Development of a Novel Device for Non-invasive Leg Fluid Volume Measurements

by

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A thesis submitted in conformity with the requirements for the degree of Master of Health Science, Clinical Engineering

Institute of Biomaterials and Biomedical Engineering

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Abstract

Bioelectrical Impedance Analysis is a non-invasive technique to measure fluid content of tissues. Based on Ohm’s law, tissue impedance is measured by injecting current into tissue and measuring the voltage. Since impedance is inversely proportional to fluid volume, this is used to estimate the fluid volume of the segment. BIA devices can be expensive and require gel electrodes, which are inconvenient and can irritate the skin. Furthermore, the typical method of measuring whole-body impedance is inaccurate due to the non-uniform current path across the body. Since fluid volume in the leg is representative of whole-body hydration, we propose a novel device that uses dry electrodes in a convenient sock format to measure fluid volume in the leg. Based on previous research of our lab, an integrated system was developed. Finally, in collaboration with an industrial partner producing smart textiles, the feasibility of dry electrodes for use in BIA was assessed.
Acknowledgements

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List of Abbreviations

DEXA - Dual Energy X-ray Absorptiometry
BIA – Bioelectrical Impedance Analysis
FM - Fat Mass
FFM - Fat Free Mass
TBW - Total Body Water
ECF - Extracellular Fluid
ICF - Intracellular Fluid
BIS – Bioelectrical Impedance Spectroscopy
AC - Alternating Current
NHANES - National Health and Nutrition Examination Survey
FAB - Fluid And Body composition software
A/D Converter – Analog to Digital Converter
ESPEN – European Society for Clinical Nutrition and Metabolism
Chapter 1

Summary

Measuring body composition is significant in the diagnosis, monitoring and management of fluid distribution and muscle and fat mass. Traditional methods of measuring body composition are timely and expensive, whereas bioimpedance of tissue offers a quick and cost effective alternative. The current bioimpedance measurement devices however, are not always user-friendly and often lack the ability to perform continuous measurements. Therefore, the objective of my research is to develop a device that is user-friendly and can continuously measure bioimpedance of the leg through a convenient electronic textile sock. To achieve this, first we performed a preliminary analysis on bioimpedance data of several hundred individuals and compared various bioimpedance regression models based on the gold standard method for measuring body composition. Consequently, we proposed a new model which has higher accuracy compared to the previous models proposed in the literature. Next, testing of textile embedded dry electrodes to assess their viability in a bioimpedance system was performed in collaboration with industry partner Myant. Finally, an integrated circuit was developed to perform the bioimpedance measures. The system, when implemented and validated in the clinical population will fill the need for a practical tool that is portable and easy to use to measure fluid volume in the leg. The proposed system can have a significant clinical role in monitoring edema and body composition in the leg.
Chapter 2

Literature Review

2.1 Introduction

The assessment of body composition is significant in the diagnosis, monitoring and management of various health outcomes such as obesity and fat distribution (1,2), edema and fluid distribution (3), muscle atrophy (4–6), fitness training assessment (7), nutritional status (8), and general health (9). In this context, assessment of body composition is important in both clinical and research settings. (10–12) Traditional methods of assessing body composition, such as dual energy X-ray absorptiometry (DEXA) (13), magnetic resonance imaging (14), computed tomography (15) and anthropometric measurements (16) are either expensive and time consuming, or are not accurate and prone to subjective measurement errors (10,13,14,17,18). Bioelectrical impedance analysis (BIA) is a relatively new technique of measuring body composition that is both cost- and time-efficient and accurate. (19)

BIA has developed to encompass a variety of methods including single-frequency BIA (20), multi-frequency BIA (10), and bioimpedance spectroscopy (21). Furthermore, BIA can be performed to assess body composition of the whole body (22) or segmentally (23). Two methods of determining body composition from BIA are discussed in this literature review. The first method utilizes regression models based on population studies to develop empirical equations, which employ the measured impedance and anthropometric features of the subject to estimate values of fat mass (FM), fat free mass (FFM) and fluid volumes. (20) The second method utilizes theoretical models considering the dimensions of the body conducting path and resistivity to estimate body composition. (23) The method used to determine body composition will play a significant role in the design of a bioimpedance device.
2.2 Body Composition

Body composition is comprised of fat mass (FM) and fat free mass (FFM). FFM is considered as everything that is not FM, including bone minerals and body cell mass, (12,18) and is comprised of intracellular mass and extracellular mass. (12,22) Total body water (TBW) is the measure of the volume of fluid inside the human body, which makes up 73% of the FFM. (17) It includes the blood plasma and interstitial fluid outside of cells making up the extracellular fluid (ECF), as well as the cytosol of the cellular mass making up the intracellular fluid (ICF). (12)

2.2.1 Techniques to Measure Body Composition

There is no agreed upon gold-standard for measuring body composition. However, several studies consider certain techniques to be more accurate and suitable for use as a ground-truth for measuring body composition. (24) These techniques include DEXA (10,13,17), magnetic resonance imaging (10,14), hydrostatic densitometry (10), radio-isotopic dilution (17,18), and computed tomography (10). These methods each have inherent limitations including: the assumption of a constant of 73.2% hydration of FFM, while this average can range from 68% to 81% (11,25); the use of harmful radiation (10,13,17,18); expensive equipment (10,13,14,17,18); long waiting periods (14,18); extensive set ups and the requirement of highly trained professionals to operate (14,18); and the inability to perform repeated measures over a short period of time. (17,18) Techniques considered as approximations include anthropometric measurements (11,12) and BIA (12,13,18,22,26,27). Anthropometric measurements lack the ability to differentiate changes in measurements due to changes in FM, muscle mass or fluid volume. (12) They are also prone to subjective measurement errors. (12) The BIA technique, which is the focus of this research, is reliable between users, and can be readily available. (19)

2.3 Bioimpedance Analysis (BIA)

BIA uses the electrical characteristics of tissue to estimate the body composition (12). Typically, skin surface electrodes are used to measure the electrical impedance of the underlying tissue. The principle is based on the inverse relationship between tissue resistance and its fluid content. (12)
Where the highly hydrated FFM is a good electrical conducting medium and the poorly hydrated FFM is mostly an electrical insulator. (13,26)

BIA offers many advantages in assessing body composition compared to alternative technologies in that it is portable (18,21,27) and non-invasive. (18,22,27) Furthermore, BIA does not emit ionizing radiation (10), and does not interfere with pacemakers or defibrillators (27). BIA is also simple, which eliminates the need for a highly trained technologist or radiologist, (10,18,27) and inexpensive when compared with other techniques such as magnetic resonance imaging or dual X-ray absorptiometry. (10,27) BIA is also fast and can be performed continuously in real time. (18,22) Finally, BIA is more precise than body mass index, anthropometric and skin fold methods (12) and offers trustworthy results, with prediction errors between 3-8% for FFM and TBW (12), reproducibility/precision between 2.7-4% (28), and coefficients of variation of 1-2% and 2-3.5% for same day and weekly measurements, respectively (19,28). As a result, BIA has been used as a reference method in measuring body composition. (12,18)

Despite the many advantages there are some limitations in BIA use and accuracy. First, the technique is not valid for subjects with body mass index over 34kg/m², due to the disproportionalities between body mass and body conductivity in the obese. (27) Another limitation is the dependence of electrolyte concentration and hydration level on tissue impedance. In healthy individuals the concentrations of electrolytes and body fluids are relatively constant making BIA suitable. However, the accuracy is compromised in a pathological population with electrolyte concentration and hydration levels outside the normal range, seen in conditions such as kidney, liver and cardiac disease, excessive edema and pregnancy. (22,27)

2.3.1 Principles of Bioimpedance Analysis

Tissue impedance is understood to be a result of the resistance of the ICF and ECF, and the capacitance due to the cell membranes. (11,18) The relationship between resistance and hydration status is explained by the mixing model theory, which assumes that the resistance of conductive fluids in tissue increases as the amount of non-conducting material suspended in the
fluid increases. Hence, resistance is inversely proportional to hydration. (18) The conventional and most commonly used electrical model for tissue includes a resistor ($R_{ECF}$) representing the ECF, in parallel with a resistor ($R_{ICF}$) representing the ICF, and a capacitor ($C_m$) representing the membrane capacitance, as seen in Figure 1a. (18) We can apply Ohm`s law to measure tissue impedance, by injecting a known value of current into a segment of tissue, record the resulting voltage across the tissue, and calculating the measured impedance. This is demonstrated in Figure 1b.

![Electrical Model of Tissue. Tissue Impedance Measurement](image)

Typically, the body is considered as a cylinder and the relationship between the measured resistance ($R$), the conducting resistivity ($\rho$), the length of the segment or the conducting path ($L$) and the conducting volume ($Vol$) is shown in equation 1. The derivation of equation 1 can be found in Appendix A.

$$Vol = \frac{\rho L^2}{R}$$ (1)

In the electrical model of tissue, when low frequency current is passed through the tissue, the cell membrane acts as a retarding force, and therefore the current only passes through the ECF (figure 2). At high frequencies the retarding effects of the membrane capacitance eventually decays to the point where it acts as a perfect conductor and the current effectively passes through both the ECF and ICF (figure 2). (10,11,17,18,22)
2.3.2 Whole Body and Segmental Analysis

Bioimpedance measurements can be performed across the whole body or segmentally. In the whole body method, skin electrodes are placed on a wrist and an ankle, such that the injected current passes through the “whole body”. (11–13,18) While whole body BIA is the simplest method for measuring body composition, it is limited by estimation errors. These errors occur due to the assumptions made about the length and cross-sectional area of the subject under measurement which assumes the body is a uniform cylinder. In reality, the body has a varying cross-sectional area, and the conductivity is not constant, as the current passes through different tissues throughout its path. (18) The resistance is largely confined to the limbs (arms and legs) with the trunk contributing as little as 10% to the whole body resistance while accounting for up to 50% of the mass. (18,26) As a result, alterations in anatomical and anthropometric features of the whole human body and segments, especially between individuals with similar heights, can cause large variations in estimated volumes. (12,18) This limitation is less of an issue in healthy subjects, as water distribution is usually well balanced throughout the body, and the resistance of any segment of the body is therefore representative of the whole body. (26)

In the segmental method, skin electrodes measure the impedance at specific segments of interest. This method is based on the shape and length of the conducting medium, where it can be used to measure tissue fluid volume of different body segments. (18) Of the BIA methods used,
Segmental BIA is considered to be the superior method in the estimation of body composition for two main reasons. (10) First, it provides information on tissue composition over smaller regions that might be of interest to the researcher. (12) Clinically segmental BIA has been shown to accurately measure ECF and ICF volume changes in patients undergoing peritoneal dialysis. (26) While whole body BIA was shown to be insensitive to these changes. (26) It has also been noted that for clinicians, it would be helpful to be able to separate the trunk into thoracic and abdominal compartments. (26) Second, it overcomes inconsistencies between impedance and the body mass of the trunk. (18) Variations in impedance in the whole body method can be due to a number of reasons, however, segmental BIA overcomes the differences in distribution of body compartments. (27) With that said, studies have shown relative errors as high as 13-17% and 10-13% of FFM for the arms and legs respectively. (18) However, most studies consider segmental BIA to be a valid and accurate tool in the assessment of body composition. (10,13)

2.3.3 Single Frequency Bioimpedance Analysis

Single-frequency whole body BIA is the typical method of body composition analysis using bioimpedance. Using a single frequency (generally 50 kHz, see Appendix A) to measure the tissue impedance, this method relies on empirical equations to calculate FFM, FM and TBW. (18) These equations are based on a ratio between the conducting length and recorded tissue impedance, and factors such as age, sex, race and other pathologies. (18) In clinical practice, while performing whole body analysis, it is easier to measure the subject’s height than their conducting length. Therefore, the empirical relationship typically relies on the measurement of $Height^2/Resistance$. (13,18)

In a review of BIA principles and methods by Kyle et al., 26 equations were compared. The results of this study found that equations 16 - 21 listed in Appendix B (Table 8) had the lowest standard error of the estimate (SEE) and can therefore be reliably implemented. (18)
2.3.4 Bioimpedance Spectroscopy

In bioimpedance spectroscopy (BIS), a broad “spectra” of frequencies are used. (11,12) BIS works on the principal that impedance at zero frequency ($R_0$) only measures ECF. While impedance at infinite frequency ($R_\infty$) measures both ECF together with ICF. (12) However due to limitations, it is not possible to physically measure the impedance at these frequencies. (12,17,18) To address this limitation, BIS takes advantage of the Cole-Cole model. The Cole-Cole model plots the resistance ($R$) against the negative reactance ($-X$) for a range of low to high frequencies (Figure 3). Using the resulting plot, the resistance at zero and infinite frequency can be extrapolated by observing that the locus of the resistance and reactance points form a circular arc, with $R_0$ and $R_\infty$ at the X-axis intercepts where reactance disappears at these frequencies, and center below this axis. (17,30) A popular BIS device on the market, the Impedimed SFB7, uses 256 frequencies between 5kHz and 1MHz to generate its Cole-Cole plot. (31)

To estimate body composition, an alternative to using empirical equations is to use the estimation of $R_0$ and $R_\infty$ from the Cole-Cole plot. By substituting $R_0$ into Equation 1, which theoretically represents $R_{ECF}$, we can calculate the ECF volume ($V_{ECF}$), as shown in Equation 2. Similarly, ICF volume ($V_{ICF}$) is estimated by assuming that $R_\infty$ is equal to $R_{ICF}$ in parallel with $R_{ECF}$ (Figure 1a). TBW is then computed as the sum of ECF and ICF. (5) Using this method we could describe $V_{ICF}$ and TBW using Equations 3 and 4 respectively.
\[ V_{ECF} = \rho_{ECF} \cdot \frac{L^2}{R_{ECF}} \]  
\[ V_{ICF} = \rho_{ICF} \cdot \frac{L^2}{R_{ICF}} \]  
\[ TBW = V_{ECF} + V_{ICF} \]

Resistivity differs between segments and sexes. Zhu et. al. found the segmental resistivities summarized in Table 1. (32)

<table>
<thead>
<tr>
<th></th>
<th>Women (Ωcm)</th>
<th>Men (Ωcm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \rho_{ECF} ) arm</td>
<td>67±14</td>
<td>67±8</td>
</tr>
<tr>
<td>( \rho_{ECF} ) trunk</td>
<td>172±21</td>
<td>159±24</td>
</tr>
<tr>
<td>( \rho_{ECF} ) leg</td>
<td>99±15</td>
<td>98±13</td>
</tr>
<tr>
<td>( \rho_{ICF} ) arm</td>
<td>191±36</td>
<td>194±29</td>
</tr>
<tr>
<td>( \rho_{ICF} ) trunk</td>
<td>266±42</td>
<td>250±38</td>
</tr>
<tr>
<td>( \rho_{ICF} ) leg</td>
<td>281±45</td>
<td>281±43</td>
</tr>
</tbody>
</table>

Since BIS utilizes the resistance values extrapolated to zero and infinite frequencies, it is considered to be more precise and accurate than BIA methods which rely on empirical relationships. (11,17,18) While few studies have tested this theory, one study found standard deviation of TBW of 6.3-6.9% and 5.7-5.9% for single frequency-BIA and BIS respectively. (33)

2.4 BIA Device Designs

2.4.1 Background

BIA devices share the common elements of their design; being an alternating current (AC) injecting source and a phase sensitive resistance measuring component (Figure 4). The phase sensitive resistance measuring component of the design is what typically differs between design methods as discussed below.
2.4.2 Quadrature Demodulation Method

The traditional technique for performing BIA involves using the quadrature demodulation method for phase sensitive resistance measuring (21,34,35). The quadrature demodulation method is based on the phase coherent demodulation of the measured signal and relies on two well matched channels consisting of an in-phase and a quadrature reference (34,35). The analog quadrature demodulation technique needs careful matching of the two channels, and small mismatches can cause large phase errors. A block diagram describing this method is shown in Figure 5. (21) The digital quadrature demodulation techniques do not require carefully matched in-phase and quadrature channels. However, this requires high speed analog to digital converters and digital signal processors, which can be expensive and have high power consumptions. (21) These traditional techniques are associated with high costs, high power consumption, and require large circuit board space and are not ideal for small portable devices. (21)

![Figure 5 - Block diagram of the analog quadrature demodulation method. I₀ is the sinusoidal AC current source; Vᵣ₀ is the in-phase (I) reference signal and Vᵣ₉₀ is the quadrature (Q) reference signal; IA is an instrumentation amplifier; M are multipliers (demodulators); LPF are low pass filters that are used to remove sidebands at 2ω₀; Zₐₐₑᵣ and Zᵢₐₚₐᵣᵣ are the real and imaginary components of the complex impedance of tissue; ADC are synchronized A/D converters. (21)]](image-url)
2.4.3 Gain-and-Phase Detector Method

A more recent method that is quickly gaining popularity is the gain and phase method (21). The Gain-and-Phase detector method measures the voltage drops across the tissue and the reference resistor, and outputs two voltages that corresponds to the magnitude ratio and phase difference between the two signals. As opposed to other BIA methods, the accuracy of the current source is not required to be perfect. This is because a reference resistor acts as a dynamic reference base for the Gain-and-Phase detector. This provides a reduction in circuit hardware and software, complexity, power consumption and cost for real-time measurements. (21) In Yang et al.’s study they designed and tested a single channel whole body BIS device using the gain and phase method. The major components of their device included a signal generator, a voltage controlled current source, two identical instrumentation amplifiers, a Gain-and-Phase detector, and a microcontroller system. (21) The design for this method is explained in further detail in the methods.

2.5 Existing BIA Devices

There is a range of commercial BIA devices; however they are expensive ranging from $2,500 up to $23,700. While these devices have a range of features, they typically lack in the ability to perform multi-segment BIA/BIS or continuous measurement of tissue composition, such as variations in ICF and ECF. The optimum device should be multi-segmental, multi-frequency, output body composition and raw impedance values, allow continuous output and be simple to operate. A summary of the available devices can be seen in Appendix C (Table 9). The SFB7 is a device that is commonly used as a reference in body composition measurements, which has all the desired specifications, however it lacks the ability to perform multi-segment analysis simultaneously and ability to measure tissue composition continuously. (31) As a result, the device can only be used on a single segment at a time, which makes tracking fluid shifts difficult and getting measures of multiple segments time consuming. All of the portable devices rely on traditional gel electrodes to measure resistance, as they provide excellent signal quality with proper application. However, these can be inconvenient for the measurement of fluid volumes
continuously as the conductive gel can dry over extended periods of time leading to signal quality degradation and the application can be uncomfortable or painful to users. (36)

2.6 Summary

Body composition is significant in the diagnosis, monitoring and management of various health outcomes. BIA is a portable, non-invasive and cost-effective method for measuring body composition. Segmental BIA gives improved resolution over whole-body methods and spectroscopy gives the added differentiation between intracellular and extracellular fluid. The gain-and-phase lock method design to measure bioimpedance is simple and effective to implement. Existing devices have a range of features, however they are either not portable or require the use of gel electrodes which can be uncomfortable and impractical to use repetitively. It would be beneficial to have a portable device with a convenient electrode configuration that can be quickly applied, can be comfortably worn for extended periods of time and is reusable for repeated and periodic measurements.
Chapter 3

Objectives

The aim of this study is to develop a portable, user-friendly and convenient device to measure bioimpedance spectroscopy to estimate intracellular and extracellular fluid volume of the leg. The device should be cost-effective and should be designed around the gain-and-phase detection method. Through an industrial collaboration with Myant, a company specializing in the development of electronic embedded textiles, we look to develop a smart sock that can be worn comfortably throughout the day to continuously monitor fluid volumes of the leg. It can be broken down into three main objectives:

3.1 Objective 1: BIA population validation study

The accuracy of whole-body BIA fat free mass measurements will be validated against the gold standard of body composition measurement, dual energy x-ray absorptiometry (DEXA), using a large population sample. Our own regression models will be created using BIA and anthropometric measurements, and demographic information. These models will be compared with existing regression models in literature. This will be used as a benchmark for accuracy of our device in estimating leg composition.

3.2 Objective 2: Dry electrode sock testing

Collaborating with industrial partner Myant, we will test a range of dry surface electrodes against a conventional gel electrode to assess their feasibility for use in a wearable sock for measuring leg fluid volumes continuously.

3.3 Objective 3: Device design and validation

To establish a prototype for a BIS device that will be validated through comparisons of impedance measurements in circuit models against those of an existing validated device, namely SFB7 (Impedimed, Australia).


Chapter 4

Objective 1: BIA population validation study

4.1 Rationale

As indicated by Kyle et. al., BIA regression models found in literature are typically formulated and validated on limited sample sizes and are targeted for specific populations. (18) Furthermore, choosing a superior regression model to use is complicated by the fact that the accuracy of models found in literature are often validated using different techniques, which can each have inherent limitations and biases, and varying sample sizes which could have different effects on the reported error. (18) Therefore it would be beneficial to be able to compare regression models found in literature fairly by comparing them on a common dataset and validation technique.

It has been suggested that the commonly used frequency of 50kHz is not scientifically based and arbitrary. (11) Furthermore, the relationship between impedance and body composition is based on anthropometric proportions which are often not taken into account other than height and weight. (18) Therefore, including impedance at multiple frequencies and additional anthropometric measurements could improve the accuracy of a BIA regression model. Furthermore, it would be clinically beneficial to have a single convenient equation that could be used for any healthy individual, without the need to find and select an equation specific to each individual. Therefore, we aim to establish an improved BIA based FFM regression model which employs additional variables.

Lastly, since whole-body BIA has found the measured impedance to be largely confined to the leg and arm with the trunk contributing as little as 10%, the regression models for whole body are closely representative of the leg and arm segments. However, this may not be true outside of a healthy population, where body fluid may not be uniformly distributed throughout the body. Therefore, to establish the accuracy of this objective as a benchmark for measuring leg composition, we need to limit the population to healthy individuals where body fluid is uniformly distributed though the body.
Our objective was to develop an improved model to estimate FFM in the general population using BIA measurements. This was achieved by creating our own regression models based on BIA, anthropometric measurements and demographic information obtained from the National Health and Nutrition Examination Survey (NHANES). The accuracy of the model was validated against DEXA and compared to existing models found in literature.

Previous authors have used the NHANES anthropometric data to generate body composition equations. However, to our knowledge only Stevens et al. has used the NHANES dataset to generate percent body fat equations which include bioimpedance measurements. In the study by Stevens et al. multiple equations are generated, however, the performance of their equations is not compared to others found in literature. To our knowledge FFM equations that include bioimpedance data have yet to be established on a large nationally representative population and compared against other equations found in literature. We hope to fill this gap with the following analysis.

4.2 Materials and Methods

4.2.1 Population Dataset

The National Health and Nutrition Examination Survey (NHANES) is an ongoing program of studies which assess the health and nutritional status of adults and children in the United States. The datasets of this survey are open source and freely available. Included are interviews, examination data and laboratory tests. Full details of the procedure NHANES followed are available. The particular datasets of interest to us include BIA, demographic, anthropometric and DEXA recordings.

4.2.2 Exclusion Criteria

The NHANES data is available in subsets of 2-year intervals. Three subsets were usable (1999-2000, 2001-2002, 2003-2004) as these were the only sets with both BIA and DEXA measures for body composition. The available laboratory, examination and survey data from NHANES was searched to identify relevant parameters. Subjects excluded from the study included: those
missing BIA or DEXA data, those found to have factors that are likely to affect BIA and DEXA readings, and those under 18 years of age.

Factors that were found to likely affect BIA and DEXA readings include kidney disease and received or receiving dialysis (42); congestive heart failure, coronary heart disease, congenital heart disease or a heart valve problem (27); cancer, anemia, emphysema, liver conditions, thyroid problems and rheumatoid arthritis. (27) Participants having any of these disorders were excluded from the analysis. It should also be mentioned that the DEXA dataset contains information for subjects who had partial or full data missing. This information was multiply imputed (replaced by estimated values). For accuracy in our study we excluded any subjects with imputed data. (43,44)

After applying the exclusion criteria, the number of subjects whose data was valid for use in the study was 5,254. The significant reduction in eligible subjects is primarily due to subjects with invalid and missing data (64.4% of total sample) and underage subjects (16.4% of total sample) as opposed to those with disease and medical conditions (2.3%). Figure 6 displays the exclusion process in further detail. BIA data was only included for subjects up to 49 years of age in the NHANES protocol. The subjects were split into men and women with sample sizes of 2,856 and 2,398, respectively. These were then split, using a random sampling function, into equal sized datasets for training and testing purposes.
Figure 6 - Exclusion Criteria Flow chart showing number of subjects.
4.2.3 Regression Modelling

The healthy adult population was partitioned based on sex, since preliminary analysis found sex to be a significant factor. This finding is consistent with the findings of Dittmar who showed that sex primarily influences variability in resistance. (45)

The datasets for men and women were then randomly partitioned into two roughly equal sized ‘training’ and ‘testing’ sets. The training set for each sex was used to create regression models for fat free mass. The testing set for each sex was then used to test the accuracy of the model created from the training set as well as the models found in literature.

While building the regression model DEXA Lean Body Mass was considered as the dependent variable. Independent variables included all anthropometric measures as well as resistance and reactance for a range of 7 selected frequencies (5kHz, 10kHz, 25kHz, 50kHz, 100kHz, 500kHz, and 1MHz). Height$^2$/Resistance ratios were also considered (for the same 7 frequencies), based on the relationship between length, resistance and volume.(18) A comprehensive list of variables considered can be seen in Appendix D.

The regression models for men and women were created using forward, backward, and stepwise regressions. The best performing model for each regression technique was chosen based on Akaike Information Criterion. For each regression, variables were removed one at a time if their corresponding p-value exceeded 0.001, starting with the variable with the largest p-value. This process was completed for each sex. Of the 3 regression models for each sex, the one with the lowest standard error and highest adjusted $R^2$ was chosen as the final model.

4.2.4 Model testing

Both the regression models created and the models found during literature review were compared using the test dataset. The root mean squared error, adjusted $R^2$, standard error, and Bland-Altman residuals of each model were found, using the DEXA Lean Body Mass as the dependent variable for comparison.
After determining the best models for men and women, three additional linear regression models were developed using the training data set with the following combination of variables. 1) By combining all the variables found in both the final models of men and women, 2) By including all variables except those related to bioimpedance, and 3) By using the variables from the top performing models found in literature (See Table 3). This was done to ensure the final regression models established were not performing better because of bias from being trained on a similar population representative of the testing set.

4.3 Results

4.3.1 Literature Search

A total of 16 male equations and 20 female equations for FFM were found during our literature review (see Appendix D). They are organized by gender and indexed using labels where ‘M’ is for male and ‘F’ is for female.

4.3.2 Population

The distribution of the subjects included in the study after the exclusion criteria can be seen in Table 2. The proportion of ethnicities is meant to represent a national US sample. The ethnicities reported were Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black and other race including multi-racial; and had sample sizes of 1,511, 249, 2,122, 1,148 and 224 respectively. The distribution of subjects for the training and testing subsets for men and women can be seen in Appendix E tables 12 – 15.

<table>
<thead>
<tr>
<th>Population Data</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>30.37</td>
<td>9.93</td>
<td>18 – 49</td>
</tr>
<tr>
<td>Weight</td>
<td>75.84</td>
<td>16.86</td>
<td>33.70 – 138.10</td>
</tr>
<tr>
<td>Height</td>
<td>169.00</td>
<td>9.72</td>
<td>133.00 – 193.90</td>
</tr>
<tr>
<td>BMI</td>
<td>26.49</td>
<td>5.27</td>
<td>15.16 – 48.10</td>
</tr>
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</table>
Sex

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>2856</td>
<td>2398</td>
</tr>
</tbody>
</table>

Race

<table>
<thead>
<tr>
<th></th>
<th>Mexican American</th>
<th>Other Hispanic</th>
<th>Non-Hispanic White</th>
<th>Non-Hispanic Black</th>
<th>Other Race Incl. Multi-racial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race</td>
<td>1511</td>
<td>249</td>
<td>2122</td>
<td>1148</td>
<td>224</td>
</tr>
</tbody>
</table>

4.3.3 Determined Equations

The final regression models developed for fat free mass appear below:

\[
Men = 0.703 + 0.174(ht) - 0.223\text{(triceps skinfold)} - 0.056\text{(sub skinfold)} - 0.181\text{(waist circ)} + 0.605\text{(wt)} + 0.127\text{(arm circ)} + 0.149\text{(arm length)} + 0.125\text{(R}_{25\text{KHz}}) - 0.153\text{(R}_{50\text{K}}) + 0.761\text{(ethnicity)} \\
\]

\[
Women = -8.753 + 0.082(ht) - 0.114\text{(triceps skinfold)} + 0.311\text{(wt)} + 0.153\text{(arm length)} - 0.021\text{(age)} - 0.155\text{(X}_{x5\text{KHz}}) + 0.145\text{(X}_{c10\text{KHz}}) - 0.019\text{(X}_{c1\text{MHz}}) + 1.368\text{(ethnicity)} + 0.254 \text{ht}^2 / \text{R}_{1\text{MHz}} \]

Where ht is height (m), triceps skinfold is triceps skinfold (mm), sub skinfold is subscapular skinfold (mm), waist circ is waist circumference (cm), wt is weight (kg), arm circ is arm circumference (cm), arm length (cm), ethnicity (non-Hispanic black = 1, Otherwise=0), age (years), \(R_{\text{MHz}}\) is resistance at subscript frequency, \(X_{\text{MHz}}\) is reactance at subscript frequency, and \(\text{ht}^2 / R_{1\text{MHz}}\) is the height squared over resistance at 1MHz ratio (m²/ohms).
Ethnicity is a categorical variable that was split into binary variables where it was found that non-Hispanic black was the only ethnicity that had a significant difference on the model.

Figure 7 shows the above equations in the visual format of a radar plot where the absolute value of the standardized regression coefficients (beta coefficients) for the men (blue) and women (pink) models are shown. The further from the center the points for a variable appear the greater impact each variable has on the model. Variables without a point in models of men or women are not included in that model. This suggests the top 3 factors for predicting fat free mass in men is weight, resistance at 50 kHz and resistance at 25 kHz. Whereas, in women the top 3 factors for predicting fat free mass is weight, height $^2$/Resistance at 1 MHz and reactance at 10 kHz.

Figure 7 - Radar plot of the normalized values of the coefficients in the regression models of men and women.
4.3.4 Testing the models

The findings above are in comparison to models found in literature where the most common variables used to predict fat free mass are weight, height\(^2\)/Resistance at 50kHz or resistance at 50kHz and height, and age. The occurrence of variables found in the literature can be seen in Figure 8 for men and women respectively.

![Figure 8 - Frequency of use of top variables in BIA equations. (blue - men, pink – women)](image)

The results for the root mean squared error for the models can be seen in Figure 9. It can be seen that our models for men and women had root mean squared errors below 1.8 kg and 1.7 kg, respectively, while the root mean square errors of all models from literature were above 2.3 kg and 2.0 kg.
The additional regression models developed using different combinations of variables (Model testing) with the training set for comparison with the final established models for men and women are compared in Table 3. It can be seen that in both men and women that a regression model made up of the combined variables performed the best, but, they were only slightly better than their respective final models. When variables taken from the model for women were used to generate a regression model for the men’s dataset, it can be seen that the model performed worse and similarly when the men’s model variables were used to generate a regression model for the women’s dataset, the model performed worse. Using the variables from the top performing models for men and women from literature and regressing using the training dataset improved the performance slightly; however both models still had a lower performance than the final models.

Table 3 - Comparison of similar equations in men and women.

<table>
<thead>
<tr>
<th>Model</th>
<th>MEN</th>
<th>Standard Error</th>
<th>Adj. R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final variables for men and women regressed on training dataset</td>
<td>1.7613</td>
<td>1.8480</td>
<td>0.9543</td>
</tr>
<tr>
<td>Final model for men</td>
<td>1.7786</td>
<td>1.7801</td>
<td>0.9590</td>
</tr>
<tr>
<td>Women’s model variables regressed on training dataset</td>
<td>1.9391</td>
<td>2.0400</td>
<td>0.9467</td>
</tr>
</tbody>
</table>
The Bland-Altman plots for the final models for men and women as well as the top five performing men’s and women’s models from literature are shown in Appendix F Figure 56 and Figure 57. The results show that the final models for men and women have a low mean difference from the gold standard and do not show any bias with increasing mean. This is in contrast to the models in literature which all show higher mean differences and most can be seen to have some bias with increase in fat-free mass. The mean difference from the gold standard is 0.016 and 0.089 in our final models for men and women respectively. Whereas the mean difference for models in literature range from 0.253 to 7.824 in men and 0.83 to 4.32 in women. Furthermore the spread of the difference two standard deviations above and below the mean

<table>
<thead>
<tr>
<th>Model</th>
<th>RMSE</th>
<th>Standard Error</th>
<th>Adj. R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>All variables excluding bioimpedance regressed on training dataset</td>
<td>1.9886</td>
<td>2.0460</td>
<td>0.944</td>
</tr>
<tr>
<td>Top model for men in literature regressed on training dataset (M10)</td>
<td>2.2013</td>
<td>2.2030</td>
<td>0.9372</td>
</tr>
<tr>
<td>Top model for men with coefficients as seen in literature (M10)</td>
<td>2.3197</td>
<td>2.3216</td>
<td>0.9303</td>
</tr>
<tr>
<td>WOMEN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>RMSE</td>
<td>Standard Error</td>
<td>Adj. R2</td>
</tr>
<tr>
<td>Final variables for men and women regressed on training dataset</td>
<td>1.6874</td>
<td>1.5610</td>
<td>0.9376</td>
</tr>
<tr>
<td>Final model for women</td>
<td>1.6936</td>
<td>1.6954</td>
<td>0.9215</td>
</tr>
<tr>
<td>Men’s model variables regressed on training dataset</td>
<td>1.7287</td>
<td>1.6130</td>
<td>0.9334</td>
</tr>
<tr>
<td>Top model for women in literature regressed on training dataset (F10)</td>
<td>1.8401</td>
<td>1.8421</td>
<td>0.9074</td>
</tr>
<tr>
<td>Top model for women with coefficients as seen in literature (F10)</td>
<td>1.9806</td>
<td>1.9828</td>
<td>0.8927</td>
</tr>
<tr>
<td>All variables excluding bioimpedance regressed on training dataset</td>
<td>2.1635</td>
<td>2.0120</td>
<td>0.8964</td>
</tr>
</tbody>
</table>
difference in our model for men is 6.99 and in women is 6.653. Whereas in literature these values range from 9.136 to 12.909 in men and 8.117 to 9.851 in women.

4.4 Discussion

While performing our literature review, we were unable to find any studies that have used the NHANES datasets to generate and compare FFM equations.

The final models established here suggest that some variables, which are not commonly recorded when performing bioimpedance analysis, can be used to improve the performance of FFM equations. As shown by Figure 8, height and weight are the most commonly used variables in the existing models. However, our findings suggest that the inclusion of additional variables such as skinfold measurements, waist and arm circumference, arm length, resistance measured at 25 kHz in men, and reactance and the height^2/resistance at 1MHz in women should be included in the models to improve accuracy.

These results are in line with the findings of Stevens et al. and Lee et al., who show that the addition of skinfold measurements in men and women, and waist circumference in men only, increase the prediction accuracy of body composition models. (38,39) Triceps skinfold measurements are only included in model M10 and model F10, and thigh circumference is only included in model M2 and model F2. None of the models include subscapular skinfold measurement, waist circumference, arm circumference, or arm length. The addition of these anthropometric measurements, along with the additional impedance measurements, over weight and height are likely the reason for the significant improvement in prediction by our models over those in the previous studies.

Many models in previous studies were created based on limited samples specific to a single ethnic population. Previous multi-ethnical studies have found no significant effect of ethnicity on body composition models. (46,47) However, other studies have suggested differences in body composition models based on ethnicity due to differences in body build, relative leg length, and frame size. (48–50) Our results show significant differences in the model for non-Hispanic
blacks only, with no significant differences in FFM between non-Hispanic white, Mexican American, other Hispanic, and other races.

From Table 3 it can also be seen that in our proposed model, the inclusion of bioimpedance measurements further improves the prediction accuracy. In men resistance at 50 kHz was found to be significant, which justifies its inclusion in most models found in previous studies. We also found that resistance at 25 kHz was significant. However, this was not the case for women. In women it was found that reactance at 5 kHz, 10 kHz, and 1 MHz, and the height²/resistance at 1 MHz were significant factors in the model. The addition of resistance and reactance at multiple frequencies are likely reducing the variability in our model which may contribute to the improved accuracy of our model over those found in previous studies. It is interesting to note that in previous studies reactance is more commonly found in models for men than for women. However, in our models reactance was found to only be a significant predictor of FFM in women.

We propose that the difference seen between men and women models is likely due to tissue distribution and the mechanisms of fat storage. Studies have shown that men generally store fat in the abdominal area and as visceral fat, and women generally store fat as peripheral fat in the extremities. (51–53) This would explain the significant role of reactance in women. Since fat acts as an insulator and would increase reactive effects, and, the impedance measured through bioimpedance analysis is known to be primarily made up from the legs and arms with the trunk contributing to as little as 10% of the measured impedance. (18,26) Furthermore, visceral fat is more innervated and vascular, therefore containing more blood and body fluid, which would further reduce its effect on reactance by reducing impedance. (54) It is therefore likely that fat in women has a more significant effect on reactance than in men as seen in the final models.

Additionally, from the Bland-Altman plots in Figures 55 and 56, we see that the models found in previous studies are prone to bias. Equations M7, M16, F17, F18, and F7 all show a clear trend of overestimating when FFM is low and underestimating when FFM is high. Our final models show little bias with a balanced distribution of differences between low and high FFM.
It may have been possible that there was an inherent bias from using the same dataset to derive and test the models. This was mitigated through the use of separate train and test subsets. Furthermore, by generating a regression model using the top performing men’s and women’s models in previous studies with the training dataset we would negate potential bias that the training dataset may have towards the test dataset. In this case, the generated models from previous studies still had inferior results compared to the models proposed in this thesis. This is due to the inclusion of additional anthropometric and impedance data in our models. Therefore, if our models were trained on the datasets used to generate the models found in previous studies, including all of our model variables, we believe they would perform better than their respective models.

In conclusion, the new regression models proposed in this study improve the estimation of FFM and provide reliable equations that can be used by clinicians. Our models outperformed those in previous studies and could be used as a new standard to estimate FFM of healthy individuals aged 18-49. This would allow clinicians and researchers to perform longitudinal studies using a single equation.

It should be mentioned that although this study is meant to form a new general equation for a large population, it could be limited in that it is formed on a population representative of the US and may not be adequate for other countries. While our models perform better in a generalized healthy population, the same may not be true for targeted populations. Furthermore, the addition of certain anthropometric measures could increase the time taken to perform this estimation beyond what a clinician may deem necessary. The inclusion of additional impedance data however, should not have any effect on the data acquisition time and effort as most new bioimpedance analyzers, such as the Impedimed SFB7, include all of the required data in a single measurement.
4.5 Future Work

The equations defined in this study may be implemented in the software for the device design covered in objective 2. As segmental impedance of the leg has been shown to be closely representative of whole body hydration in healthy subjects, these equations may be used to perform whole body analysis along with segmental analysis to establish relationship between segmental impedance and body composition. Furthermore, the excluded populations from this study may be looked at further to establish relevant equations using the same methods which would aid in clinical and diseased populations.
Chapter 5

Objective 2: Dry electrode socks

5.1 Description

As our collaboration with Myant involved an iterative process for producing and assessing the dry electrode socks, this chapter is split into an initial exploratory sock test section followed by a small validation study. In the initial sock test, the first sock produced was tested on a single subject to determine whether bioimpedance data could be measured. Following the initial sock test, Myant produced three pairs of bands with different electrode coatings which were then compared to gel electrodes, as the gold standard, in a validation study on five subjects.

5.2 Exploratory Sock Test

5.2.1 Methods

To test the feasibility of using dry electrodes to measure bioimpedance of the calf we proposed the electrode configuration seen in Figure 10. This configuration is used in a previous study by Vena et al. (55) In this configuration, we tested our current method of performing BIA on the leg, by placing the electrodes just below the tibial tuberosity and just above the malleolus with the voltage measuring electrodes 2cm within the current injecting electrodes. Our industrial partner, Myant, fabricated the configuration into socks with embedded electrodes made of silver yarn and a conductive polymer coating as seen in figure 11.
The data acquisition was performed using the Impedimed SFB7 which has previously been used in our lab to measure fluid volume of the leg. During testing we measured the resistance and reactance of the leg segment at a range of frequencies between 1kHz and 1MHz in a single subject while seated. This measurement was performed using the sock electrode configuration with no conductive gel, the sock with conductive gel applied between the electrodes and the skin, and finally with conventional gel electrodes which were considered the true value for comparison and validation. The impedance, phase angle and Cole-Cole plot for the dry electrodes and the gel electrodes were visually compared for the initial validation stage. The tests were performed sequentially minimizing time and movement between the tests to reduce the potential for differences in measurements caused by altering the leg fluid volume.
Following the testing on the first sock produced by Myant, we were then provided with three different dry electrode bands to compare. The electrodes were manufactured as bands as opposed to a sock for ease of application during the testing phase. The bands were produced using different techniques and polymer electrode coatings as determined by Myant. The details of the differences in electrode coating techniques were not disclosed by Myant, however the goal of testing the three bands were to identify whether they affect the ability to perform bioimpedance analysis and if one of the styles is more suited to collecting BIS measurements. These bands were again compared on their resistance, reactance and Cole-Cole plots with the data obtained from the Impedimed SFB7.

5.2.2 Results

The results for the data acquisition performed on the first dry electrode sock can be seen in Figures 12, 13, and 14. The plots compare the dry electrode sock when no conductive gel is applied, when a conductive gel is applied between the electrode and the skin, and when using traditional gel electrodes. Figure 12 shows the impedance plotted against the frequency between 1kHz and 1Mhz, and Figure 13 shows the impedance phase angle plotted against frequency between 1kHz and 1Mhz. Figure 14 shows the Cole-Cole plots.
Figure 12 - Impedance vs frequency of the first dry electrode sock with and without conductive gel compared to conventional gel electrodes.

Figure 13 - Phase angle vs frequency of the first dry electrode sock with and without conductive gel compared to conventional gel electrodes.
Figure 14 - Cole-Cole plot showing the resistance vs -reactance of the first dry electrode sock with and without conductive gel compared to conventional gel electrodes.

Following from the results of the first electrode sock the results for the data acquisition performed on the three electrode bands can be seen in Figures 15, 16, and 17. Figure 15 shows the impedance plotted against the frequency between 1kHz and 1Mhz, and figure 16 shows the impedance phase angle plotted against frequency between 1kHz and 1Mhz. Figure 17 shows the Cole-Cole plots for the three different bands.
Figure 15 - Impedance vs frequency of three different dry electrode bands.

Figure 16 - Phase vs frequency of three different dry electrode bands.
5.2.3 Discussion

From the results of the pilot testing it can be visually seen that there is very little difference in the impedance characteristics of the dry electrodes in comparison to traditional gel electrodes. The dry electrodes have slightly higher reactance than the gel electrodes, most likely caused by added capacitance at the skin electrode interface. The addition of conductive gel at this interface however did not reduce the reactance and appeared to increase the magnitude of reactance.

When comparing the three different electrode bands, they all performed similarly. Band 1 had the lowest resistance and reactance suggesting that it may be the best band, while band 3 had the highest resistance and reactance suggesting that it is the worst performing band.

The results from the pilot tests support the use of the dry electrodes for use in a convenient dry electrode textile system. It should be noted however that these initial pilot results were only performed on a single subject. Therefore, to validate their feasibility for use in a BIA system we needed to perform further analysis on a larger sample size in a pilot study.
5.3 Validation Study

5.3.1 Aim

The aim of this study is to compare and validate three different types of dry electrode textile bands against the gold standard (gel electrodes) for use in bioimpedance analysis of the calf.

5.3.2 Objectives

1) **Compare the spectral impedance characteristics of the electrode bands.**
   This will be achieved using the Impedimed SFB7 data acquisition system to measure calf impedance in subjects using the band and gel electrodes at 256 discrete frequency points logarithmically spaced from 1kHz to 1MHz.

2) **Compare the temporal characteristics of the electrode bands.**
   This will be achieved using the Biopac data acquisition system which measures impedance continuously at 50kHz. We will measure the change in impedance as the calf is raised, which will cause a change in both calf shape and fluid volume in the calf.

5.3.3 Methods/Protocol

Healthy subjects were recruited from the lab to participate in the study. Each subject followed the protocol detailed below:

1) Subject was asked to wear loose shorts and remove footwear, and then seated for the test procedure.
2) The order of electrode bands to be tested was randomized.
3) The first electrode band was placed on the calf and its position marked in order to align the gel electrodes and have consistent placement when testing the other electrode bands.
4) Gel electrodes were placed on the same calf in line (radially) with the electrode bands. (Figure 18 a).
5) Upper and lower calf diameter, in line with the voltage measuring electrodes, were measured and recorded. Then calf length, between the voltage measuring electrodes, were measured and recorded.
6) The Impedimed SFB7 was connected to the electrodes and 5 measurements (256 frequency points between 1kHz and 1MHz) at 1 second intervals were recorded for the gel electrodes first followed by the band electrodes (in randomized order).
7) The SFB7 was disconnected and the Biopac was then connected to the electrodes.
8) Biopac recording started (at 50kHz, current injected through the gel electrodes, voltage measured with both electrodes). (Figure 18 b).
9) The subject then sat stationary for 30 seconds.
10) 3 calf raises were performed: The calf was raised with toes still touching the ground and held for 10 seconds. The calf was then lowered and held for 10 seconds. This process was repeated 3 times.
11) The subject then sat stationary for 30 seconds.
12) Steps 8 to 11 were repeated with the current now injected through the band electrodes. (Figure 18 c).
13) Steps 6 to 12 were repeated until each of the three electrode bands had been tested.

![Figure 18 - Positioning of the dry band and gel electrodes (a). In b) the gel electrodes inject the current while voltage is measured in both gel and dry electrodes. In c) the dry electrodes inject the current while voltage is measured in both gel and dry electrodes.](image)

Following data collection, the data is then analyzed in Matlab to plot the impedance, phase and Cole-Cole features of the SFB7 data and the changes in impedance over time for the Biopac data. For the change in impedance over time, the average of the first 0.5 seconds of impedance measurement is set as the baseline and the change in impedance over time is measured relative to this value. Finally, the calf fluid volume for each band is estimated using the data recorded from the SFB7 using Equation 7. (56,57) Where \( V \) is the volume of extracellular fluid in the calf (L), \( \rho \) is the resistivity of the calf (\( \Omega \cdot \text{cm} \)), \( L \) is the length of the calf between the voltage measuring electrodes (cm), \( C_1 \) and \( C_2 \) is the top and bottom circumference at the two voltage measuring electrodes (cm), and \( R_e \) is the extracellular resistance of the calf measured by the SFB7 (\( \Omega \)).
\[ V = \frac{\rho^{2/3}}{3(4\pi)^{1/3}} \cdot \left( \frac{L}{C_1 C_2 R_e} \right)^{2/3} \cdot L(C_1^2 + C_2^2 + C_1 C_2) \] (7)

5.3.4 Results and Discussion

5 healthy men participated in the study and had measurements made on their right calf. Subject’s demographic information can be found in Table 4.

| Table 4 - Subject details and calf dimension measurements. |
|---------------------------------|--------|--------|--------|--------|--------|
| **Sex**                        | **Subject 1** | **Subject 2** | **Subject 3** | **Subject 4** | **Subject 5** |
| Calf Side Measured             | Man    | Man    | Man    | Man    | Man    |
| Lower circumference (cm)       | 24.25  | 27.25  | 21     | 23.5   | 25     |
| Upper circumference (cm)       | 36     | 38.75  | 33     | 36.5   | 38     |
| Length (cm)                    | 12     | 15     | 14     | 14.75  | 19     |

Figures 19, 20 and 21 show the spectral characteristics recorded from the 5 subjects. The impedance, phase, and Cole-Cole plot (resistance vs. reactance) are shown respectively. It can be seen that subject 1 shows the best results, subject 2 has acceptable results for band 2, and subjects 3-5 have poor results for each of the electrode bands. While the impedance and phase characteristics of the three bands degrade progressively from subject 1 to subject 5, the traditional gel electrodes retain their functionality.

From the spectral results of subject 1, it can be seen that each of the bands performs similar to the gel electrode. The performance of the bands cannot be validated in subjects 2-5 as the results are highly variable and inconsistent.

Figure 22 shows the temporal results of testing the bands with calf raises. Only the case of the bands injecting the current has been shown, since the case where the gel electrodes were injecting the current had even worse results and more drift in the signals. It can once again be seen that in subject 1 the results are as expected, in subject 2 the results are close to expected, and in subjects 3-5 the results are inconsistent and the signals are variable. It can be seen that in
some cases there is a problem of drift in the band electrode signal, and in some cases, there is poor to no response in the electrodes with the calf raise. It is unclear whether the poor signal quality is due to bad connections between the electrodes and the attached wiring, poor impedance characteristics of the electrodes at the skin electrode interface, degradation in the electrode after the first use, or other physiological differences between subjects.
Figure 19 – Impedance vs Frequency for the 3 textile electrode bands and traditional gel electrodes.

Subject 1

Subject 2

Subject 3

Subject 4

Subject 5
Figure 20 - Phase vs Frequency for the 3 textile electrode bands and traditional gel electrodes.

Subject 1

Subject 2

Subject 3

Subject 4

Subject 5
Figure 21 – Cole-Cole plot for the 3 textile electrode bands and traditional gel electrodes.

Subject 1

Subject 2

Subject 3

Subject 4

Subject 5
Figure 22 – Change in impedance during three 10 second calf raises for the three difference electrode bands. Blue: Gel Electrode (Gold Standard). Red: Band Electrodes.

<table>
<thead>
<tr>
<th>Subj</th>
<th>Band 1</th>
<th>Band 2</th>
<th>Band 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="Graph" /></td>
<td><img src="image2" alt="Graph" /></td>
<td><img src="image3" alt="Graph" /></td>
</tr>
<tr>
<td>2</td>
<td><img src="image4" alt="Graph" /></td>
<td><img src="image5" alt="Graph" /></td>
<td><img src="image6" alt="Graph" /></td>
</tr>
<tr>
<td>3</td>
<td><img src="image7" alt="Graph" /></td>
<td><img src="image8" alt="Graph" /></td>
<td><img src="image9" alt="Graph" /></td>
</tr>
<tr>
<td>4</td>
<td><img src="image10" alt="Graph" /></td>
<td><img src="image11" alt="Graph" /></td>
<td><img src="image12" alt="Graph" /></td>
</tr>
<tr>
<td>5</td>
<td><img src="image13" alt="Graph" /></td>
<td><img src="image14" alt="Graph" /></td>
<td><img src="image15" alt="Graph" /></td>
</tr>
</tbody>
</table>
Using the impedance values recorded from the SFB7 and the calf measurements taken, we tried to estimate fluid volume of the calf using equation 7. The results are shown for each subject and electrode band compared with the gel electrodes in Table 5. It must be noted however, that since the fluid volume estimation is calculated using the resistance obtained from the Cole-Cole plots, which were found to be unreliable, the fluid volume estimations do not provide an accurate or reliable comparison. In subject 1 who had the closest to acceptable results, it can be seen that the difference in fluid volume between the gel and band electrodes was as low as 0.65% in band 1 and up to 4.93% in band 2. In the other subjects this difference was as high as 84.56%.

Table 5 – Estimated calf fluid volume based on the impedance measured from gel and three different band electrodes.

(Difference in estimated calf fluid volume based on the impedance measured from the gold standard gel electrodes.)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gel (mL)</th>
<th>Band 1 [mL (Δ%)]</th>
<th>Band 2 [mL (Δ%)]</th>
<th>Band 3 [mL (Δ%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>197.72</td>
<td>199.01 (0.65)</td>
<td>207.47 (4.93)</td>
<td>199.61 (0.95)</td>
</tr>
<tr>
<td>2</td>
<td>241.65</td>
<td>319.96 (32.40)</td>
<td>285.77 (18.26)</td>
<td>337.98 (39.86)</td>
</tr>
<tr>
<td>3</td>
<td>196.19</td>
<td>310.62 (58.32)</td>
<td>362.10 (84.56)</td>
<td>270.47 (37.86)</td>
</tr>
<tr>
<td>4</td>
<td>253.15</td>
<td>264.92 (4.65)</td>
<td>187.26 (26.03)</td>
<td>454.00 (79.34)</td>
</tr>
<tr>
<td>5</td>
<td>453.63</td>
<td>648.85 (43.04)</td>
<td>701.46 (54.63)</td>
<td>647.69 (42.78)</td>
</tr>
</tbody>
</table>

5.3.5 Observations and Recommendations

In conclusion the bands do not appear to be reliable at present for measuring bioimpedance in the calf. In response to this, some observations and recommendations for future iterations have been laid out in the following section.

1) The electrode pairs are too close to each other (Figure 23). The electrodes should be spaced at least 2cm apart from their closest edges. Currently the gap is approximately 1.5cm. Kyle et al. recommends a minimum of 5cm between electrodes as part of the ESPEN Guidelines. (27) It was also found when testing the first generation socks that when the electrodes that were further apart were tested, the impedance characteristics improved.
2) Band 3 electrodes arrived coated in a sticky residue (presumably a conductive gel). It was found that this can cause the electrode material to peel away from the textile if they make contact with one another. Furthermore, the residue wore off after a few tests, which would negate its conductive properties. This could add to inconsistent impedance measurements over time which would be an undesirable property.

3) The electrode wires were very thin, which made connecting them to measuring equipment challenging. Furthermore, the wires had an inconsistent contact with the conductive textile and in one case did not have any contact, which gave inconsistent results. Potentially an alternative solution such as a sew-on ECG style snap connector (Figure 24) could provide a better contact with the conductive electrode material. (58)

4) In the current testing it was observed that swapping the bands during testing causes such a large difference in signal quality that these changes outweigh any ability to quantify which band performs better.
5.4 Conclusion

With the implementation of the recommendations above it may be possible to use dry electrode textiles for bioimpedance analysis. These textiles could then be used alongside the device developed in objective 3 to measure bioimpedance data and estimate body composition and fluid volume.

It may also be seen with further testing that the model used to calculated tissue impedance (figure 1) might no longer be valid, as the impedance and capacitance of the dry electrodes could be large enough to no longer be negligible in the model. More advanced alternative models which account for the effects of electrode impedance and capacitance may be required. In this case figure 1 may be modified to figure 25 to account for the resistance and capacitance of the dry electrodes indicated by R-Ex and C-Ex respectively.

Figures 25 – Modified tissue impedance model to account for dry electrode resistance and reactance.
The current testing method requires the subject to be still as movement will cause significant noise to the measurements. Electromyographic signals are much larger than the ones measured during BIA. For studies subjects should be required to refrain from movement during recording. In future implementations of a textile-based BIA device with continuous measurements of fluid volume it could be feasible to include a way of detecting when the subject is moving to identify potentially unusable data. This could be achieved with the inclusion of an accelerometer. Furthermore later iterations of the sock may be able to use stretch sensors embedded in the sock to calculate the diameter of the calf and would be able to dynamically update this value to improve fluid volume estimates.
Chapter 6

Objective 3: Device design and validation

6.1 Methods

Figure 26 shows a diagram of the proposed system design. The design is separated into five sections; a current generator, leg segment voltage recording sensor sock, a data acquisition unit, a data transmission unit and a smart processing unit. This means that each section can be developed independently but will work together to achieve the common end goal. The current generator will provide current at multiple frequencies to the body. The voltage recording sensor placed on the leg will record the voltage difference just below the tibial tuberosity and just above the malleolus. The data acquisition unit will amplify and filter the raw sensor data. This data will be passed through a phase and gain detector, which is then converted to a digital value by an analog-digital converter. Finally, this digital value is converted to an impedance estimate for the leg segment at every frequency. The impedance values are then transmitted by the data transmission unit to a processing unit. A smart processing unit will use software to analyze the collected data and estimate the intracellular and extracellular fluid volumes of the leg.
A test system was designed by a previous MHSc student, Bojan Gavrillovic, as a test of concept. Our aim was to develop a prototype system based on this design. While objective 2 aimed to fulfil the voltage recording sensors stage of this design. This objective will focus on the design of the current source, data acquisition unit and smart processing software.

The design of the system was implemented in an iterative process involving testing, troubleshooting and revising the system design. The design methods and evaluation results are therefore presented in a chronological sequence to best describe how we concluded the final design.
6.2 Design and Validation

6.2.1 Proof of Concept Circuit

The design is segmented into a current source, unity gain inverting amplifier, the device under test, a reference resistor, differential amplifier, current buffer, gain and phase detector and analog to digital converter (A/D converter). The output of the current source is filtered by the unity gain inverting amplifier. The filtered signal then passes through the device under test (tissue or phantom) with the positive lead of the current source connected to the top of the circuit. The ground lead of the current source was connected to the bottom of the circuit which was also connected by a reference resistor to ground. Voltage recording was performed across the device under test and across the reference resistor. The two recorded voltages are connected to filters that feed into the gain and phase detector to measure the gain and phase shifts between the two signals. The output of the gain and phase detector was recorded by an A/D converter connected to a computer which uses software to record the output and saved these measures for analysis. At this first stage of design, Matlab code was then run on the recorded measures to convert the paired gain and phase measurements to resistance and reactance measurements, which could then be plotted to form a Cole-Cole plot and extrapolated to calculate \( R_0 \) and \( R_\infty \). This process is described in Figure 27. The test set up first established by Bojan Gavrillovic can be seen in Figure 28. (59)

![Diagram](image)

Figure 27 – Electrical design overview of the system.
Initial tests were performed to compare setup of the proof of concept device against the ImpediMed SFB7 (considered as the gold standard throughout). The test involved using a phantom circuit as in Figure 1a, with $R_E = 508\,\Omega$, $R_I = 1075\,\Omega$, $C_M = 2.2\,nF$. The results of this test can be seen in Table 6. The SFB7 matches very closely to the actual values of $R_0$ and $R_\infty$ with errors of only 0.2 Ω and 0.3 Ω respectively, while our design has much larger errors of 13.3 Ω and 134.1 Ω respectively.

<table>
<thead>
<tr>
<th></th>
<th>$R_0$</th>
<th>$R_\infty$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Theoretical</strong></td>
<td>508 Ω</td>
<td>345 Ω</td>
</tr>
<tr>
<td><strong>ImpediMed SFB7</strong></td>
<td>507.8 Ω</td>
<td>344.7 Ω</td>
</tr>
<tr>
<td><strong>Proof of concept circuit</strong></td>
<td>494.7 Ω</td>
<td>210.9 Ω</td>
</tr>
</tbody>
</table>

The results above were calculated using the measured recordings from the initial circuit design considering frequencies between 1kHz and 200kHz. It was found that at frequencies above 200kHz the Cole-Cole plot did not behave as expected and had a sudden increase in the reactance magnitude at high frequencies (above 200Hz) as seen in Figure 29. It was suspected that this is a result of stray capacitances from the long wires currently in use as suggested by the findings of De Lorenzo. (60)
Hypothesized sources of error include hardware and software errors. Hardware errors could be a result of stray capacitances in the multitude of long wires connecting components as well as low tolerance electrical components leading to imperfect matching between the measured signals. Furthermore, as the gain and phase detector relies on close matching of its components, mismatches could exacerbate errors seen in the measurements. Furthermore, the SFB7 uses calibrations that could account for inherent measurement biases, whereas our current method uses the raw recorded data.

6.2.2 Initial Printed Circuit Board

To address the above concerns, a small printed circuit board was developed, for the amplifier and buffer sections of the system, to allow the incorporation of high tolerance components and eliminate some of the stray capacitances of the long wiring. The first revision of this board (revision A) can be seen in Figure 31 and Figure 32 with the relevant schematic in Figure 30.
Figure 30 – PCB Revision A schematics.
Figure 31 – Printed Circuit Board Revision A Design (6x5 cm).

Figure 32 - Printed Circuit Board Revision A.
A high precision prefabricated gain and phase development board with matching components was used to replace the current gain and phase setup, in an attempt to minimize errors from mismatched components.

A microcontroller (STM32F103RB, STMicroelectronics) was implemented to control the current source and to record the gain and phase output signals. The microcontroller was programmed in the C programming language using the Eclipse integrated development environment. The previous current source which was controlled using a USB connection to a laptop was replaced with a small board (AD9833, Analog Devices) which was controlled with the serial peripheral interface of the microcontroller. The recording of the gain and phase signals previously done by a stand-alone analog to digital converter connected to a laptop has been replaced by the onboard 12-bit analog to digital converter of the microcontroller.

As seen with the proof of concept circuit, the board was tested using a phantom RRC circuit representing whole body impedance. The resulting recorded Cole-Cole, impedance, and phase plots can be seen in Figure 33, Figure 34 and Figure 35 respectively. From this testing it can be seen that the large phase shift above 200kHz is still prevalent. This ruled out the previous theory that the long and messy wiring was causing stray capacitances.

![Cole-Cole plot of recording with PCB Revision A.](image)
After a thorough examination of the circuit schematics and components, a new theory to explain the unexpected behavior at higher frequencies was considered. It was believed that the TL072 op-amps (61) being used were limiting the operational bandwidth of the op-amp to below the 1 MHz requirements of the system. The datasheet reports the unity-gain bandwidth of the op-amp to be 3 MHz. Therefore, operating as a unity-gain amplifier the op-amp has an effective bandwidth of 3 MHz. However, as the gain of the op-amp is increased the effective bandwidth is
decreased. This concept is illustrated in figure 3.6. The bandwidth can be calculated using equation 8, where the unity-gain bandwidth is equal to the gain bandwidth product divided by a closed loop gain of 1. Since we use gains up to 2.047 in our system, our effective bandwidth is reduced to 1.47MHz. While this value is above the 1MHz that our system operates up to, it must be noted that the bandwidth is measured as a loss of -3dB, therefore there are still some losses below the rated bandwidth. To address this, an op-amp with a much higher bandwidth was selected, which also retains the same footprint and pinout as the previous TL072 chips to eliminate the need to redesign the PCB. The LM6172 op-amp was selected as this had a wide unity-gain bandwidth of 100MHz. (62)

\[ \text{Bandwidth (Hz)} = \frac{\text{Gain Bandwidth Product (Hz)}}{\text{Closed Loop Gain}} \]  \hspace{1cm} (8)

Figure 3.6 – Illustration of the effective bandwidth of an op-amp as the gain is increased. (63)

The TL072 op-amp chips on the board were replaced with the wide bandwidth LM6172 chips. After replacing the chips, the same test as performed previously was repeated. The results of this test are shown in figure 37, figure 38 and figure 39. It can be seen that the performance of the system was significantly improved, with the Cole-Cole plot showing the expected curve with no phase shift at 200 kHz. Upon closer look at the phase vs frequency plot in figure 39, it can be seen that there is still a flattening of the phase around 400 kHz and eventually a phase shift.
around 900 kHz. However, this does not significantly affect the Cole-Cole plot and still allows for much more accurate estimation of $R_0$ and $R_\infty$ as seen in Table 7.

Figure 37 - Cole-Cole plot of recording with PCB Revision A using the new op-amps.

Figure 38 - Impedance vs Frequency of recording with PCB Revision A using the new op-amps.
Figure 39 - Phase vs Frequency of recording with PCB Revision A using the new op-amps.

Table 7 - Extrapolated $R_0$ and $R_\infty$ using the circuit with the old TL072 versus the new LM7162 op-amps.

<table>
<thead>
<tr>
<th></th>
<th>$R_0$</th>
<th>$R_\infty$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical</td>
<td>508 Ω</td>
<td>345 Ω</td>
</tr>
<tr>
<td>Old Op Amps (TL072)</td>
<td>510.2 Ω</td>
<td>283.4 Ω</td>
</tr>
<tr>
<td>New Op Amps (LM7162)</td>
<td>509.7 Ω</td>
<td>352.1 Ω</td>
</tr>
</tbody>
</table>

6.2.3 Final Design

Following the successful results in testing the new op-amps a final revision of the system was designed. This revision included the current source, filtering, and gain and phase circuits integrated into a single printed circuit board. This circuit board also includes header pins to quickly and easily mount the STM32F103 Nucleo microprocessor; voltage regulators to attach two 9v batteries to power the device; and four ports to attach cables to the electrodes. The schematics for this design can be seen in figure 40 and figure 41. The printed circuit board with the mounted microcontroller and cables can be seen in figure 42 and figure 43. Finally, a 3D printed housing for the system was designed in SolidWorks which can be seen in figure 44 and figure 45.
Figure 40 - Integrated System PCB Final Design (1/2).
Figure 41 - Integrated System PCB final design (2/2).
Figure 42 - Integrated System PCB final design.

Figure 43 - Integrated system PCB with mounted microcontroller and electrode cable connectors.
Figure 4 - Bioimpedance system mounted within its 3D printed housing. The white microcontroller circuit is on the left with USB cable to connect to a computer, the green PCB is on the right with four cables to attach to electrodes, not seen here in the bottom section of the housing is two 9V battery connectors.

Figure 45 - Enclosed bioimpedance system. (130x100x58 mm)
In the initial design for the integrated circuit board, coaxial cables were chosen, to connect the electrodes to the circuit, under suggestion that the grounded outer cable would act as shielding to reduce electromagnetic noise carried through the cables. During testing it was found that the grounded cables introduced a high level of noise. However, when the outer conducting ring of the coaxial cable was disconnected from ground, the signal quality increased significantly. Therefore, in the final revision of the board design, the coaxial cables were left ungrounded. The results of testing the board using grounded compared with the ungrounded cables can be seen in figures 46 - 51.

Figure 46 - Impedance vs Frequency of PCB using grounded coaxial cables.
Figure 47 - Phase vs Frequency of PCB using grounded coaxial cables.

Figure 48 - Cole-Cole plot of PCB using grounded coaxial cables.
Figure 49 - Impedance vs Frequency of PCB using ungrounded coaxial cables.

Figure 50 - Phase vs Frequency of PCB using ungrounded coaxial cables.
After solving the noise issue from the grounded coaxial cables, the system was then tested using the RRC circuit from previous tests. This was compared with the results from the SFB7. The Cole-Cole, impedance vs frequency, and phase vs frequency plots can be seen in figure 52, figure 53, and figure 54 respectively. It can be seen in the Cole-Cole plot that the curve closely matches that of the SFB7. However, it can be seen in the phase and impedance plots that there is a slight deviation from the results of the SFB7 at high frequencies.
Figure 52 - Cole-Cole plot of our circuit board (red) and the SFB7 (blue).

Figure 53 - Impedance vs frequency of our circuit board (red) and the SFB7 (blue).
6.2.4 Smart Processing Software

Collecting data using the circuit board requires running the firmware in debugger mode, copying the collected A/D Converter values into a CSV file and then processing the values in MATLAB. To simplify this process, a user-friendly GUI was designed in Visual Studio. The Fluid And Body composition software (FAB) was designed to establish a connection to the device over a USB COM port. After successfully connecting to the device the user selects a save location for data storage and can then select to do a single or continuous measure. Following each measure, the FAB software extrapolates $R_0$ and $R_\infty$ and displays the Cole-Cole, impedance vs frequency, and phase vs frequency plots. Details for operating the software can be found in Appendix G. The FAB software interface after completing a measure is shown in figure 55.
6.3 Recommendations

While the results of the current design support its use as a BIA device, there are still some deviations at high frequencies. It may be possible to implement a calibration method on the raw measurements to account for these deviations at higher frequencies. Further testing of different reference resistor values could also improve the accuracy of the measurements.

The current device was tested on an RRC circuit representative of whole body impedances. Testing against impedances representative of the calf to validate its performance in measuring calf fluid is recommended. Furthermore, to improve the performance when measuring different body segments the reference resistor should be adjusted according to the expected resistance of the body segment being measured.

It may be possible to include ports for multiple electrode cables to attach to the circuit board and then have a digital switch controlled by the microprocessor to switch which set of cables are
connected to the circuit and therefore measure fluid of multiple segments sequentially. This would allow researchers to monitor fluid shifts between body segments over time.

To improve the portability of the device as a wearable technology, the size of the circuit board needs to be reduced. The current board design has ample room to be reduced in size. The passive component sizes and trace dimensions could be significantly reduced. The current plug-in microcontroller development board could be incorporated into the circuit board. Power management chips could be implemented to use a single battery as the power supply instead of two separate batteries for positive and negative supplies. Finally, a wireless module could eliminate the need for a physical USB connection to a computer running the FAB software.
Chapter 7

Conclusions and Significance

The successful completion of the single channel prototype will lead towards the development of a commercial, fully integrated BIA sock that will be able to continuously monitor fluid volume in the leg throughout the day. In moving forward with this research, the system could be used along with the established Impedimed SFB7 to measure fluid in healthy subjects with various maneuvers to determine how reliable it is, how reproducible it is and what the range of error of the system is. Further research into applying calibration techniques may also be tested to improve the system measurements. Following this it is feasible that research trials in patients and various clinical populations of interest may be carried out.

This research and the future research that may be carried out as a result of this may aim to fill the need for a practical tool that is portable and easy to use. In our lab we are interested in monitoring fluid volume in the legs through the day and at night and under different levels of exertion to assess the impact of fluid shifts on sleep apnea. Outside of our lab the device could be used as a multidisciplinary tool to assess and monitor body composition such as:

- Fluid levels and edema in patients with fluid retaining disorders including heart failure and renal failure.
- Muscle mass in the elderly and frail population.
- Fat mass in the obese.

Furthermore, as research and testing of the textile based dry electrodes continues we hope to expand from the sock to a suite of integrated textiles to measure body composition and fluid volume in different segments of the body. Designing the device such that it is user-friendly and intuitive will encourage participatory use by patients and clinicians to actively monitor body composition and see their results. Having this feedback mechanism will encourage patients to pursue improved body composition which will ultimately assist with the prevention of disease.
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77. Chapman NC, Bannerman E, Cowan S, MacLennan WJ. The relationship of


87. Deurenberg P, Kooij K van der, Evers P, Hulshof T. Assessment of body composition by


Appendix A: Electrical Impedance

Electrical impedance (Z) is a measure of the opposition of electrical current through a material. It consists of two elements; resistance (R) which is passive and has a constant value with respect to frequency, and reactance (X) whose value changes with respect to frequency. (12,64) Impedance is represented by a complex number, with the real component representing the resistance and the imaginary component representing the reactance. This rectangular form of impedance is represented in equation 9. (12,64)

\[ Z = R + jX \]  
(9)

Measuring electrical impedance uses the basic principles of Ohm’s law, as seen in equation 10. Z is the measured impedance of the object, I is the current injected into the object, and V is the voltage difference measured between two points on the object. By using an alternating current (AC) as the source of electrical current, we can measure the impedance and associated phase shift. (64)

\[ Z = \frac{V}{I} \]  
(10)

Resistance of an object is determined by its shape and material properties. Resistance (R) is affected by the objects length (L), cross sectional area (A) and resistivity (\( \rho \)) as seen in equation 11. Reactance of an object is the opposition to a change in voltage across the object due to its capacitive properties. Reactance (X) is affected by the frequency (f) and capacitance (C) of the object as seen in equation 12. (12)

\[ R = \rho \cdot \frac{L}{A} \]  
(11)

\[ X = \frac{1}{2\pi \cdot f \cdot C} \]  
(12)

This relationship between resistance (R), resistivity (\( \rho \)) and the length (L) of a homogeneous material with a uniform cross-sectional area (A), is described in equation 13. (12)

\[ R = \frac{\rho L}{A} \]  
(13)
If the body is considered as a cylinder then the volume can be considered as the cross-sectional area multiplied by the length and we arrive at equation 14.

\[ R = \frac{\rho L^2}{\text{Vol}} \]  

(14)

Finally, if we rearrange equation 14 the measure of volume is realized as seen in equation 15.

\[ \text{Vol} = \frac{\rho L^2}{R} \]  

(15)

In clinical practice it is easier to measure a subject’s height (Ht) than their conducting length from the ankle to the wrist. Therefore, the empirical relationship typically relies on the measurement of \( \frac{Ht^2}{R} \). (13,18)

Almost all BIA devices performing SF-BIA use 50kHz as the frequency of choice, (12,17,18,22) which has no theoretical basis and has been reported to be scientifically “unsound”. (11,17,18) It is thought this may be due to the fact that day-to-day coefficient of variation increases for frequencies lower than 50kHz. (18)
Appendix B: Recommended Body Composition
Equations from Literature

Table 8 – Summary of lowest standard error of the estimate (SEE) body composition equations from literature. (18)

<table>
<thead>
<tr>
<th>Equation</th>
<th>SEE</th>
<th>Eq. #</th>
</tr>
</thead>
<tbody>
<tr>
<td>( FFM = -4.104 + 0.518 Ht^2/R_{50} + 0.231 Wt + 0.130 X_{50} + 4.229\text{Sex} )</td>
<td>1.8</td>
<td>(16)</td>
</tr>
<tr>
<td>( FM = -23.58 + 20.03 (R_{50} Wt)/Ht^2 + 0.29 \text{thigh circ} )</td>
<td>3.8</td>
<td>(17)</td>
</tr>
<tr>
<td>( TBW = 6.69 + 0.34573 Ht^2/Z_{100} + 0.17065 Wt - 0.11 \text{Age} + 2.66 \text{Sex} )</td>
<td>1.73</td>
<td>(18)</td>
</tr>
<tr>
<td>( ECF = 2.30 + 0.19528 Ht^2/Z_{100} + 0.06987 Wt - 0.02 \text{Age} )</td>
<td>0.98</td>
<td>(19)</td>
</tr>
<tr>
<td>( ICF = 9.182 + 0.285 Ht^2/Z_5 + 7.114 PA_5 + 2.113 \text{Sex} )</td>
<td>0.9</td>
<td>(20)</td>
</tr>
<tr>
<td>( BCM = 1.898 Ht^2/X_{50} - 0.051 Wt + 4.180 \text{Sex} + 15.496 )</td>
<td>1.71</td>
<td>(21)</td>
</tr>
</tbody>
</table>

Abbreviations: FFM – fat free mass, fm – fat mass, TBW – total body water, ECF – extracellular fluid, ICF – intracellular fluid, BCM – body cell mass, Ht – height, R_{50} – resistance measured at 50kHz, Wt – weight, X_{50} - reactance measured at 50kHz, thigh circ – thigh circumference, Z_{100} – impedance at 100kHz, Z_5 – impedance at 5kHz, Age – age, PA_5 – phase angle at 5kHz, and Sex is 1 for men and 0 for women.
**Appendix C: Commercial BIA Devices**

Table 9 – Commercially available BIA devices.

<table>
<thead>
<tr>
<th>Device (REF)</th>
<th>Electrode Style*</th>
<th>Frequencies</th>
<th>Continuous Output</th>
<th>Portable</th>
<th>Outputs Raw Impedance</th>
<th>ECF/ICF Capability</th>
<th>Cost (CAD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIA450 (Biodynamics Corp) (65)</td>
<td>Whole Body</td>
<td>50kHz</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>$2,500</td>
</tr>
<tr>
<td>Quantum V (RJL Systems) (66)</td>
<td>Five segment method</td>
<td>50kHz</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>$4,350</td>
</tr>
<tr>
<td>MC-980Uplus (Tanita) (67)</td>
<td>Five segment method</td>
<td>1.5,50,250, 500,1000 kHz</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>$16,000</td>
</tr>
<tr>
<td>SFB7 (ImpediMed) (31)</td>
<td>Single Channel</td>
<td>4 kHz – 1 MHz</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>$7,500</td>
</tr>
<tr>
<td>InBody 770 (InBody) (68)</td>
<td>Five segment method</td>
<td>1.5,50,250, 500,1000 kHz</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>$23,700</td>
</tr>
<tr>
<td>mBCA515 (Seca) (69)</td>
<td>Five segment method</td>
<td>1 kHz – 1 MHz</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>ECF Only</td>
<td>$12,995</td>
</tr>
<tr>
<td>Multiscan 5000 (Bodystat) (70)</td>
<td>Single Channel</td>
<td>5 kHz – 1 MHz</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>$17,495</td>
</tr>
<tr>
<td>BodyComp MF Hexa (SMT Medical) (71)</td>
<td>Whole Body</td>
<td>5,50,100,150, 200,250 kHz</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>ECF Only</td>
<td>$6,400</td>
</tr>
</tbody>
</table>

*Segmental BIS is performed by measuring the impedance across specific segments of the body. The practices for achieving this are explained here. In the five segment method the whole body method is supplemented by placing additional electrodes on the opposite wrist and ankle of the body which segments the body into five segments consisting of two arms, two legs and the torso. (12,13,18) The disadvantage of this five segment (arm, arm, leg, leg, torso) method is that the current still passes through the entire body when measuring the impedance of each segment and is therefore still prone to inconsistencies between impedance and the body mass of the trunk. The second method also supplements the whole body method by placing additional electrodes on the shoulder and hip which segments the body into an arm, torso and leg. (12,13,18) The final method is achieved by moving a single channel of current injecting and voltage sensing electrodes between segments of interest. (12,13,18) The disadvantage of this method is that apart from being tedious and time consuming, you cannot measure each segment simultaneously, precluding the measure of fluid shifts between segments.
## Appendix D: Fat-Free Mass Equations

Table 10 and Table 11 shows all the equations for fat free mass found in the literature. They are organized by gender and indexed using labels (Modelm/f#, where ‘m’ for male and ‘f’ for female, and # is the equation number) (20,47,72–84)

### Table 10 - Male fat-free mass equations in literature.

<table>
<thead>
<tr>
<th>Population</th>
<th>Source</th>
<th>Equation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Subjects aged 12-71</td>
<td>Boulier et al. (85)</td>
<td>M1 = 6.37 + 0.64(wt) + 0.4(ht²/Z₁MHz) - 0.16(age) - 2.71</td>
</tr>
<tr>
<td>Elderly aged 65-94</td>
<td>Baumgartner et al. (83)</td>
<td>M2 = -1.732 + 0.28(ht²/R₅₀kHz) + 0.27(wt) + 4.5(sex) + 0.31(thigh circ)</td>
</tr>
<tr>
<td>Elderly</td>
<td>Dey et al. (82)</td>
<td>M3 = 11.78 + 0.499(ht²/R₅₀kHz) + 0.134(wt) + 3.449</td>
</tr>
<tr>
<td>Healthy subjects aged above 16</td>
<td>Durenberg et al. (86)</td>
<td>M4 = -12.44 + 0.34(ht²/R₅₀kHz) + 0.1534(ht) + 0.273(wt) - 0.127(age) + 4.56</td>
</tr>
<tr>
<td>Healthy subjects aged 33-65</td>
<td>Heitmann (80)</td>
<td>M5 = -14.94 + 0.279(ht²/R₅₀kHz) + 0.181(wt) + 0.231(ht) - 0.077(age) + 0.064</td>
</tr>
<tr>
<td>Elderly aged 60-83</td>
<td>Deurenberg et al. (87)</td>
<td>M6 = 3.9 + 0.672(ht²/R₅₀kHz) + 3.1(sex)</td>
</tr>
<tr>
<td>Healthy adults aged 18-94</td>
<td>Kyle et al. (20)</td>
<td>M7 = -4.104 + 0.518(ht²/R₅₀kHz) + 0.231(wt) + 0.130(Xc) + 4.229(sex)</td>
</tr>
<tr>
<td>Elderly</td>
<td>Roubenoff et al. (88)</td>
<td>M8 = 5.741 + 0.4551(ht²/R₅₀kHz) + 0.1405(wt) + 0.0573(Xc) + 6.2467(sex)</td>
</tr>
<tr>
<td>Elderly aged 60-83</td>
<td>Deurenberg et al. (87)</td>
<td>M9 = 7.0 + 0.360(ht²/R₅₀kHz) + 4.5(sex) + 0.359(wt) - 0.2(thigh circ)</td>
</tr>
<tr>
<td>Adults aged &gt;75</td>
<td>Chapman et al. (89)</td>
<td>M10 = 0.409(wt) - 0.217(tri skinfold) + 0.241(ht) - 27.553 + 2.583(sex) + 0.202(ht²/R₅₀kHz)</td>
</tr>
<tr>
<td>Healthy, ethnic divers</td>
<td>Kotler et al. (90)</td>
<td>M11 = 0.49 + 0.5(ht¹.₅⁶/₅₀kHz) (1.0/1.21) + 0.042(wt)</td>
</tr>
<tr>
<td>Healthy Subjects aged 12-94</td>
<td>Sun et al. (46)</td>
<td>M12 = -10.678 + 0.652(ht²/R₅₀kHz) + 0.262(wt) + 0.015(R)</td>
</tr>
<tr>
<td>Healthy adults aged 18-29</td>
<td>Lohman (91)</td>
<td>M13 = 5.32 + 0.485(ht²/R₅₀kHz) + 0.388(wt)</td>
</tr>
<tr>
<td>Healthy adults aged 30-49</td>
<td>Lohman (91)</td>
<td>M14 = 4.51 + 0.549(ht²/R₅₀kHz) + 0.163(wt) + 0.092(Xc)</td>
</tr>
<tr>
<td>Healthy Adults aged 50-70</td>
<td>Lohman (91)</td>
<td>M15 = -11.41 + 0.600(ht²/R₅₀kHz) + 0.186(wt) + 0.266(Xc)</td>
</tr>
<tr>
<td>Elderly</td>
<td>Roubenoff et al. (88)</td>
<td>M16 = 9.1536 + 0.4273(ht²/R₅₀kHz) + 0.1926(wt) + 0.0667(Xc)</td>
</tr>
</tbody>
</table>

*variables: ht = height (cm), wt = weight (kg), age = age (years), R = Resistance (ohm, frequency identified in subscript. If no frequency found 50kHz was assumed), Xc = Reactance (ohm, frequency identified in subscript. If no frequency found 50kHz was assumed), Z = Impedance (ohm, frequency identified in subscript. If no frequency found 50kHz was assumed), thigh circ = thigh circumference (cm), ankle circ = ankle circumference (cm), tri skinfold = triceps skinfold (cm), sex = 1 for men, 0 for women
Table 11 - Female fat-free equations in literature.

<table>
<thead>
<tr>
<th>Population</th>
<th>Source</th>
<th>Equation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Subjects aged 12-71</td>
<td>Boulier et al. (85)</td>
<td>F1 = 6.37 + 0.64(wt) + 0.4(ht²/Z₄MHz) + 0.16(age) − 5.42</td>
</tr>
<tr>
<td>Elderly aged 65-94</td>
<td>Baumgartner et al. (83)</td>
<td>F2 = −1.732 + 0.28(ht²/R₅kHz) + 0.27(wt) + 4.5(sex) + 0.31(thigh circ)</td>
</tr>
<tr>
<td>Elderly</td>
<td>Dey et al. (82)</td>
<td>F3 = 11.78 + 0.499(ht²/R₅kHz) + 0.134(wt)</td>
</tr>
<tr>
<td>Healthy subjects aged above 16</td>
<td>Durenberg et al. (86)</td>
<td>F4 = −12.44 + 0.34(ht²/R₅kHz) + 0.1534(ht) + 0.273(wt) − 0.127(age)</td>
</tr>
<tr>
<td>Healthy subjects aged 33-65</td>
<td>Heitmann (80)</td>
<td>F5 = −14.94 + 0.279(ht²/R₅kHz) + 0.181(wt) + 0.231(ht) − 0.077(age)</td>
</tr>
<tr>
<td>Elderly aged 60-83</td>
<td>Deurenberg et al. (87)</td>
<td>F6 = 3.9 + 0.672(ht²/R₅kHz) + 3.1(sex)</td>
</tr>
<tr>
<td>Healthy adults aged 18-94</td>
<td>Kyle et al. (20)</td>
<td>F7 = −4.104 + 0.518(ht²/R₅kHz) + 0.231(wt) + 0.130(Xc) + 4.229(sex)</td>
</tr>
<tr>
<td>Elderly</td>
<td>Roubenoff et al. (88)</td>
<td>F8 = 5.741 + 0.4551(ht²/R₅kHz) + 0.1405(wt) + 0.0573(Xc) + 6.2467(sex)</td>
</tr>
<tr>
<td>Elderly aged 60-83</td>
<td>Deurenberg et al. (87)</td>
<td>F9 = 7.0 + 0.360(ht²/R₅kHz) + 4.5(sex) + 0.359(wt) − 0.2(thigh circ)</td>
</tr>
<tr>
<td>Adults aged &gt;75</td>
<td>Chapman et al. (89)</td>
<td>F10 = 0.409(wt) − 0.217(tri skinfold) + 0.241(ht) − 27.553 + 2.583(sex) + 0.202(ht²/R₅kHz)</td>
</tr>
<tr>
<td>Healthy, ethnic divers</td>
<td>Kotler et al. (90)</td>
<td>F11 = 0.07 + 0.88(ht¹⁹⁷/Z₄²⁷₉)(1.0/22.22) + 0.081(wt)</td>
</tr>
<tr>
<td>Healthy Subjects aged 12-94</td>
<td>Sun et al. (46)</td>
<td>F12 = −9.529 + 0.696(ht²/R₅kHz) + 0.168(wt) + 0.016(R₅kHz)</td>
</tr>
<tr>
<td>Healthy adults aged 18-29</td>
<td>Lohman (91)</td>
<td>F13 = 5.49 + 0.467(ht²/R₅kHz) + 0.295(wt)</td>
</tr>
<tr>
<td>Healthy adults aged 30-49</td>
<td>Lohman (91)</td>
<td>F14 = 11.59 + 0.493(ht²/R₅kHz) + 0.141(wt)</td>
</tr>
<tr>
<td>Healthy Adults aged 50-70</td>
<td>Lohman (91)</td>
<td>F15 = 6.34 + 0.474(ht²/R₅kHz) + 0.180(wt)</td>
</tr>
<tr>
<td>Elderly</td>
<td>Roubenoff et al. (88)</td>
<td>F16 = 7.7435 + 0.4542(ht²/R₅kHz) + 0.1190(wt) + 0.0455(Xc)</td>
</tr>
<tr>
<td>Overweight women aged 25-45</td>
<td>Jakicic et al. (74)</td>
<td>F17 = 2.04 − 0.02(R₅kHz) + 0.19(wt) + 2.63(ethnicity) + 0.2583(ht)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Caucasian = 0, African American = 1)</td>
</tr>
<tr>
<td>Overweight women aged 25-45</td>
<td>Jakicic et al. (74)</td>
<td>F18 = 2.68 + 0.20(ht²/R₅kHz) + 0.19(wt) + 2.55(ethnicity) + 0.1157(ht)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Caucasian = 0, African American = 1)</td>
</tr>
<tr>
<td>Women aged 18-60</td>
<td>Stolarczyk et al. (92)</td>
<td>F19 = 20.05 − 0.04904(R₅kHz) + 0.001254(ht²) + 0.1555(wt) + 0.1417(Xc) + 0.0833(age)</td>
</tr>
<tr>
<td>Adults aged 18-70</td>
<td>Jebb et al. (93)</td>
<td>F20 = 13.96674 + 0.348613(ht²/Z) + 0.168998(wt)</td>
</tr>
</tbody>
</table>

*variables: ht = height (cm), wt = weight (kg), age = age (years), R = Resistance (ohm, frequency identified in subscript. If no frequency found 50kHz was assumed), Xc = Reactance (ohm, frequency identified in subscript. If no frequency found 50kHz was assumed), Z = Impedance (ohm, frequency identified in subscript. If no frequency found 50kHz was assumed), thigh circ = thigh circumference (cm), ankle circ = ankle circumference (cm), tri skinfold = triceps skinfold (cm), sex = 1 for men, 0 for women
## Appendix E: Sample Population Distribution

### Table 12 - Distribution of sample population in men’s training dataset.

<table>
<thead>
<tr>
<th>Population Data</th>
<th>Scale</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>years</td>
<td>29.97829</td>
<td>9.759847</td>
<td>18 - 49</td>
</tr>
<tr>
<td>Weight</td>
<td>kg</td>
<td>80.19</td>
<td>15.80412</td>
<td>42.3 - 133.8</td>
</tr>
<tr>
<td>Height</td>
<td>cm</td>
<td>175</td>
<td>7.670925</td>
<td>152.3 - 193.5</td>
</tr>
<tr>
<td>BMI</td>
<td>Kg/m²</td>
<td>26.13</td>
<td>4.624671</td>
<td>16.01 - 44.65</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Race</th>
<th>Mexican American</th>
<th>Other Hispanic</th>
<th>Non-Hispanic White</th>
<th>Non-Hispanic Black</th>
<th>Other Race Incl. Multi-racial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>410</td>
<td>58</td>
<td>561</td>
<td>329</td>
<td>70</td>
</tr>
</tbody>
</table>

### Table 13 - Distribution of sample population in men’s testing dataset.

<table>
<thead>
<tr>
<th>Population Data</th>
<th>Scale</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>years</td>
<td>30.14671</td>
<td>9.939733</td>
<td>18 - 49</td>
</tr>
<tr>
<td>Weight</td>
<td>kg</td>
<td>78.78</td>
<td>14.71503</td>
<td>43.6 - 128.4</td>
</tr>
<tr>
<td>Height</td>
<td>cm</td>
<td>175</td>
<td>7.497532</td>
<td>151.6 - 193.9</td>
</tr>
<tr>
<td>BMI</td>
<td>Kg/m²</td>
<td>25.66</td>
<td>4.213194</td>
<td>15.16 - 44.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Race</th>
<th>Mexican American</th>
<th>Other Hispanic</th>
<th>Non-Hispanic White</th>
<th>Non-Hispanic Black</th>
<th>Other Race Incl. Multi-racial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>419</td>
<td>61</td>
<td>571</td>
<td>312</td>
<td>65</td>
</tr>
</tbody>
</table>
Table 14 - Distribution of sample population in women’s training dataset.

<table>
<thead>
<tr>
<th>Population Data</th>
<th>Scale</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>years</td>
<td>30.10509</td>
<td>9.876143</td>
<td>18 - 49</td>
</tr>
<tr>
<td>Weight</td>
<td>kg</td>
<td>70.39</td>
<td>16.51452</td>
<td>33.7 - 128.7</td>
</tr>
<tr>
<td>Height</td>
<td>cm</td>
<td>161.9</td>
<td>6.899792</td>
<td>133 - 181.3</td>
</tr>
<tr>
<td>BMI</td>
<td>Kg/m²</td>
<td>26.87</td>
<td>6.021442</td>
<td>16.18 - 48.10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Race</th>
<th>Mexican American</th>
<th>Other Hispanic</th>
<th>Non-Hispanic White</th>
<th>Non-Hispanic Black</th>
<th>Other Race Incl. Multi-racial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>346</td>
<td>67</td>
<td>481</td>
<td>257</td>
<td>48</td>
</tr>
</tbody>
</table>

Table 15 - Distribution of sample population in women’s testing dataset.

<table>
<thead>
<tr>
<th>Population Data</th>
<th>Scale</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>years</td>
<td>30.43939</td>
<td>10.11963</td>
<td>18 - 49</td>
</tr>
<tr>
<td>Weight</td>
<td>kg</td>
<td>65.42</td>
<td>12.86947</td>
<td>37.3 - 118.8</td>
</tr>
<tr>
<td>Height</td>
<td>cm</td>
<td>162.2</td>
<td>6.769382</td>
<td>143.3 - 183.7</td>
</tr>
<tr>
<td>BMI</td>
<td>Kg/m²</td>
<td>24.83</td>
<td>4.510867</td>
<td>15.56 - 39.48</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Race</th>
<th>Mexican American</th>
<th>Other Hispanic</th>
<th>Non-Hispanic White</th>
<th>Non-Hispanic Black</th>
<th>Other Race Incl. Multi-racial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>336</td>
<td>63</td>
<td>509</td>
<td>250</td>
<td>41</td>
</tr>
</tbody>
</table>
Appendix F: FFM Bland Altman Plots

1) Figure 56 - Bland Altman plot for final male model and top five models in literature (1- final male model; 2- M10; 3- M7; 4- M13; 5- M16; 6- M11)
Figure S7 - Bland Altman plot for final female model and top five models in literature (1- final female model; 2- F10; 3- F17; 4- F18; 5- F7; 6- F13)
Appendix G: Fluid and Body Composition Software GUI

1) Start State: select the COM port the device is connected to.

![Initial screen](image1.png)  
**Figure 58 - Fluid and Body composition software initial screen.**

2) COM port is connected and software is waiting on confirmation of connection from the device.

![Connection screen](image2.png)  
**Figure 59 - Fluid and Body composition software establishing COM port connection.**
3) Once device is connected (not shown here, connection status bar will show connection), select the location to save measurement data files.

![Fluid and Body composition software select file save location.](image)

**Figure 60** - Fluid and Body composition software select file save location.

4) Once the save location and name has been entered you can start a single or continuous measurement.

![Fluid and Body composition enter file save name.](image)

**Figure 61** - Fluid and Body composition enter file save name.
5) The measurement data points will be plotted and the best fit circle will be found along with information on the estimate impedance at 0 and $\infty$ frequency. The data points and calculated values will be stored to a .csv file.

![Figure 62 - Fluid and Body composition software successful measure.](image-url)