800μA alternating current, at 1kHz, 50kHz and 100kHz, was measured by the specifically constructed impedance instrument (see Chapter 4.1).

Before measuring the blood impedance, the impedance analyser was calibrated, using set external calibration resistors of 100Ω, 220Ω, 330Ω, 470Ω and 562Ω. The power supply was also checked. The instrument then was connected to the blood sample, and an AC current was injected across the electrodes. The drop in voltage across the detector electrodes was measured in duplicate by the instrument, and proportionately displayed as the impedance, in Ω, to the injected current.

The statistical analyses performed on the data were similar to those performed on the data obtained in the above-mentioned preliminary study. In summary, the nature of the data was considered by assessing normalcy, the sample size, and the repeatability, reliability and validity of the measurement set. The relationship between the impedance values and the fluid and ionic concentrations was also considered.

4.3.2. Results

a) *Data Description*

A summary of the data is presented in Tables 4.4 and 4.5. Values presented in Table 4.4 indicate the mean, standard deviation (STD), and coefficient of variation (v) for the measurements of blood impedance and blood ionic composition for the unmanipulated data set, whilst Table 4.5 presents the same values for the two manipulated data groups, i.e., the sample group with NaCl added, and the sample group with distilled H_2O added. Both summary tables include the values of the mean of the duplicate measurements.

Table 4.4: Summary of data of unmanipulated blood measurements

	10m*l* sample (n=40)			5m*l* sample (n=40)		
	MEAN*	STD†	v (%)‡	MEAN	STD	v (%)
Blood Z (Ω)						
1 kHz	42.8	4.2	9.8	41.5	3.6	8.7
50 kHz	132.7	8.9	6.7	133.5	8.0	6.0
100 kHz	286.1	14.4	5.0	284.1	13.8	4.9
Na^+ (mmol.l^{-1})	141.5	5.6	4.0	142.5	5.8	4.1
K^+ (mmol.l^{-1})	4.6	0.4	8.7	4.6	0.5	10.9
Cl^- (mEq.l^{-1})	105.4	4.8	4.6	104.5	4.8	4.6
Osmolarity (mmol.kg^{-1})	288.4	6.3	2.2	290.4	6.5	2.2

*: measurement mean
†: measurement standard deviation
‡: coefficient of variation

Table 4.5: Summary of data of manipulated blood measurements

	NaCl added (n=20)			H_2O added (n=20)		
	MEAN*	STD†	v (%)‡	MEAN	STD	v (%)
Blood Z (Ω)						
1 kHz	35.1	3.9	11.1	45.5	3.2	7.0
50 kHz	113.4	8.5	7.5	145.4	8.9	6.1
100 kHz	241.0	15.5	6.4	308.7	15.5	5.0
Na^+ ($mmol.l^{-1}$)	167.0	6.0	3.6	131.6	7.0	5.3
K^+ ($mmol.l^{-1}$)	4.4	0.6	13.6	4.2	0.5	11.9
Cl^- ($mEq.l^{-1}$)	123.3	4.7	3.8	95.6	4.9	5.1
Osmolarity ($mmol.kg^{-1}$)	339.3	11.5	3.4	268.4	11.6	4.3

*: measurement mean
†: measurement standard deviation
‡: coefficient of variation

b) *Type of Distribution*

In view of the parametric nature of the statistical procedures performed on the collected data, it was considered prudent to assess the degree the sample set approximates a normal distribution. To compare the mean, median and mode of each of the variables measured, each of the three measures of central tendency were plotted. The result of this plot was very similar to the plots shown in Chapter 2.1, indicating no marked difference between the mean, median or mode of any of the variables measured. This would qualitatively indicate that the data collected are reasonably approximated by a normal probability distribution.

c) *Sample Size Adequacy*

For the measurements performed on the unmanipulated blood samples, the sample size of n = 40, for both the 10m*l* and the 5m*l* samples, was within the limits needed for a probability level of $\alpha \leq 0.05$. Also, at this 5% probability level, the sample size of n = 20 for the 5m*l* manipulated samples was also adequate, except for the measurements of plasma K^+ in the sample with NaCl added. At a probability level of $\alpha \leq 0.075$, the sample size of n = 20 was adequate for all measurements, including the NaCl sample measurements of K^+.

d) *Repeatability of Measurements*

All measurements obtained were performed in duplicate. This was done to assess the reliability and repeatability of the sampling techniques and measurement methods used in this study. Because only one investigator was responsible for the sampling and measurement of all data, it was not necessary to assess inter-observer reliability. To assess the amount of error variance, a calculation of the standard error of the measurements (SEM) was performed, and to assess the proportion of error variance in relation to true variance, a correlation analysis was performed, resulting in a variance (R^2) statistic. Table 4.6 indicates the SEM and R^2 values obtained for these two tests. The true variance was proportionately high in relation to error variance, ranging from $R^2 = 0.9915$ to $R^2 = 0.8920$ (i.e. 99.2% to 89.2%). This low proportion of error variance indicates that the measurements were reliable indications of the true values, and were repeatable.

Table 4.6: Reliability assessment of impedance and ionic measurements

	Z1	Z50	Z100	Na^+	K^+	Cl^-	Osmol
10ml - Plain							
R^2	0.9356	0.9785	0.9802	0.9213	0.9894	0.9843	0.9430
SEM	2.9488	3.3183	8.5406	11.3192	0.0675	2.1081	17.2038
5ml - Plain							
R^2	0.9420	0.9810	0.9765	0.9044	0.9915	0.9908	0.9328
SEM	2.4912	2.6710	8.8422	9.9756	0.0455	1.3445	18.3866
5ml - NaCl							
R^2	0.9209	0.9733	0.9688	0.8932	0.9862	0.9914	0.8920
SEM	2.8079	3.4109	8.4312	14.8498	0.0834	1.2329	26.5919
5ml - H_2O							
R^2	0.9148	0.9708	0.9696	0.8990	0.9899	0.9872	0.9064
SEM	3.3347	4.3781	7.7118	11.1899	0.0761	1.7202	22.1587

e) *Reliability and Validity of Blood Impedance Measurements*

The two unmanipulated samples (10m*l* and 5m*l*) were compared for significant differences in order to indicate whether two separate measurements of essentially the same sample were representative of each other. The means and standard deviations of the two sample groups were used to calculate the *t*-score, in a paired *t*-test, considering the difference between the two sample groups. Table 4.7 shows the calculated t-score for each of the variable pairs, as well as the probability level of significance.

Table 4.7: Results of 2-sample paired *t*-test between blood variables

	| t | - Score	Critical Value*	Probability†
Z1 - Z1	1.4119	2.0227	0.1619
Z50 - Z50	0.4345	2.0227	0.6651
Z100 - Z100	0.6312	2.0227	0.5298
Na^+ -Na^+	0.7929	2.0227	0.4302
K^+ -K^+	0.4504	2.0227	0.6536
Cl^- - Cl^-	0.8901	2.0227	0.3761
Osmolarity-Osmolarity	1.4149	2.0227	0.1615

*: critical value of | t | at $\alpha = 0.05$
†: probability that sample group difference is explained by chance alone.

It is evident from the results presented in Table 4.7 that, for $\alpha = 0.05$, no significant differences exist between the measurements of the 10ml plain sample and the 5ml plain sample.

f) *Data Analysis*

The associations between the blood impedance values, and the blood ionic composition indicators were investigated. The most obvious of these, the linear association, was considered. To assess the linearity between blood impedance and blood ions, Pearson product-moment correlation coefficients were performed calculated on the 10m*l* data, the 5m*l* unmanipulated data and the 5m*l* manipulated data. The resultant correlation coefficients (R) were assessed for significance for $\alpha = 0.05$. At this level of significance, for $n = 40$, and $n = 20$, all R values greater or equal to 0.3845 and 0.4440, respectively, are significantly different from $R = 0$. The method of assessing the correlation coefficient significance is described in detail in Appendix 2. Table 4.8 shows the correlation matrix of corrected correlation coefficients obtained.

Table 4.8a: Corrected correlations between unmanipulated blood impedance & ions* - 10ml sample

	Z1	Z50	Z100	Na^+	K^+	Cl^-	Osmol
Z1	1.00						
Z50	0.97	1.00					
Z100	0.99	0.97	1.00				
Na^+	-0.87	-0.76	-0.86	1.00			
K^+	-0.79	-0.69	-0.78	0.79	1.00		
Cl^-	-0.82	-0.73	-0.81	0.81	0.72	1.00	
Osmol.	-0.78	-0.69	-0.77	0.79	0.68	0.69	1.00

*: all corrected R values significant for $\alpha = 0.05$

Table 4.8b: Corrected correlations between unmanipulated blood impedance & ions* - 5ml sample

	Z1	Z50	Z100	Na^+	K^+	Cl^-	Osmol
Z1	1.00						
Z50	0.98	1.00					
Z100	0.95	0.97	1.00				
Na^+	-0.84	-0.85	-0.88	1.00			
K^+	-0.53	-0.61	-0.65	0.61	1.00		
Cl^-	-0.71	-0.73	-0.76	0.66	0.53	1.00	
Osmol.	-0.71	-0.73	-0.74	0.70	0.55	0.55	1.00

*: all corrected R values significant for $\alpha = 0.05$

Table 4.8c: Corrected correlations between manipulated blood impedance & ions* - NaCl added

	Z1	Z50	Z100	Na^+	K^+	Cl^-	Osmol
Z1	1.00						
Z50	0.98	1.00					
Z100	0.93	0.92	1.00				
Na^+	-0.76	-0.65	-0.75	1.00			
K^+	-0.59	-0.51	-0.63	0.75	1.00		
Cl^-	-0.78	-0.72	-0.88	0.78	0.70	1.00	
Osmol.	-0.86	-0.80	-0.79	0.84	0.72	0.73	1.00

*: all corrected R values significant for $\alpha = 0.05$

Table 4.8d: Corrected correlations between manipulated blood impedance & ions* - H_2O added

	Z1	Z50	Z100	Na^+	K^+	Cl^-	Osmol
Z1	1.00						
Z50	1.00	1.00					
Z100	0.86	0.83	1.00				
Na^+	-0.86	-0.86	-0.73	1.00			
K^+	-0.73	-0.76	-0.56	0.65	1.00		
Cl^-	-0.77	-0.79	-0.58	0.64	0.58	1.00	
Osmol.	-0.51	-0.51	-0.56	0.60	0.59	0.51	1.00

*: all corrected R values significant for $\alpha = 0.05$

4.3.3. Discussion

This study attempted to provide an indication of the effect current frequency has on impedance measurements. Problems associated with different frequencies, i.e., increased skin impedance at low frequencies and the possibility of a shell effect at high frequencies, are discussed at the beginning of this chapter (page 159). The results obtained in this study were very similar to those obtained in the previous study of this nature (Chapter 2.1). The obtained values were similar to those in the preliminary study, having relatively normal ionic concentration ranges for the unmanipulated data set. The manipulated data set does deviate significantly from the mean values of the unmanipulated blood. This was a desired response as a deviation from the normal ionic concentrations in either direction was necessary for the purposes of this study. The data collected were also found to be adequately normally distributed, of sufficient sample size, and repeatable, reliable and valid.

Again, as in the preliminary study, a significant linear relationship between the blood impedance measurements (at three frequencies), and the fluid and ionic concentrations of the unmanipulated and manipulated blood was found. An interesting result, which added to the validity of the impedance measurements of the blood, was the fact that the three impedance measurements correlated very strongly with each other ($R = 0.83$ to $R = 0.99$). This positive relationship did decrease slightly after manipulation of the blood sample. Also, in terms of reliability and validity, the different fluid and electrolyte concentrations were significantly related to each other ($R = 0.51$ to $R = 0.84$). Again, this relationship seemed to decrease with the addition of H_2O, and was slightly stronger with the addition of

NaCl - these changes were to be expected as the H_2O diluted the ionic concentrations and the NaCl added was an accurately measured, uniform amount.

For the manipulated and the unmanipulated samples, the relationship between the blood impedance and the ionic concentrations showed a strong negative correlation between the lower frequencies (1kHz and 50kHz) and the Na^+ and Cl^- ions and osmolarity (Table 4.8). A similar correlation was found between the higher frequency impedance measurement (100kHz) and the K^+ ion concentration. An exception to this relationship was the blood with H_2O added, which for the 100kHz frequency impedance measurement, the correlations were all relatively weaker. Likewise, the correlations were found to be slightly higher in the NaCl-manipulated blood - this, again, was to be expected. These correlations for the different frequencies of measurement were interesting in that they indicated a stronger relationship between the extracellular ions (Na^+ and Cl^-) and the lower frequencies and the intracellular ion (K^+) and the higher frequencies. The relationships found between frequency and ionic concentrations are probably based on the premise of the ability of ions to move as an electrical current. It is possible that the size of the ionic molecules would influence the degree of conduction specific ions exhibit.

With reference to solid-state conductor theories on which BIA is currently based, it would be expected that the impedance values at low frequencies are greater than those at higher frequencies ($Z_{low} > Z_{high}$). The results of blood impedance measurement indicate the opposite relationship ($Z_{high} > Z_{low}$). Impedance measurements of the whole body may be influenced by many factors such as skin impedance, electrode contact, conduction pathway, cell membrane capacitance and

the heterogeneous nature of tissue. Blood impedance measurements may show different relationship to the whole body as impedance may be influenced by the ability of ions to move. Ionic flux is an essential component of frequency-dependent conduction of an AC current. The cell membranes and fluid volume may provide no effect on conduction at different frequencies. As the frequencies used in this study were limited in terms of numbers and range, the ionic flux involvement may be premature speculation, but the results from this study do provide a preliminary indication that the solid-state conductor theory of bio-electrical conductance is erroneous.

In summary, the results of this study indicate a strong relationship between the electrical conductance and impedance of the body and the fluid and electrolyte nature of the total body water.

4.4) Muscle Activity Indication by Electrical Impedance

From the data obtained in the preceding studies, it was becoming more apparent that ionic involvement is an important factor in bio-electrical impedance measurements. *In vivo* empirical evidence regarding this issue is difficult to obtian. Impedance measurements should ideally made in a active physiological system such as during nerve conduction, muscle contraction, or ion movement across the gut wall or kidney epithelium. It was noticed during the data collection and measurement of the main study (Chapter 3) that an artefact in the whole body impedance measurement could be elicited. In particular, the impedance recording fluctuated significantly and immediately if the subject, lying in the supine position, began moving. This movement included any flexion or extension of the limbs and neck, isometric muscle contraction - as in the tensing of the neck; thoracic or abdominal muscles, and deep respiratory action.

The initial explanation for this was a possible change in the conductor length to change the impedance recording. This explanation was, however, rejected after observing the same changes with isometric contraction and deep respiratory actions - both of which do not involve any significant changes in conductor length. With the idea that Z is possibly influenced by ions and ionic flux, it was postulated that this movement-induced Z change was due to some electrical event involved in the contraction of muscle - in this case, voluntary skeletal muscle.

On consideration of previously published literature, it was found that the first study reporting electrical signal property change at sustained muscular contraction

was that of Piper (1912). These observations led to the development and application of the currently used technique of measuring muscle activity, namely electromyography, or EMG (Viitasalo & Komi, 1977; Hagberg, 1981; Kilbom *et al.*, 1983). Theoretically, with skeletal muscular contraction, motor unit action potentials are evident and are measured by surface electromyographic power spectra (Hägg, 1992). An increase in contraction will result in the increased recruitment of motor units with larger fibres and action potentials with higher velocities. Obviously various factors will influence the electrical activity of the contracting muscle, such as muscle lactate concentrations and changing pH during sustained contractions (Tesch *et al.*, 1983; Brody *et al.*, 1990). An obvious involvement of the electrical changes in contracting muscle is the direct effect of ionic changes across the nerve and muscle membrane, and the physiological mobilisation of Ca^{2+} during excitation-contraction coupling. It was thought that these increased electrical events could explain the changes in whole body electrical impedance measurements that were observed in the main study.

It was recently reported that the measurement of bio-electrical impedance is influenced by excitable tissue activity (Holder, 1989). A study was performed recently, describing the ability of electrical impedance measurements to predict the muscle and fat areas of the upper arm (Brown *et al.*, 1988). Although this study did not consider the effects of muscle contraction on impedance measurement, it did provide a methodological basis for the present study.

Due to the ease of measurement, the possibility of simple controlled muscle contraction against different loads, and that there is literature on the upper arm, the muscle group of choice was the anterior compartment of the arm (including biceps

brachii, brachialis and coracobrachialis). The problem of standardisation was thought to be of primary concern in assessing whether the electrical events during muscle contraction specifically influence the impedance recordings. Essentially, the electrical events of muscle contraction were desired, independent from the effects on impedance measurement of conductor length, and possibly volume. It was decided to separate the arm into two compartments - the fat compartment and the fat-free compartment (Jelliffe, 1966). The fat compartment is broadly considered to be the subcutaneous fat surrounding the arm - of which the thickness is easily assessed by standard skinfold thickness measurements, whilst the fat-free compartment includes bone, muscle and other non-adipose tissue (Trowbridge *et al.*, 1982; Campbell & Borrie, 1988; Reid *et al.*, 1992). To standardise for the differences in individual muscle masses and conductor lengths, the electrodes used in recording impedance, were placed at a predetermined length on the ventral surface of the upper arm, in the longitudinal plane of the anterior muscle compartment. This set length of the impedance measurement technique effectively standardises measurements for conductor length. The upper arm muscle area was calculated from arm circumferences and skinfold-thickness measurements (Heymsfield *et al.*, 1982). In order for the effects of impedance to be statistically investigated, the muscle area was controlled. In doing this, the effects of individual variation of muscle mass are negated. The electrical impedance to an injected current was then measured along the anterior muscle compartment during isotonic contraction against different loads. Impedance recordings were performed at three frequencies to assess whether the frequency of the injected current would play any role in the

determination. It has been reported that the frequency of the current, coupled with the orientation of the muscle fibres, does influence the electrical impedance recording (Epstein & Foster, 1983; Zheng *et al.*, 1984).

In summary, the effect of electrical activity of excitable tissue on impedance measurements was assessed by means of standardised electrical impedance measurement of skeletal muscle during controlled contraction.

4.4.1. Subjects, Materials and Methods

Fifteen apparently healthy volunteers, 9 male and 6 female, participated in this study, which was approved by the University of the Witwatersrand Committee for Research on Human Subjects - Medical (Protocol No. 16/7/91). The volunteers were all students or staff from the University, and were between the ages of 20 years and 28 years. Before commencing the study, all volunteers read and signed an informed consent declaration.

a) *Protocol*

After measuring the height and weight, and other anthropometric variables of each subject, bio-electrical impedance to a 800μA alternating current across the right anterior-arm muscle compartment was performed, using the instrument developed for this study. The impedance measurements were performed whilst the arm was passively extended, and whilst under isotonic contraction against different loads, i.e., 1kg, 2kg, 3.5kg, and 5kg.

b) *Anthropometry*

After measuring the subject's height and weight using the equipment and techniques described earlier (Chapter 3.2), the arm circumference (in mm) at the midpoint between the elbow and the shoulder was measured. Skinfold thickness measurements over the triceps and biceps brachii muscles were also performed.

c) *Muscle Impedance*

After swabbing the area clean with alcohol, a pair of silver silver-chloride disposable ECG skin electrodes (Promed, South Africa) were applied to the dorsal surface of the right arm, 2cm proximal to the cubital fossa. A second pair of skin electrodes was then applied to the site 15cm proximal to the first one (see Figure 4.3). Impedance measurements across this length were then performed whilst the subject was sitting in position in gymnasium equipment designed to isolate the action of the anterior-arm muscle compartment (arm flexion). The impedance of the injected alternating current was measured at frequencies of 1kHz, 50kHz and 100kHz. All three impedance measurements were performed on each subject whilst their arm was passively extended, and isotonically contracting and lifting increasing loads of 1kg, 2kg, 3.5kg and 5kg.

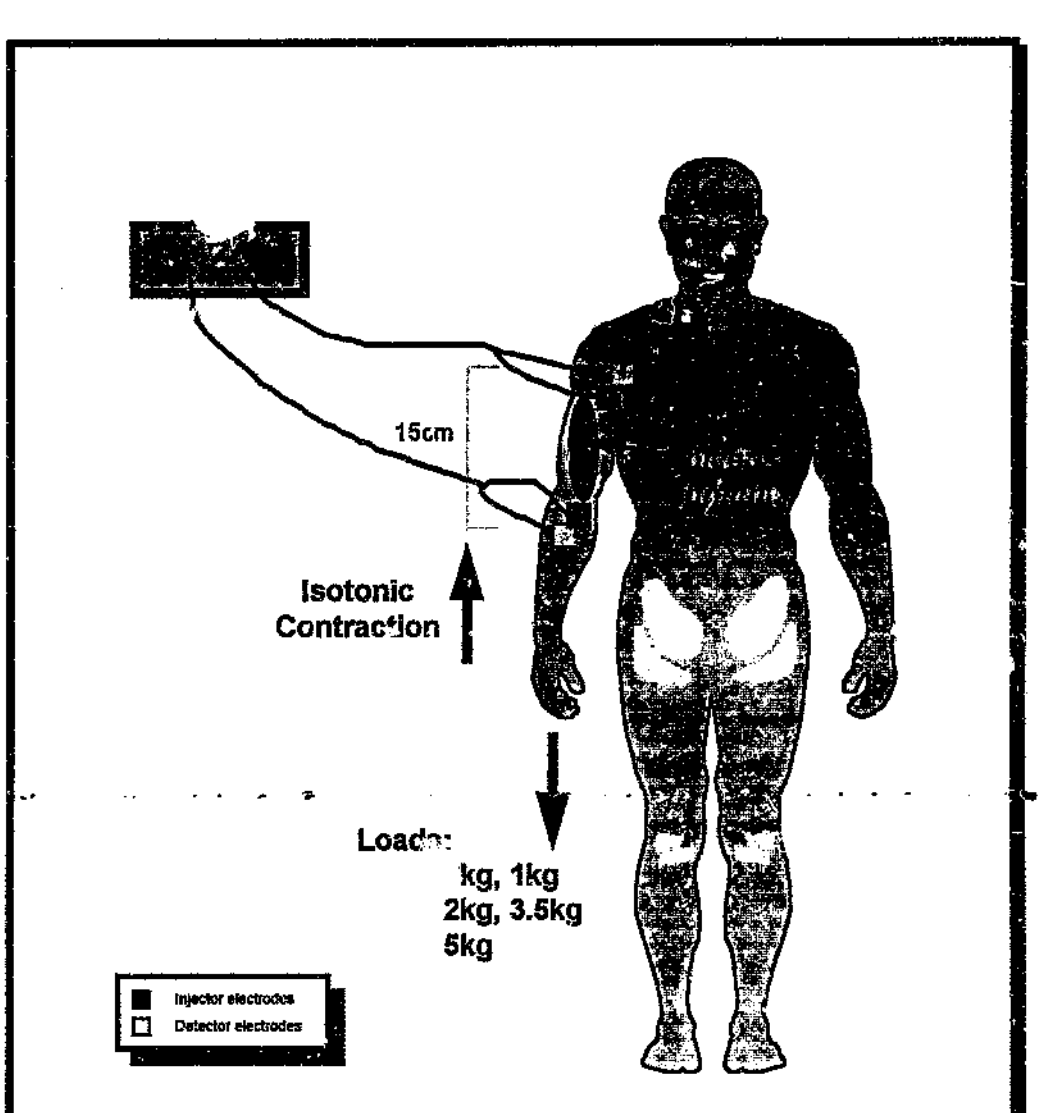


Figure 4.3: Placement of electrodes and isotonic contraction against various loads.

d) *Muscle Volume Standardisation*

Using the methods described by Heymsfield *et al.* (1982), an attempt was made to standardise the muscle volume in the 15 volunteers. From a measurement of the circumference of the mid-arm (C_{arm}), the mid-arm radius (R_{arm}) was calculated, using:

$$R_{arm} = \frac{C_{arm}}{2\pi} \quad \text{.....(4.1)}$$

A mean of the two skinfold thickness measurements, i.e., biceps (BCP) and triceps (TCP) indicates the radius of the fat layer surrounding the upper arm (R_{fat}). This R_{fat} was then subtracted from R_{arm} to indicate the radius of the muscle and bone compartments ($R_{fat\text{-}free}$). i.e , substituting equation 4.1,

$$\begin{aligned} R_{fat-free} &= R_{arm} - R_{fat} \\ &= \left(\frac{C_{arm}}{2\pi}\right) - \left(\frac{BCP + TCP}{2}\right) \end{aligned} \quad \text{.....(4.2)}$$

Assuming the bone mass to be relatively constant, and the length of the conductor (15cm) to be the same in all subjects, a muscle volume indicator was thus provided in the calculation of the mid-arm fat-free area (AMV):

$$\begin{aligned} AMV &= \pi(R_{fat-free})^2 \\ &= \pi \times \left[\left(\frac{C_{arm}}{2\pi}\right) - \left(\frac{BCP + TCP}{2}\right)\right]^2 \end{aligned} \quad \text{.....(4.3)}$$

e) *Statistical Analyses*

To provide an indication of whether the impedance measurements of the arm are related to load, a partial correlation analysis was performed between impedance measurement and load lifted. The estimation of arm muscle volume was standardised to be constant for all subjects in this partial correlation analysis. The correlation coefficients obtained for this analysis were assessed for significance.

To indicate whether the correlation coefficient values obtained for either the 1kHz, or the 50kHz or the 100kHz measurements were significantly different from the other correlation coefficients, a statistical analysis for difference between correlation values was performed (see Appendix 2 for details of statistical analyses).

4.4.2. Results

a) *Data Description*

Table 4.9 presents the means and standard deviations of the summary data for the measurements of the volunteers. Table 4.10 presents the combined values and standard deviations of the duplicate impedance values obtained for the three frequencies, measured whilst the arm was passively extended, and whilst the arm was isotonically contracting against different loads.

Table 4.9: Summary data of subjects

	MEAN	STD	v (%)
Age (yrs)	24.1	2.3	9.54
Height (cm)	178.0	6.5	3.65
Weight (kg)	74.4	7.7	10.35
Biceps s.f. (mm)*	47.7	6.5	13.63
Triceps s.f. (mm)	92.0	7.7	8.37
Arm Circum. (mm)	27.6	6.0	21.74
Arm Muscle Vol.†	29.5	5.3	17.97

*: s.f. = skin folds
†: index calculated as described in the text

Table 4.10: Impedance values for different loads lifted

	Z1		Z50		Z100	
LOAD	**MEAN**	**STD**	**MEAN**	**STD**	**MEAN**	**STD**
passive	75.1	12.5	62.9	7.0	40.8	5.0
1 kg	67.2	7.0	55.0	9.4	37.0	8.0
2 kg	61.4	7.2	50.6	11.0	30.0	7.8
3.5 kg	57.7	8.5	45.0	10.0	28.9	7.2
5 kg	54.3	10.3	42.9	11.0	27.0	8.0

b) *Measurement Reliability*

The standard error of measurement and the correlation between the two measurements of muscle impedance were calculated to assess the amount and the proportion of the error variance respectively. Table 4.11 presents the values of the amount of variance (SEM), and the proportion of the error variance relative to true variance (R^2). From these statistics, it is evident that all measurements of muscle impedance are reliable and valid.

Table 4.11: Reliability scores for impedance and anthropometry measurements

	Measurement	R^2	SEM
	height	0.9998	0.1790
	weight	0.9999	0.0746
	biceps	0.9903	0.0478
	triceps	0.9915	0.0932
	arm circum.	0.9877	0.0292
passive			
	Z1	0.9879	1.0238
	Z50	0.9890	0.7834
	Z100	0.9694	0.4887
1 kg			
	Z1	0.9749	0.8126
	Z50	0.9902	0.7036
	Z100	0.9897	0.4504
2 kg			
	Z1	0.9799	0.7599
	Z50	0.9856	0.6385
	Z100	0.9901	0.3561
3.5 kg			
	Z1	0.9769	0.6820
	Z50	0.9895	0.5753
	Z100	0.9860	0.3712
5 kg			
	Z1	0.9906	0.6904
	Z50	0.9833	0.6246
	Z100	0.9690	0.3995

c) *Statistical Analyses*

The results of the correlation coefficient calculations are presented in Table 4.12. Shown are the corrected correlation coefficients (R) between the loads lifted and the impedance values at the three frequencies. A partial correlation analysis, with arm muscle volume considered constant, was also performed, and the correlation coefficients (R_p) for this analysis are also presented. All corrected correlation and partial correlation coefficients are significant for $\alpha = 0.05$

Table 4.12: Corrected correlation and partial correlation analysis of load and impedance

	Load	Z1	Z50	Z100
Load*	1.0000			
Z1†	-0.6060	1.0000		
Z50‡	-0.5817	0.9011	1.0000	
Z100§	-0.5551	0.8977	0.8909	1.0000
Load (R_p)**		-0.8103	-0.7846	-0.7276

*: load lifted
†: impedance at 1kHz
‡: impedance at 50kHz
§: Impedance at 100kHz
**: partial correlation coefficient, with arm volume constant

To assess the type of relationship of the muscle impedance with the load lifted, a regression analysis was performed, considering both a linear and an exponential model. Table 4.13 gives the results obtained for both models. From these results, it appears that the relationship is not entirely linear.

Table 4.13: Regression of impedance on load in linear and exponential models

		Y = a + bX	Y = exp(a + bX)
Z (1kHz)			
	Intercept	72.2654	4.2753
	Slope	-3.9629	-0.0633
	Correlation coeff.	-0.6060	-0.6417
	SEE*	9.3727	0.1364
	Rss†	6412.9214	1.3579
Z (50kHz)			
	Intercept	60.2115	4.0939
	Slope	-3.8864	-0.0789
	Correlation coeff.	-0.5817	-0.6405
	SEE	9.7911	0.1704
	Rss	6998.1191	2.1209
Z (100kHz)			
	Intercept	39.0621	3.6554
	Slope	-2.7466	-0.0876
	Correlation coeff.	-0.5551	-0.5912
	SEE	7.4160	0.2154
	Rss	4014.8022	3.3860

*: standard error of estimation
†: residual sum of squares

4.4.3. Discussion and Conclusions

The changes that were seen in the whole body impedance recording on movement of the body were quite possibly due to the involvement and influence of excitable tissue. As is the case in EMG recordings (Hägg, 1992) and in the impedance changes due to nervous tissue activity (Cole & Curtis, 1939; Holder, 1989), this study found that gross skeletal muscle contraction influenced electrical impedance recordings. For all three frequencies used, the impedance measurement across the anterior-arm muscle compartment decreased with an increasing load of contraction. Table 4.12 shows the correlations between the load and the impedance values. Two more interesting points to note from this correlation analysis are the high degree of positive association between the impedance values at the three frequencies (R = 0.89 to R = 0.90), and the strong negative partial correlation between the load lifted and the impedance values with the arm muscle volume held constant (R = -0.73 to R = -0.81). The fact that the latter correlation is negative substantiates the theory that on muscular contraction a high concentration of ions are liberated and cause an increase in conductivity with a concomitant decrease in electrical impedance. The liberation of the ions is probably due to Na^{+} ions exiting neural and muscular cells during action potentials and due to Ca^{2+} ions being mobilised from the sarcoplasmic reticulum during muscle contraction.

The regression analysis showed an interesting result in that both linear and exponential models produced a high predictive ability for load lifted using impedance at three frequencies (R = -0.56 to R = -0.64). The exponential model does provide a better indication of load lifted when compared with the linear model. This is

possibly justified by the fact that neurone recruitment and muscle fibre involvement are not linearly related to the strength of contraction (Guyton, 1986). It is also interesting that the load predicted by impedance is more accurate for the low frequency of 1kHz, and diminishes with an increase in frequency (from R = -0.81 to R = -0.73). This may be due to the lack of difference between extracellular and intracellular fluids caused by parallel and longitudinal arrangement of the muscle fibres. This would mean that the capacitance effect of cells that was observed in Chapter 4.3 would be reduced and there would be a lesser tendency of the higher frequencies to provide better ionic indication. The anatomic volume of the muscle is unchanged during contraction, and in this study, the different upper arm volume of the volunteers was statistically kept constant. Volume therefore has no influence on the interpretation of the results obtained in this study.

In summary, this limited study would indicate that the impedance of the body, whole or segmental, is influenced by the electrical activity of excitable tissue. The ionic nature of this electrical activity would tend to support the view that the impedance measurements of the body depend on the concentrations of the conducting media - the ionised electrolytes. The influence of muscle contraction on impedance measurements adds further substance to the hypothesis that electrical conduction is dependent on ionic concentration and flux, and not on conductor volume.

4.5) Summary of Further Investigations

The results of the investigations that were inspired by the findings of the main study further added to the understanding of the nature of electrical impedance measurements of the body.

The design, development and construction of a 'triple-frequency' impedance instrument were successfully accomplished. This instrument was favourably validated against known impedance electrical resistors, and performed satisfactorily when used in a biological setting. A concerning point was noticed in that a discrepancy exists between different commercial impedance instruments. This concern is highlighted by the fact that large numbers of studies report various specific prediction equations, and purport that further investigations may use these equations, with impedance values obtained from different instruments. The lack of standardisation is concerning to say the least.

The measurements of blood impedance in Chapter 4.3, at different current frequencies indicate similar results to those found in Chapter 2.1. Impedance of blood, at any of the frequencies used, is influenced by concentrations of the fluid and electrolytes of the blood. There did exist an effect due to changing frequency, which suggests that the lower frequencies indicate the extracellular fluid. Furthermore, the higher frequency current, able to overcome the capacitance of cell membranes, is conducted in both the extracellular and intracellular fluid compartments. This finding was further supported by the fact that specific ions in each compartment were reasonably indicated at the different frequencies used.

The activity of muscle during contraction was found to influence and affect impedance measurements. It was postulated that the ionic changes and movements during the excitation and contraction of skeletal muscle influenced impedance recordings. The frequency of the measurement did have an effect on the conduction and impedance of the injected current.

From these results, further proof exists that the ionic nature and concentration of the fluids of the body are significantly involved in electrical impedance measurement. Furthermore, impedance is a poor indicator of any body fluid "volume".

CHAPTER 5

DISCUSSION AND CONCLUSION

Numerous studies, including the main study in Chapter 3, three pilot studies reported in Chapter 2, and three further investigations reported in Chapter 4, attempt to answer the original question proposed: "what aspect of the composition of the body do electrical impedance measurements indicate?"

The validity of using the various selected methods and instruments to measure total body water, ionic concentration, osmolarity, whole body impedance, segmental body impedance and blood impedance was assessed in the pilot studies performed (Chapter 2). The results obtained showed that all techniques, methods and instruments provided valid and reliable measurements, and were acceptable for this study.

The validity and accuracy of commercial impedance instruments were investigated in Chapter 4. Disconcertingly, large significant differences were found between two commercial instruments and a specifically constructed instrument. The results of this investigation support the views expressed by other investigators that the impedance results obtained from different instruments are not interchangeable (Graves *et al.*, 1989; Newby *et al.*, 1990).

The theoretical basis of measuring the electrical impedance of body tissue, in this case blood, to indicate the ionic composition of that tissue was investigated on a preliminary basis. Chapter 2.1 reports the results of impedance measurements performed on blood of different ionic concentration. The results indicated that a good relationship existed between blood impedance measurements and measured body solvent and electrolyte concentrations. This would tend to support the hypothesis that the electrical impedance of the body is determined by the electrolyte concentrations. These electrolytes in solution in the total body water comprise the conductive compartment of the body.

The proposed hypothesis that the electrical impedance of the body depends on the conduction compartment alone, was assessed in the main study (Chapter 3). The results obtained in this study support this hypothesis.

As tentatively predicted, the inclusion of impedance in the BIA technique does not improve body composition assessments significantly. It appears that the anthropometric variables used in BIA adequately provide the largest proportion of the predictive strength in body composition assessment. This phenomenon regarding impedance measurements is supported by other studies. These studies have also questioned the need for impedance to predict aspects of body composition such as fat mass, fat-free mass, and total body water (Campos *et al.*, 1989; Houtkooper *et al.*, 1989; Hergenroeder & Klish, 1990; Deurenberg *et al.*, 1991; Gregory *et al.*, 1991; Mazess, 1991a, 1991b; Forbes *et al.*, 1992). The results of Chapter 3 show that the measurement of impedance, used in BIA, is a very poor

predictor of total body water. It seems that the success of BIA in predicting total body water relies heavily on anthropometric inclusions.

The results further show that whole-body impedance measurements are not good indicators of body fluid volume and hydration status. Rather, these measurements are influenced by the electrolytes in the conductive fluid compartment. The influence of muscle movement on impedance recordings supports this finding (Chapter 4.4). It appears that an increased involvement of ions in muscle contraction causes the impedance recordings to change. When the degree of muscle contraction increases, a greater number of ions associated with mechanisms of muscle contractility are released, and electrical impedance concomitantly decreases.

Although it was not adequately assessed in the studies performed, it appeared that ICF and ECF ionic distribution would affect the path of current though selected tissue. The frequency of the electrical alternating current used would seem to influence the impedance recordings (Deurenberg & Schouten, 1992).

In conclusion, this study shows that whole-body impedance measurements, used in the BIA technique to assess body composition, are pointless. The impedance of the body to an electrical current is influenced by the fluid compartment with its constituent ions. These results are not definitive, but only serve to raise further questions and point to areas of future research. A basic question that needs to be addressed in far more detail is whether the body acts as a true electrical solid-state conductor, or whether it should be considered in the light of an electrolyte solution. Perhaps the body exists as both, with specific tissue favouring one or the

other electrical models. The present technological advances in impedance recording instruments allow the recording of impedance to multiple frequency alternating currents. These instruments function as scanning frequency recorders, and may provide further information regarding the body impedance saga. The standardised segmentation of the body for impedance measurements may also provide an avenue for further research. There does seem to be an indication for the move towards a more holistic view of the body. The separation of the body into the fat and fat-free masses does led to broad generalisations that are misleading. The division of the body into five levels of functional compartments seems to be worth further investigation. Regarding the use of electrical impedance to assess characteristic of the body, simple impedance measurements must take precedence over the involved and ambiguous BIA technique.

Interest in the study of the body composition will provide sufficient motivation and momentum for the further assessment and development of the basic nature and subsequent application of electrical impedance characteristics of the human body.

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APPENDIX 1

BODY FLUID STUDY
MAIN STUDY : Data Sheet

Name:	A. Fiction			Study No:	008			Date:	20/6/1992
DOB:	21/8/1971	EG:	W	Sex:	F	Ht:	173.7	Wt:	66.6
Skinfold:	tcp 10.6			bcp 8.5		sscap 7.2		spil 10.4	

IMPEDANCE MEASUREMENTS			
	B. eline	Dehydrated	Rehydrated
total body	458	459	468
lateral calf (20cm)	131	132	138
forearm (15cm)	114	114	125
abdomen (15cm)	56	55	59
back (30cm)	65	66	65
blood	130	129	135

BLOOD ANALYSES				
	Sample	Baseline	Dehydrated	Rehyrdated
Tritium (DPM)	232477	36.50	780.42	762.18
TBW (l)			48.87	50.16
Osmolarity		290.8	291.2	282.7
Na^+		145.0	145.6	141.2
K^+		5.3	5.2	4.9
Cl^-		107.9	107.8	106.3
Haematocrit		0.42	0.41	0.36

APPENDIX 2

STATISTICAL TECHNIQUES

The following statistical tests were performed on the data collected. Most of the conventional statistical methods, e.g., Pearson correlation coefficient calculations, *t*-tests, Z-tests, etc., are described in most elementary statistics textbooks. The tests included below were those that are not commonly used, or are not regularly described in standard textbooks.

Coefficient of Variation

The coefficient of variation (v) was used to give an quantitative indication of the amount of standard deviation of the measurements performed.

$$v = \frac{MEAN}{STD} \times \frac{100}{1}$$

Size of Sampling Error

The sample sizes were assessed for statistical adequacy using the following test.

Where E = the percent of sampling error allowed, e.g., 5%,

α = level of significance

n = sample size

Z = Z distribution value,

$$E = Z_{\frac{\alpha}{2}}\left(\frac{STD}{\sqrt{n}}\right)$$

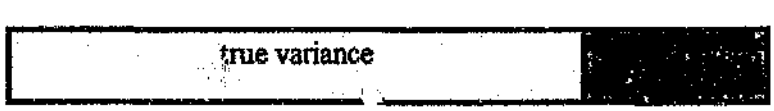
$$n = \frac{(Z_{\frac{\alpha}{2}})^2 \times STD^2}{E^2}$$

Reliability of Measurements (test-retest scores)

The amount and the proportion of the total variance which is error variance was used to assess the reliability of the duplicate measurements performed in these studies. The information used in this test is obtained from duplicate measurements which were performed.

Two measurements, a and b, will have a total variance of σ^2

This total variance is made up of true variance (σ_t^2) and error variance (σ_e^2)



Two indications of the reliability of measurement are therefore:

1. The amount of error variance, which is indicated by the standard error of measurement (SEM):

$$\sigma_{t_m} = \sqrt{\frac{\sum d^2}{n}}$$

where d is the difference between duplicate measurements, and

n is the pairs of duplicate measurements.

2. The proportion of error variance relative to total variance is taken to be the square of the correlation coefficient calculated for two duplicate measurements, i.e., R^2.

Correlation Coefficient Correction

Using the R^2 values calculated in the reliability studies, the correlation coefficients calculated in the different studies were corrected to take into account the technical errors.

$$R_{correct} = R_{xy} / (R_{xx}.R_{yy})^{0.5}$$

where x and y are the duplicate measurements

Correlation Coefficient Significance

The correlation coefficients calculated in the present studies were assessed for significance ($\alpha = 0.05$) using the following test.

Using H_0: $R = 0$,

and the *t*-statistic,

$$t_\alpha = \frac{R\sqrt{n-2}}{\sqrt{1-R^2}} \quad \text{or,}$$

$$R_{min} = \sqrt{\frac{t_\alpha^2}{n-2+t_\alpha^2}}$$

Differences Between Correlation Coefficients

Some correlation coefficients were compared to others, and the following test assessed whether their was any significant difference between the calculated coefficients.

H_0: $R_1 = R_2$ $\qquad$ H_1: $R_1 < R_2$

using the upper confidence limit of one correlation coefficient (R1) and the lower confidence limit of the other correlation coefficient (R2),

i.e., $\qquad R_1 + 1.96E_{R1} < R_2 - 1.96E_{R2} \qquad (1.96 = \alpha_{0.05})$

using $\qquad E_Z = 1 / (n-3)^{0.5}$ the R values need to be transformed

APPENDIX 3

3-FREQUENCY INSTRUMENT DESIGN

The body fluid analyzer can be divided into two distinct sections, the instrumentation for current injection, and the instrumentation for voltage detection. These are described below, and are shown in the circuit diagram below.

The first part of the circuit is made up of integrated circuits (IC's) U5 and U6. U5 and accompanying components make up a Weim bridge oscillator (Schwan & Sittel, 1953). This oscillator produces low-distortion sinusoidal signals. Resistance values R1 through R6 with C1, and resistance values R8 through R12 with C2 will set the required frequencies by ensuring that the voltage gain from the noninverting input to the op-amp is exactly 3. This gain needs to be controlled. This is accomplished by LP1 acting as a variable resistance. As the output level varies so the current through the lamp varies resulting in it either heating or cooling. The resulting voltage output is applied to U6.

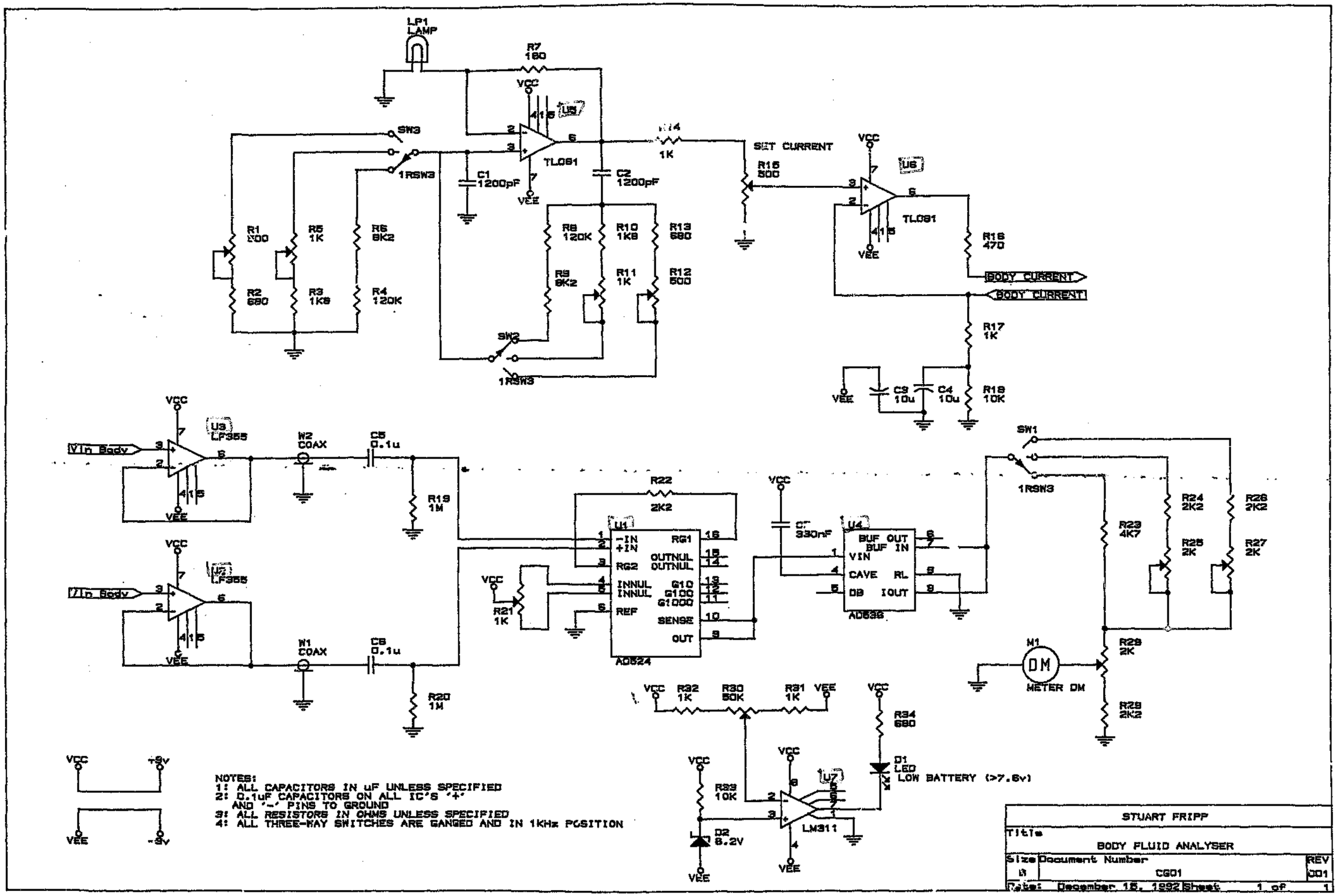
U6 and its accompanying components is a voltage-to-current converter. The voltage applied to the input of this op-amp is controlled by a variable resistor R15 which determines the output current. This current generated is injected into the body.

The second part of the circuit consists of U1, U2, U3, U4 and their accompanying components. The voltage that is detected across the body is applied to the inputs of op-amps U2 and U3. These inputs are of high impedance ensuring that no loading on the detected signal occurs. The output of these buffers are applied to the differential inputs of U1. This IC is a precision instrumentation amplifier with an extremely high common mode rejection. This is required so that any stray noise will not affect the detected signal. The differential signal is amplified by U1. The resulting output is applied to the RMS-to-DC converter IC U4. The function of this device is to convert the sinusoidal voltage to a DC signal which then drives a digital panel meter. This display indicates to the user the effective body impedance.

As the body fluid analyzer is a portable device it runs off batteries. Because of this a low battery indicator has been included. This is done around the voltage comparator U7.

List of Parts

Item	Quantity	Reference	Part
1	2	C1,C2	1200pF
2	2	C3,C4	10u
3	2	C5,C6	0.1u
4	1	C7	330nF
5	1	D1	LED
6	1	D2	6.2V
7	1	LP1	LAMP
8	1	M1	METER DM
9	3	R2,R13,R34	680
10	2	R3,R10	1K8
11	7	R5,R11,R14,R17,R21,R31, R32	1K
12	2	R6,R9	8K2
13	1	R7	180
14	3	R1,R12,R15	500
15	2	R4,R8	120K
16	1	R16	470
17	1	R18	10K
18	2	R19,R20	1M
19	4	R22,R24,R26,R29	2K2
20	1	R23	4K7
21	3	R25,R27,R28	2K
22	1	R30	50K
23	1	R33	10K
24	3	SW1,SW2,SW3	1RSW3
25	1	U1	AD524
26	2	U2,U3	LF355
27	1	U4	AD536
28	2	U5,U6	TL081
29	1	U7	LM311
30	2	W1,W2	COAX


LP1
LAMP
R7
180
VCC
U5
TL081
SW3
1RSW3
C1
1200pF
C2
1200pF
VEE
R1
500
R5
1K
R6
8K2
R2
680
R3
1K8
R4
120K
R8
120K
R10
1K8
R13
680
R9
8K2
R11
1K
R12
500
SW2
1RSW3
R14
1K
SET CURRENT
R15
500
VCC
U6
TL081
VEE
R16
470
BODY CURRENT
BODY CURRENT
R17
1K
C3
10u
C4
10u
VEE
R18
10K
VCC
U3
LF355
VIn Body
W2
COAX
C5
0.1u
R19
1M
VEE
VCC
U2
LF355
VIn Body
W1
COAX
C6
0.1u
R20
1M
VEE
R22
2K2
U1
AD524
-IN
+IN
RG1
RG2
OUTNUL
OUTNUL
INNUL
INNUL
G10
G100
G1000
REF
SENSE
OUT
VCC
R21
1K
VCC
C7
330nF
U4
AD536
BUF OUT
BUF IN
VIN
CAVE
RL
DB
IOUT
SW1
1RSW3
R23
4K7
R24
2K2
R25
2K
R26
2K2
R27
2K
M1
DM
METER DM
R29
2K
R28
2K2
VCC
R32
1K
R30
50K
R31
1K
VEE
R34
680
D1
LED
LOW BATTERY (>7.6v)
VCC
U7
LM311
R33
10K
D2
8.2V
VEE
VCC
+9v
VEE
-9v
NOTES:
1: ALL CAPACITORS IN uF UNLESS SPECIFIED
2: 0.1uF CAPACITORS ON ALL IC'S '+'
AND '-' PINS TO GROUND
3: ALL RESISTORS IN OHMS UNLESS SPECIFIED
4: ALL THREE-WAY SWITCHES ARE GANGED AND IN 1KHz POSITION
STUART FRIPP
Title
BODY FLUID ANALYSER
Size
A
Document Number
CG01
REV
001
Date: December 15, 1992 Sheet 1 of 1

APPENDIX 4

RESEARCH ETHICAL CLEARANCE

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 (Registry)

CLEARANCE CERTIFICATE **PROTOCOL NO:** 16/7/91

PROJECT: HYDRATION LEVELS OF THE BODY: THE VALIDITY OF USING BIOELECTRIC IMPEDANCE ANALYSIS AS A PREDICTOR OF BODY WATER LEVELS

INVESTIGATORS: MR C GRIEVE

DEPARTMENT: ANATOMY & HUMAN BIOLOGY, MEDICAL SCHOOL

DATE CONSIDERED: 26 JULY 1991

RECOMMENDATION OF THE COMMITTEE: APPROVED

Kindly advise how body impedance will be done.

Date: 1 AUGUST 1991 Chairman:.....
Prof P E Cleaton-Jones

* Guidelines for written "Informed Consent" attached where applicable.

DECLARATION OF INVESTIGATOR/S

To be completed in duplicate and **ONE** copy returned to Miss S M Boshoff at Room 10-002, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorised to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee.

Date:..... Signature:.....

Informed Consent

Dear volunteer,

Thank-you for volunteering to take part in this study. Please be aware that your participation in this study is TOTALLY voluntary, and you may withdraw from this study at any time you wish. Having said that, any non-participation in this study will NOT disadvantage you in any way at all.

The study is attempting to find out the potential uses of a Body Impedance Analyzer (BIA) in clinical, sporting and weight-management areas. It has been suggested that BIA measures the amount of water in the various parts of the body. Hopefully, our measurements will determine whether this is true or not.

You will be required to do the following:

1) Slightly dehydrate your body by sitting in a sauna for ± 30-40 minutes.
2) Ingest, by mouth, a mixture of slightly radioactive water and a chemical (NaBr). Please note, the radioactive water is NOT dangerous, having a lower intensity than a chest X-ray. Also, the NaBr, or sodium bromide is not toxic at all at the levels we are using it.

3) Your body impedance will then be measured using a BIA machine. This involves passing a tiny current (800μAmps, 50kHz), too small to even feel, though your body, and measuring the impedance.

4) After about 1½ hours after drinking the 'mixture', three small blood samples will be taken, one per hour, by an experienced, qualified medical technologist. These blood samples will be the total amount of discomfort you will be likely to experience

If you have any queries or problems at all, please contact me at 339-4366 (X321), or 647-2204.

Thank-you again,

Clayton Grieve BSc (Hons)
Department of Anatomy & Human Biology

I,_____, consent to my participation in the study being completed by Clayton Grieve. I have been informed as to the nature of the study, and of the requirements my participation will involve.

SIGNED:_____

DATE:_____

STRICTLY CONFIDENTIAL

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

ANIMAL ETHICS SCREENING COMMITTEE

CLEARANCE CERTIFICATE NO:	92	117	2b

APPLICANT: Mr C Grieve

DEPARTMENT: General Physiology

PROJECT TITLE: Measurement of total body water and extracellular fluid by chemical dilution and blood impedance.

SPECIES	NUMBER	DATE OF EXPIRY
Sprague-Dawley Rats	20	9 March 1995

The use of these animals is subject to AESC Guidelines for the use and care of animals, to the procedures specified in the application form, and to:

SIGNED _____ DATE 9 March 1993

(Chairman: Animal Ethics Screening Committee)

PLEASE SEE ATTACHMENT

Author: Grieve Clayton Lance.
Name of thesis: Hydration levels of the body as measured by bioelectrical impedance.

PUBLISHER:
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