Midyear Report

Hydration Monitoring Using Impedance Techniques

Harvey Mudd College Engineering Clinic

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ABSTRACT

Proteus Digital Health, Inc. is interested in using impedance methods to measure human hydration. Proteus desires measurements taken on a scale such that the measurement can be easily integrated into their product, a wearable patch that measures biological signals. The Proteus Clinic Team at Harvey Mudd College has implemented a testing system that uses the Bodystat Quadscan 4000, an impedance measurement device, to determine whether local impedance measurements on the torso may be correlated with hydration. This document presents research used to determine which measurement techniques and electrical parameters would be best for the design. In the first semester, the team found that local four-electrode measurements at 50 kHz and 800 μA on the stomach produced the lowest variance (3.4%, collective) and highest correlations with full body impedance measurements (0.8215 for Subject 1, and 0.7967 for Subject 2) compared to other tested configurations. The data indicate that it may be feasible to track relative hydration changes over periods of at least three hours. In the spring, the team anticipates taking further impedance measurements to optimize the wear location and measurement frequency for such measurements. Additionally, the team plans to create a hydration measurement device that can be integrated into the wearable patch.

TABLE OF CONTENTS

INTRODUCTION

Proteus Digital Health, Inc. is sponsoring a Harvey Mudd Clinic project for the 2015-16 academic year. This section will provide information about Proteus Digital Health, and explain the goals for the project.

1.1. PROTEUS DIGITAL HEALTH, INC.

Proteus Digital Health, Inc. is entering the biomedical marketplace with their wearable technology that takes physiological measurements. The company is a proponent of “digital medicine” and has designed an ingestible chip that can be integrated into pills to detect whether patients have taken their medication [1]. The ingestible sensor interacts with a wearable patch that also takes physiological measurements on the wearer, such as heart rate and body temperature. The goal of this clinic project is to extend the functionality of this patch to other useful measurements, such as hydration and blood pressure, so the patch may be more useful to a larger population of consumers.

1.2. PROJECT STATEMENT

The Proteus Clinic Team will develop a prototype for a device that will use impedance techniques to measure hydration levels in the body. The team will also extensively test the prototype to determine its effectiveness and feasibility. The team may also integrate the prototype in to Proteus’ wearable patch, or, develop additional prototypes that measure metrics such as blood pressure.

1.2.1. Objectives

Objectives for the hydration measurement device include:

- Measures hydration consistently

- Measures hydration accurately
- Correlates impedance measurements with a widely used hydration-measuring device
- Produces meaningful measurements under a variety of environmental conditions

1.2.2. Constraints

The test device must meet the following constraints:

- Uses impedance measurements
- Follows IEC 60601 safety requirements
- Uses the current patch's electrode dimensions and placement
- Use a wear location on the torso

1.2.3. Functions

The design should perform the following functions:

- Measures impedance within the human body and correlates measurements with body hydration level

1.3. DELIVERABLES

By the end of the fall semester, the team will deliver:

- A literature review from the existing hydration and impedance research
- Test results/report on correlation between local skin impedance and hydration
- If feasible, a hydration-measuring prototype
- Project documentation and presentations including:

    - Work Plan
    - Three design reviews
    - Midyear Report

By the end of the spring semester, the team will deliver:

- An optimized hydration-measuring prototype or a setup for measuring an alternate biological signal
- Test results
- Project documentation and presentations including:
    - Final Report (including engineering drawings)
    - Spring semester presentation at HMC
    - Projects Day presentation
    - Presentation at Proteus Digital Health

2. BACKGROUND

The purpose of this section is to compile the existing research that involves hydration and impedance measurements in order to decide which techniques and devices will be most useful to the project.

2.1. CLINICAL HYDRATION MEASUREMENTS

The two main hydration measurement methods used in clinical and commercial settings are impedance-based and fluid-based.

2.1.1. Fluid-Based Measurements

The fluid-based measurements are often the most accurate and widely accepted techniques. However, these techniques require a laboratory setting. These tests take a blood or urine sample of the patient and measure ion concentrations. Of the laboratory tests, the most common comparator is tritium dilution. According to early research on the topic, tritium dilution was able to accurately measure water mass in vitro, but had a 4.6% overestimation of water in vivo for chukar partridges [3]. Tritium dilution relies on the assumption that the concentration of the ingested tritium is approximately equal throughout the body. Therefore, the concentration of tritium in a urine sample would equal the total body concentration. Similar tests involve a dose of deuterium (D2O) or sodium bromide (NaBr). The NaBr tests will only produce ECW measurements [30]. Although, it would be impossible to incorporate such testing types into a patch form, if other devices can correlate their measurements with the results of a laboratory test, they will be able to determine hydration more accurately.

2.1.2. Impedance-Based Measurements

Lastly, impedance-based techniques inject a current into the body and determine the impedance of the body by measuring the voltage differences at the entrance and exit of the current. Many commercial and clinical devices are currently available that utilize this method in order to calculate body composition. The body composition measurements tend to include percent body fat and hydration.

For impedance measurements, hydration will typically be represented as total body water. This metric is the total volume or weight of water in the patient's body. Most often, this would be represented in liters, however, some devices such as the Tanita scale (manufactured by the Tanita Corporation of America, Inc.) will output a total body water percent, where an average female should have 45-60% water and an average male should have 55-65% water. Other devices, such as InBody (designed by InBody Co., Ltd.) will output hydration in terms of the intracellular and extracellular water weight in pounds. In terms of a person's health, InBody considers the ratio of extracellular to intracellular water. This value, ECW/ICW, should be between 0.36 and 0.39 for a healthy patient.

2.1.3. Research Studies with Hydration Devices

The Tanita scale is a hydration and body fat monitoring device that utilizes impedance techniques. The company offers consumer and professional scales of various models that output different performance metrics of the patient. The Tanita Corporation sponsored tests of the actual performance of these scales in relation to hydration measurements. The results in these studies indicate a correlation between the patient's height squared over the measured resistance to the tritium dilution volume. For two studies, the results were $n = 29$, $r = 0.88$, p

<0.001 [23], and n = 14, r = 0.78, p<0.001 [24]. Both studies used the TBF 105 & 305 Tanita scale models. Another Tanita-sponsored study was conducted to evaluate the performance of the scale at estimating body fat. The test found that the scale underestimates body fat compared to a four-compartment model. Additionally, the results indicate that the Tanita scale is slightly less correlated to the model than another impedance-based device, produced by Bodystat Ltd. (r = 0.933, compared to r = 0.952) [13].

In another of their studies, Tanita researchers found that correlation coefficients are lower for leg-to-leg than for arm-to-leg methods. However, by factoring in age, gender, and waist/hip circumference, they were able to achieve more significant results [25]. Based on this information, the electrode placement for measuring impedance will likely have a significant effect on the accuracy of the resulting data. All commercial products that were found in this research using the impedance techniques measure the impedance from two distant points rather than something localized to a patch. Although impedance measurements can easily be taken locally, further research is required to determine the feasibility of converting this number into a measure of hydration.

Researchers tested the accuracy of three InBody models (S10, 720, and 520) by comparing the measurements to results from deuterium (D2O) dilution testing. Each of the three InBody models overestimated total body water compared to D2O testing. In men, InBody overestimated by about 12% and in women, in body overestimated by about 13%. The model closest to D2O was the InBody S10, which uses electrodes as opposed to a scale like the 720 and 520 models [2]. Based on this information and the Tanita studies, a better comparator would be one that measures impedance from the wrist to ankle rather than from leg-to-leg.

Medical researchers tested the Bodystat 1500 and Bodystat Dualscan 2005 (no longer available) devices for accuracy by comparing the output measurements of the devices to tritium dilution results. The Dualscan product measured resistance at frequencies of 5 and 200 kHz while the 1500 just measured at 50 kHz. The correlation between each device and tritium dilution was $r^2 = 0.96$, $P<0.0001$. On the absolute scale, the single frequency device underestimated by about 1 L while the dual frequency device underestimated by about 5 L compared to tritium dilution [30].

2.2. IMPEDANCE TECHNIQUES

There are a variety of methods that are used to measure physiological impedance.

2.2.1. Basic Impedance Techniques

A commonly used and cited technique for measuring hydration using impedance is bioelectrical impedance analysis (BIA). In this technique, an AC current at a single frequency (often 50 kHz) is injected into the body. The impedance across certain parts of the body is then measured by dividing the output voltage by input current. This gives insight to the body composition or body water level because water is conductive, and so more hydrated tissues like muscle have a lower impedance than less hydrated tissues like fat [4]. The usefulness of BIA depends on the measurement setup and model used to convert impedance to a quantity of interest. Calculating hydration or body composition of the whole body is usually done by placing multiple electrodes on the hands and feet or just the feet so the current can run through the entire body. The commercial comparators use this approach. The body is then modeled either as a uniform cylinder or as multiple cylindrical segments. Equations have been developed to determine total body water given impedance and other factors like height and BMI. There are usually different equations for people of different genders, races, etc. [1]. Measurements conducted at a single frequency can measure total body water (TBW) as a weighted sum of extracellular water (ECW) and intracellular water (ICW), but cannot distinguish between the two [14].

If either ECW or ICW is of interest, it then becomes desirable to use BIA with multiple frequencies. As described in section 3.2, signals at high frequencies can penetrate cell membranes and signals at low frequencies flow around cells, so the different signals can measure ECW or TBW separately. One can take measurements at even more frequencies to construct a transfer function of body impedance at a certain frequency, allowing for perhaps a more accurate value of body impedance at a certain time [4]. This method is called bioelectrical impedance spectroscopy (BIS), and has been used to determine relative changes in hydration for an individual [34].

Another method of using impedance to measure hydration is bioelectrical impedance vector analysis (BIVA). This differs from BIA in that both the magnitude and phase angle of the impedance are measured, allowing for a vector plot of the body's current state to be constructed. Instead of correlating an impedance value to an absolute body water level, as in BIA, the vector plot constructed using BIVA can be given meaning by superimposing ellipses constructed probabilistically that point to whether the patient is hydrated or not.

BIVA then provides a relative measurement of hydration, rather than an absolute measurement of TBW. It is a common method used in many clinical devices because it can quickly calculate an output describing the relative health of the individual, which is relevant to caregivers [8]. It has also been suggested that it can be useful to combine BIA and BIVA to monitor the health of patients with conditions like COPD. BIA gives a good measure of body composition, while BIVA provides a picture of overall hydration. Combining these two measures can indicate whether a patient is dehydrated or malnourished [33].

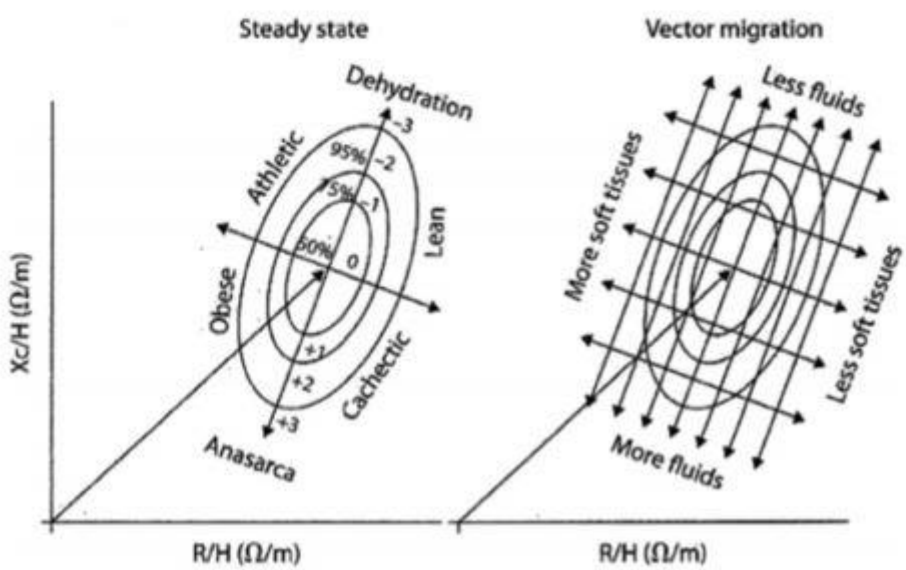


Figure 1: Sample results using the BIVA technique. [15]

EIT requires solving nonlinear partial differential equations using finite element modeling. The “inverse” process of determining the conductivity of the region from electrode potentials is known to be ill-posed and is especially difficult to solve. This method might require more computer power than a small processor can handle. This technique also requires a large number of electrodes, but some results suggest that only partially surrounding a body segment with electrodes may be sufficient to create a picture [6].

2.2.2. Electrical Models for Hydration

AC impedance can be used to calculate hydration by employing certain models of the human body. One model assumes that the body consists of a cylindrical impedance with resistive and capacitive components in series as seen in Figure 2. Equations used in this model rely on the entire impedance value. The equations used to calculate hydration in these situations are empirically calculated, and also factor in the height and weight of the patient.

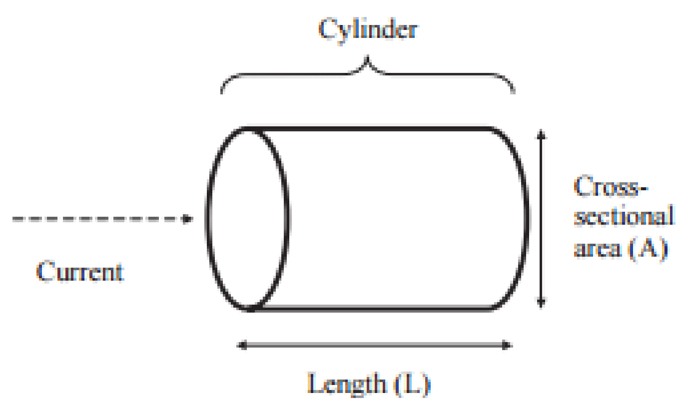


Figure 2: Depiction of a cylindrical model for the human body. [14]

An alternative model, called the Cole Model, has resistive and capacitive components in parallel, as shown in Figure 3. This model accounts for the effect of varying frequencies in the body. Because the model assumes that cell membranes are capacitive, at high frequencies, current flow is modeled through both the water outside of the cells and as well as through the cells themselves. In contrast, at low frequencies, current is modeled as only flowing through the extracellular water. Thus the Cole Model uses the circuit below (in Figure 3) to explain the impedance of tissue.

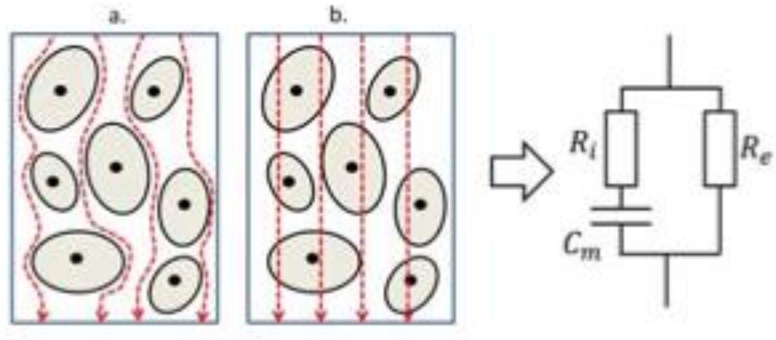


Figure 3: Cole Model diagrams and equivalent circuit. The far left image (a) shows that at low frequencies, current travels around cells through extracellular water. The middle image (b) shows that at high frequencies, the current travels through cells and around them. The far right image shows the circuit that models this behavior. [34]

For the Cole Model, equations that rely on the intracellular and extracellular resistance were determined empirically to calculate hydration. To use these equations, a low and a high frequency must be used to extract R_i and R_e separately.

2.2.3. Electrode Quantity and Placement

Based on studies evaluating EIT and BIA with slight variations in electrode position, the team expected to see statistically significant differences in absolute measurement values when the patch is moved more than 2 cm. One study employing EIT to measure lung resistivity tested the effects of slight variations in electrode position as shown in Figure 4. For this study, lung resistivity was measured with the EIT band of electrodes at different heights around the lower part of the sternum, called the xiphoid process. The study found no significant difference between measurements at the 4 and 5 cm positions, but found statistically significant variations between all other sets of electrodes, demonstrating the high degree of sensitivity of EIT techniques to electrode placement [19].

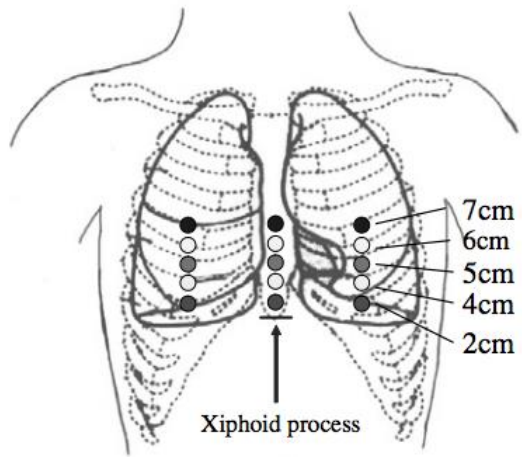


Figure 4: Variation of electrode placement from an EIT research study. [19]

A second study measured full body impedance between the ankle and wrist using the standard four-electrode, or tetrapolar, method [9]. The authors then moved each electrode to six new positions, each further up the arm or leg, with a maximum movement distance of 6 cm. The findings are summarized in Figure 5 below.

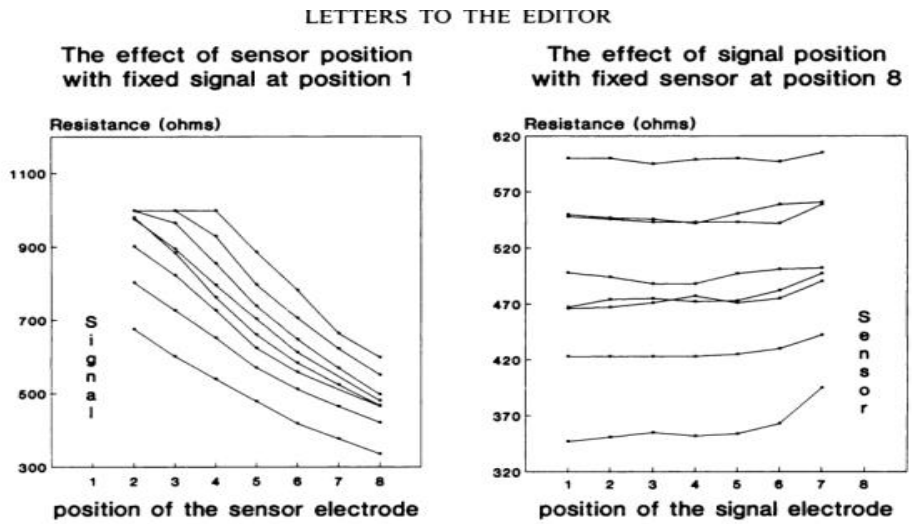


FIG 1. Effect of electrode position on bioelectrical impedance analysis measurement in newborns. Resistance was measured by using an AKERN (Firenze, Italy) analyzer. Positions 1 and 2 correspond to the standard positions in adults. The other positions correspond to electrodes (1.2 cm wide) placed side by side along the arm or leg from position 2 and above. The sensor electrode at position 8 was 6 cm from position 2. The value 999 Ω is the maximum of the machine.

Figure 5: Results from a study on electrode position. [9].

The authors found that the position of the sensor electrode, which determines the length of conductor, but not the position of the signal electrode, affects the impedance measurement. This demonstrates that the team will need to consider and experimentally test the effects of electrode “slippage”, as movement of even 1 cm may have an effect on measurements.

2.2.4. Two- and Four- Electrode Measurements

The current Proteus patch has two electrodes for measuring local body impedance. However, this impedance measurement is only used to determine whether the patch is in contact with the skin. A two-electrode impedance measurement, as shown in Figure 6, is commonly considered to be inadequate for measuring small impedances. The impedance of interest (R_test) cannot be measured directly with this setup, so the voltages across the electrodes are also included in the measured output voltage. The electrodes themselves have relatively large resistance, and any variation in their resistance can affect the overall output voltage measurement, leading to an inaccurate measurement of the impedance of interest.

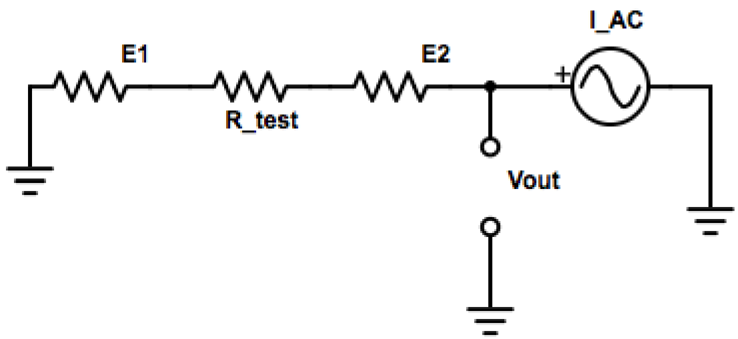


Figure 6: Two-electrode measurement setup, with resistors representing the two electrodes (E1 and E2).

An alternative approach is to use four electrodes and an op-amp, as shown in Figure 7. With this setup, two of the electrodes transmit the current signal through the body, and the other two detect the voltage difference across the test resistance. Because no current flows through E3 and E4 (in an ideal op amp model), we assume that those electrodes are not affecting the voltage difference across the test resistance, which can be measured directly.

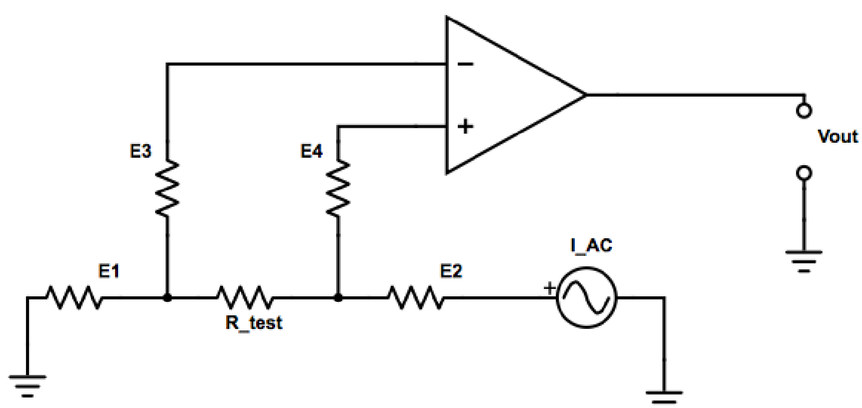


Figure 7: Four-electrode measurement setup.

2.2.5. Impedance with Localized Electrodes

Local measurements may be meaningful, but may not represent events in other regions of the body. For example, regional edemas will probably not have a measurable effect on torso impedance measurements.

This conclusion is first supported by a study examining changes in impedance measured across the lower leg in the progression of ACL injury and healing. The authors found up to a 27% decrease in resistance, R, as a result of increase in blood flow to a torn calf muscle [21]. This resistance reduction the researchers directly attributed to hydration increases. Additionally, the study found a high degree of tester reproducibility: “the mean value of R was 39.6 Ω with a SD of 0.6 Ω, and for Xc the mean value was 14.2 Ω with a SD of 0.5 Ω, with coefficients of variation of 1.4 and 3.2%, respectively” [21].

A second study provided further evidence of the success of local impedance techniques, with higher applicability to the purposes of this project [7]. The authors found large, significant changes in the impedance vector with hydration status caused by local edema, shown in Figure 8. However, the authors found no evidence of the edema in the patient’s other leg. Because there were no changes in the control leg, the results suggest that a regional edema may not be detectable by a local measurement on the torso. The study also compared measurements before and after surgery to clear the edema fluid and had the same conclusions about the applicability and accuracy of segmental BIA for edema in the leg.

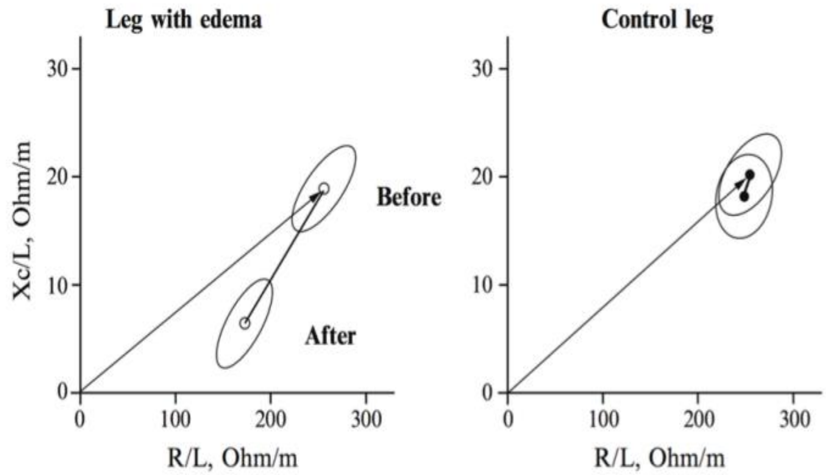


Fig. 16.11 Identification of edema in the leg with segmental BIVA. The mean impedance vector is normalized by the length of the leg (i.e., *Z/L= R/L, Xc/L*). A significant vector displacement (separate 95% confidence ellipses) was observed after the appearance of edema on the leg that underwent the surgical procedure compared to the non-affected leg. (Reprinted from Codognotto et al. (2008). With permission)

Figure 8: BIVA results from a research study using local impedance techniques. [7]

Finally, one study used local impedance techniques conducted on the torso to measure changes in fluid volume at different stages of peritoneal dialysis, a procedure where fluid is added in the abdomen and then later flushed out [20]. The researchers found a correlation between impedance and the amount of fluid drained, added, and levels of blood triglycerides, a fat in high quantities increases risk of heart disease, and albumin, a protein that in low quantities can indicate kidney or liver disease. The measurements were made between points 12 and 13 on the human model in Figure 9 below.

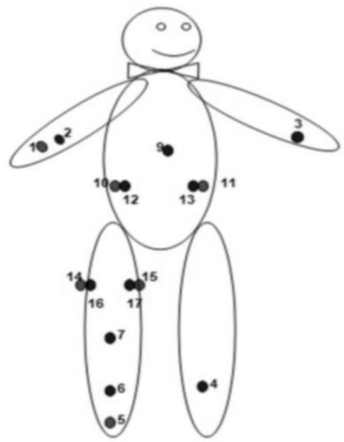


Figure 9: A model showing placement for localized electrode measurements. [20]

Specifically, the authors found a statistical significance of Rho = 0.672, P = 0.001 for correlation with triglycerides, and Rho = 0.562, P = 0.008 for correlation with albumin, suggesting that general health measurements may be possible.

2.2.6. Carrier Frequency and Signal Strength

Based on the literature, 50 kHz is the optimal choice for carrier frequency due to its superior signal to noise ratio, optimal reactance-to-resistance ratios, and the wealth of studies that have used this specification. However, these justifications might not hold for local measurements and the team may be unable to use the previously established regression equations or population statistical distributions from full body for local measurements. The team will use a current of 400-800 µA, with most machines in literature using 800 µA ([15], [21], [7], [17], [30], and [11]). Although the academic standard is the tetrapolar setup, feasibility testing will still be conducted with the two-electrode, or bipolar, system.

A *Nature* paper reviewed a number of previous studies on different BIA techniques, which supported these decisions [28]. The authors argued that that single frequency analysis is just as accurate as multiple-frequency analysis, with 50 kHz being the optimal frequency because it provides the optimal signal to noise ratio. They also argued “intra- and extracellular current flow causes equivalence of information based on functions of R and X_c measurements made at 50 kHz versus other frequencies” [27]. Specifically, the authors found no loss of information when switching from BIA spectroscopy, which uses multiple frequencies, to BIVA vector analysis, which uses a single frequency.

3. IMPACT

The noninvasive, local measurement of tissue impedance has many applications if it can be shown to correlate well with local or systemic human hydration. Primarily, such measurement can be used to monitor the hydration status of stable geriatric patients who may be unfit to do so themselves. Such scenarios might arise in nursing homes where it can be unrealistic or difficult for caregivers to monitor patient hydration. There is no simple measurement for hydration. Instead, to get at such information, potentially overburdened caregivers must monitor patients' fluid intakes around the clock, watch for signs of dehydration such as irritability, or look for regions of swelling that may indicate edema.

A passive measuring device would offer 24/7 measurement of hydration that can be monitored remotely by doctors. Detection of dehydration before it becomes severe would improve quality of life, reduce costs, and prevent some cases that can put patients in the hospital for long periods of time. And, such a sensor would also aid early detection of edema. A sensitive sensor might pick up small systemic changes in hydration that occur because of edema in the limbs, but a local sensor placed on the upper torso may also identify the more severe pulmonary edema present in the lungs. Pulmonary edema occurs rapidly and is often fatal if not treated. Similarly, edema presenting in the abdomen indicates poor heart function and may signal an upcoming cardiac event.

Furthermore, other demographics serve to profit from a noninvasive, simple hydration measurement. Current BIA devices are expensive, inconvenient to carry around, and require approximately 10 minutes to set up and take a measurement. A local device placed under the shirt could continuously measure hydration more conveniently and provide information on trends throughout the day. Such a device would be useful to athletes, people exercising with conditions that must be monitored, and researchers testing anything that might cause or depend on hydration changes.

4. TECHNICAL APPROACH

This section describes the options that the team will consider for the project. It also contains a test plan for investigating hydration correlations.

4.1. EXPERIMENTAL TESTING

4.1.1. Impedance Measurements

As described in Section 2.2, there are four main methods of measuring hydration using impedance. Each method was evaluated for its potential to meet the objectives and constraints detailed in Section 1.2.

For EIT, several electrodes surround a body segment such as a leg or torso in order to generate a topographical map of the cross section of the body segment. Although hydration and other biological signals can be derived from this method, the requirements for the number and placement of electrodes in EIT hinder the possibility of this method being integrated into the Proteus patch. Because the patch is not long enough to fully surround a body segment, and because Proteus desires the patch to be placed on the torso, EIT cannot be used for this project.

BIA and BIS techniques are commonly used in commercial devices. Each involves measuring the magnitude of impedance at one or more frequencies, and then calculating hydration using experimentally determined linear regression equations for different populations. BIS uses more frequencies than BIA to differentiate between intracellular water (ICW), extracellular water (ECW), and total body water (TBW). Because local measurements may behave differently than the usual full body measurement, if BIA or BIS techniques are to be used, it is necessary to investigate several frequencies to determine which ones are ideal for the local impedance measurements.

The last technique, BIVA, uses the measured values of resistance and reactance to calculate impedance and phase angle of the body, and then constructs a vector plot. For full body measurements, this vector plot has been correlated to a probabilistic distribution of hydration data to determine whether a person's hydration level is healthy. BIVA creates a relative rather than absolute measurement of the amount of water in the body because it does not directly calculate hydration. However, it can be used to monitor an individual's change in hydration over time.

Because relative measurements do not depend on patient factors such as height, gender, etc. like the absolute measurements obtained through BIA, the team wanted to pursue a BIVA approach for the interpretation of impedance data. However, because there is not already a distribution of hydration data for local measurements, in order to interpret measurements with BIVA, a large, unrealistic amount of data would need to be collected for various demographics. The team decided to use BIA methods to first establish if there is a correlation between local and full body impedances and hydration.

4.1.2. Hydration Device Alternatives

The team needed to purchase a commercial device that would produce an accurate hydration reading to use in conjunction with the raw impedance measurements. In addition to this, the team decided to purchase a device that would perform the impedance measurements that was already approved for use on human test subjects to gather data. This ensures that the test subjects will be safe and that the measurements will be accurate. The impedance measurements from the commercial device also provide a potential comparison for circuitry designed by the team that will be described in detail in Section 4.3.

4.1.2.1. Tanita Scale

The first option is a Tanita scale. The basic Tanita scale uses four electrodes on the surface of the scale to measure the impedance through the user’s two legs. The scale sends a 50 kHz, 500 µA AC signal through the user and then calculates the total body water percentage by weight [10]. The device is considered accurate, and correlates well with traditional laboratory hydrations tests as discussed in Section 2.1. Other Tanita scales have additional electrodes that are held in the hands while the user stands on the scale. . However, the device only outputs a hydration value rather than specific impedance values, and studies indicate that the scale method of measurement is less accurate than those that place electrodes on the hands and feet.

4.1.2.2. Bodystat

The Bodystat devices consist of four electrodes and a computational unit. Two electrodes are placed on the wrist and two are placed on the ankle. The Bodystat1500 uses a single carrier frequency of 50 kHz and outputs both the impedance values and the calculated hydration measurement. The Bodystat1500MDD takes measurements at both 5 and 50 kHz. It then outputs the impedance at each frequency, and the resistance, reactance, and phase angle from only the 50 kHz input. The device also calculates total body water from these measurements. A third model called the Bodystat Quadscan4000 provides impedance values at 5, 50, 100, and 200 kHz, along with the resistance, reactance, and phase angle measurements at 50 kHz and the total body water hydration result [5]. Based on the studies of Section 4.1.2, the Bodystat devices are well correlated with other hydration tests. Additionally, because the Bodystat devices allow users to place the individual electrodes, the device can output the accurate impedance, resistance, reactance, and phase angle measurements for any electrode configuration within reach of the cables. Additionally, many medical device companies make similarly structured four electrode devices. The team's research, though, indicates that the Bodystat provides the most useful output forms for its price.

4.1.2.3. Comparison of Devices

Both devices would be able to be used as a hydration comparator. Unlike Tanita scales, however, the Bodystat provides raw impedance measurements for multiple carrier frequencies that could be used to obtain accurate data safely and without having to purchase separate devices to perform impedance measurements. And because the Bodystat electrodes could be placed anywhere, the device can be used to take local measurements. Additionally, based on research studies, the Bodystat technology is more closely correlated to tritium dilution hydration results than the Tanita technology. For these reasons, the team chose to perform both impedance and hydration testing with a Bodystat device.

Of the available Bodystat devices, the 1500MDD or Quadscan 4000 would provide more useful results than the 1500 because the 1500MDD and Quadscan output the measured resistance, reactance, and phase angle in addition to the impedance value that the 1500 displays. The team decided that the Bodystat Quadscan would be more appropriate for testing because it comes with software that generates BIVA graphs, and measures impedance at more frequencies than the 1500MDD. Having more frequencies available to test is important for understanding the optimal electrical parameters for local impedance measurements. After purchasing the device, the team decided that the BIVA graph software would not be useful to the study because its capabilities were limited and could not display the extreme angle and magnitude differences exhibited on local measurements. However, the team still chose to examine the effect of phase angle in order to determine if a BIVA analysis would produce any meaningful results.

4.2. TESTING DESIGN

This section contains details on the type of tests completed and to be completed. Detail on the exact test procedure is provided in Appendix A. IRB Information. The industry standard for measuring hydration using impedance techniques is to place two electrodes on the wrist and two on the ankle on the same side of the body (the right side is recommended). This produces a full-body, four-electrode measurement. All local measurements that the team performed are compared to a full-body measurement taken at approximately the same time. The team focused on the following areas of study for testing.

1. Number of Electrodes: The team has tested how two- and four-electrode local measurement methods compare with each other and correlate with the industry standard of four-electrode, full-body, 50 kHz measurements.

2. Local Measurements: The team has tested how well local (with approximately 6 cm between sensing electrodes) measurements correlate with the industry standard, while holding all other test variables constant.

3. Patch Placement: The team has tested how well local measurements in different positions and orientations on the torso correlate with the industry standard. The positions tested are as follows: centered around the bellybutton, along the right side lower ribcage line, and on the right side pectorals below the clavicle. Left side measurements will not be studied due to possible concerns of the current interfering with the function of the heart, although any electrical signal inserted into the body should meet strict safety requirements.

4. Electrode Surface Area and Connection Type: The team has compared the normal Bodystat electrodes with half-sized electrodes generated by reducing the size of the normal electrodes. Further studies may be conducted on electrodes of a different type.

5. Variability and Reproducibility: The team has tested the variability and reproducibility of impedance measurements. The team will consider how the measured impedance ranges affect the calculated hydration variability and reproducibility, and whether such ranges are acceptable.

6. Carrier Frequency: Although literature indicates that 50 kHz is the optimal frequency for full body measurements, the team has also considered 5, 100, and 200 kHz frequencies (the range of the Bodystat Quadscan 4000) because such tests have never been conducted locally to the team's knowledge.

7. Miscellaneous Variables: The team has extended its variability and reproducibility measurements to include variability and reproducibility with the introduction of miscellaneous conditions that may affect the reliability of measurements. This has included the effect of eating and drinking prior to testing. Some tests have also investigated the effect of sweat and wear over time. Further ambient conditions may be tested, such as humidity, temperature, or dryness of skin.

While waiting for the arrival of the Bodystat, the team began by mathematically modeling the difference between the two- and four-electrode measuring techniques with the intent of obtaining preliminary information on the feasibility of the two-electrode technique. Specifically, a statistical analysis was employed to see what degree of variation of the electrodes would be acceptable to discern separate body impedance values. The results from this simulation are described in Section 5.4.1.

4.3. CIRCUITRY DESIGN

While conducting tests using the Bodystat device, the team began developing their own impedance measurement circuitry that could possibly be integrated into the Proteus patch. A literature search provided many references to using the AD5933 chip and evaluation board as part of this circuitry, which the Proteus liaison also suggested. The AD5933, produced by Analog Devices, is a 12-bit impedance converter and network analyzer that is capable of analog-to-digital conversion (ADC), computing the discrete Fourier transform (DFT), and digital signal processing (DSP) [35]. The AD5933 can also be purchased with an evaluation board, AD5933EB, which includes all of the circuitry necessary to power the chip, attach an impedance of interest, and interface with a computer through USB. The board can be powered through USB by a laptop that is not plugged into the wall, which is a safer way to power a device that will inject current through a human subject [36].

The AD5933EB, as purchased, is set up for two-probe measurement. The device injects a controlled voltage into the impedance under test so that the impedance may be measured. For this project, the team requires a four-probe measurement to increase the accuracy of the data in addition to a controlled current source to ensure that the signal injected into a human subject is safe. Several bioimpedance research papers have suggested an analog front end (AFE) circuit that can be used with the AD5933 to adapt the measurements to four-electrode measurements with a controlled current source. The block diagram of this circuit is shown in Figure 10 [37].

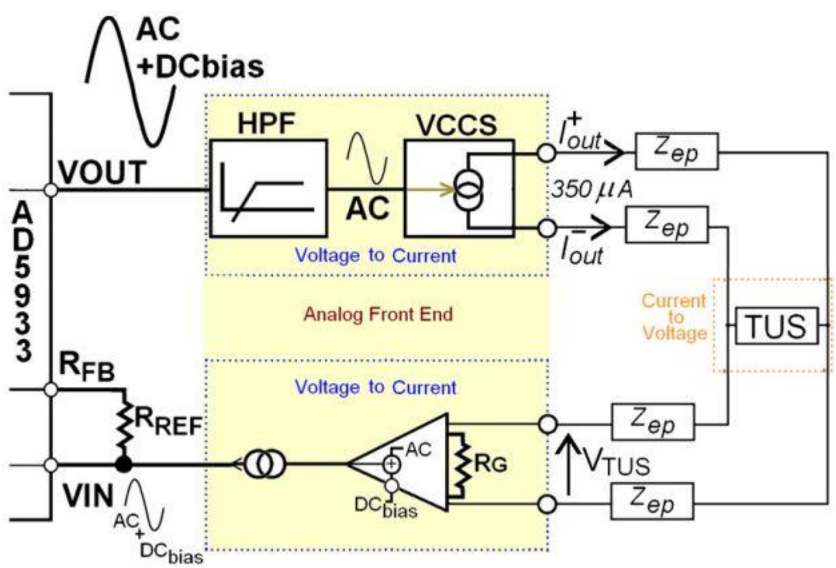


Figure 10: Block diagram for adapting AD5933 for four-electrode measurements. [37]

The output voltage signal from the AD5933 must be run through a high pass filter to remove the DC component because DC current can be dangerous to humans. Then the AC voltage needs to be converted into a constant AC current using a voltage-controlled current source (VCCS). The resulting signal is sent out through the two signal electrodes and the resistance under test (denoted in Figure 10 as TUS, or “tissue under study”). The two sensing electrodes then detect the voltage across the resistance under test, and a differential amplifier sends the detected voltage difference back into the AD5933 to be converted into an impedance measurement.

This analog front end has been implemented by several research groups. One study presented at a 2015 conference on bioimpedance reported the components used for this circuit, and also compared the effectiveness of two different VCCS circuits, the mirrored modified Howland current source and the load-in-the-loop current source. The tests conducted in this paper suggest that the modified Howland source has generally less error in measuring the impedance and phase than the load-in-the-loop source, but both circuits are good enough to be used for bioimpedance measurements [38].

5. RESULTS & ANALYSIS

5.1. PRELIMINARY TESTS

5.1.1. Sitting Up vs. Lying Down

The Bodystat Quadscan 4000 instructions say that a user should lie down for three to five minutes prior to measurement. The exact reason for this requirement is not explained, but it may be because the body is more active when standing or sitting up, and noise is reduced when the user lies down. Additionally, the body has a different blood pressure and heart rate when standing than when lying down, and these factors could also affect measurement. However, the Proteus patch may be taking measurements when a person is standing or seated. Therefore, the team wanted to determine whether measurements could be consistent (and therefore usable) when a person was sitting up.

For these tests, the four-electrode, full-body Bodystat setup was used because this is how the Bodystat is meant to be used, and therefore gives the most accurate determination of whether hydration measurement accuracy changes with position. One member of the team wore four electrodes: two on the ankle, and two on the wrist, as instructed. Measurements were then taken with the individual sitting up, and then five minutes after lying down. This data is shown in Table 1 below. Additionally, a comparison was made between limbs touching and not touching, as this is another factor that the Bodystat instructions advise to avoid, but that might be encountered during Patch use.

Table 1: Preliminary Bodystat test results performed on a team member who sat up and then laid down for five minutes. Repeated measurements were taken for the measurements taken lying down with limbs spread to form a baseline, since this is the way the Bodystat is meant to be used.

	Sitting Up	Lying down, Limbs touching	Lying down, Limbs not touching (Baseline 1)	Baseline 2	Baseline 3	Baseline Average
TBW (%)	68	64.4	63.4	63.2	63.1	63.23
Impedance at 5 kHz (Ω)	445	484	449	504	504	485.7
Impedance at 50 kHz (Ω)	375	410	420	422	423	421.7
Impedance at 100 kHz (Ω)	348	387	392	395	395	394
Impedance at 200 kHz (Ω)	328	374	372	374	374	373.3

For measurements at 50 kHz, impedance changes by about 11% between a measurement with the user sitting up and one with the user lying down with their limbs apart. When three measurements were repeated for this standard baseline, there is a difference of only 0.7%. This suggests that the impedance values for the different positions are too different from each other to ignore. The data also show that when limbs are touching and a full-body measurement is taken, the impedance at 50 kHz may vary by roughly 2.8%. This is perhaps due to the fact that the current takes a different path through the body when limbs are in contact. While this isn't a large amount, it is still significant compared to how much the baseline itself varies. Because of these findings, the team found it best to take measurements the way the Bodystat was meant to be used for initial testing, but to keep these problems in mind for later, more advanced testing. The testing protocol was amended so that participants lie down for five minutes before taking tests, and ensure that their limbs are not touching during measurement. This practice will standardize the testing procedure and likely improve results.

5.1.2. Ingestion

Ingestion was another variable that the team investigated. Because the local measurements were generally taken on the stomach, the team hypothesized that ingestion of food or drink could affect the measured impedance and therefore give an inaccurate hydration measurement. Therefore, measurements in general were standardized by waiting at least two hours after eating or drinking.

Some tests were done to analyze this hypothesis. One team member took some Bodystat data over a span of almost three hours, taking four-electrode measurements on the full-body and the stomach, in order to analyze what would happen with ingestion. Three data points were taken before the participant had eaten or drunk that day, and then three more points were taken after the participant had drunk a smoothie. The results for this experiment are shown in Figure 11 and Figure 12. In Figure 11, full-body impedance was normalized in order to compare it to local impedance.

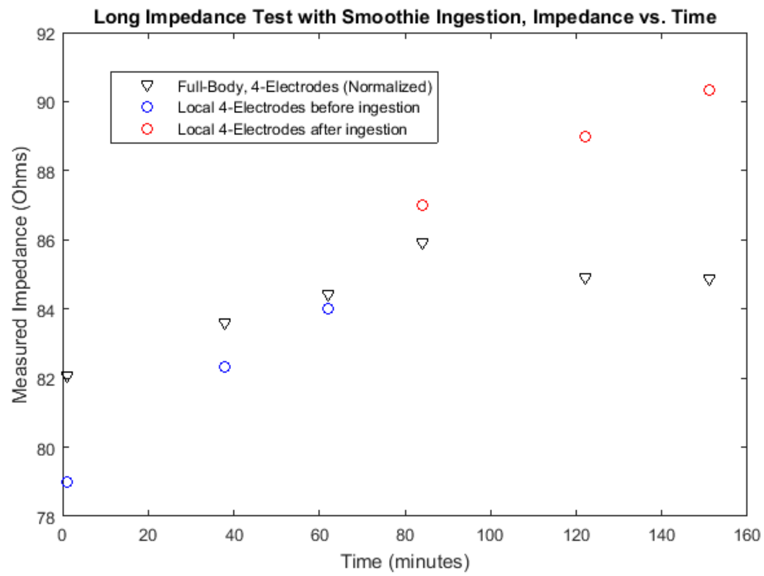


Figure 11: Impedance measurements taken over a span of 160 minutes. Points in black represent the full-body impedance. The other points represent local impedance taken on the stomach, where red points were measured after the participant had drunk a smoothie.

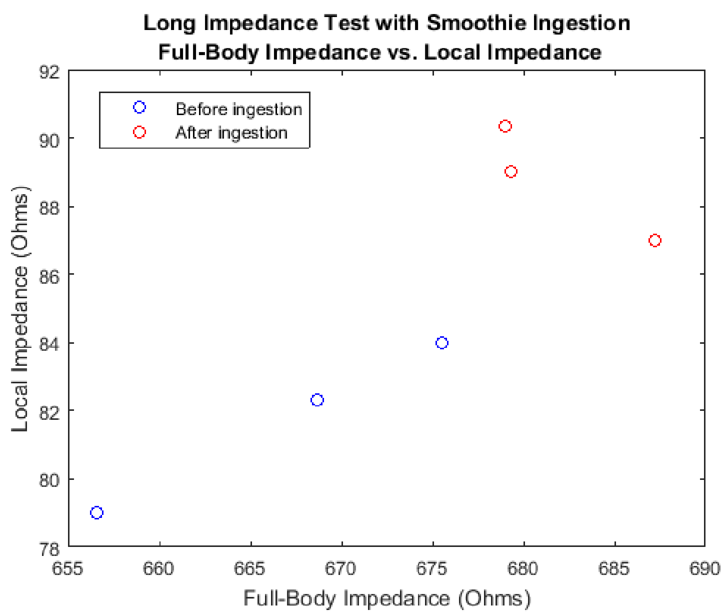


Figure 12: Impedance measurements taken over a span of 160 minutes. Points in red were measured after the participant had drunk a smoothie.

The data shows some interesting results. Both graphs show that the points taken before ingestion are very consistent. Impedance increases over time for both measurements, and these measurements are well correlated. This could be due to the participant becoming more dehydrated, or due to electrode degradation. After ingestion, the full-body impedance decreases while the local impedance increases. It is possible that the stomach measurements continue to be affected by electrode degradation or have not seen any changes in the body as a result of hydration. The full-body impedance decreases, suggesting that hydration increases from the liquid that was drunk. This results in a poor correlation between the two impedance measurements.

It can therefore be concluded that ingestion may have some effect on the measurements that is not desired. At this stage, it is difficult to tell whether impedance from the full-body or the local measurement is more adversely affected by ingestion. The team would like to pursue more testing in this area to understand these changes. However, for most tests, it is advised to avoid ingestion at least three hours before taking measurements.

5.2. FULL BODY IMPEDANCE AND HYDRATION

The Bodystat outputs values for total body water in liters and as a weight percent in addition to the raw impedance data that it measures. These values are calculated based on regression equations that rely on all of the measured impedance values and the inputted demographic information of the patient. The team wanted to establish a correlation between the impedance measurement and the total body water calculation to ensure that correlating the local impedance to the full body impedance would be sufficient and to confirm the integrity of the device. All measurements of total body water and full body impedance were compiled into Figure 13 below.

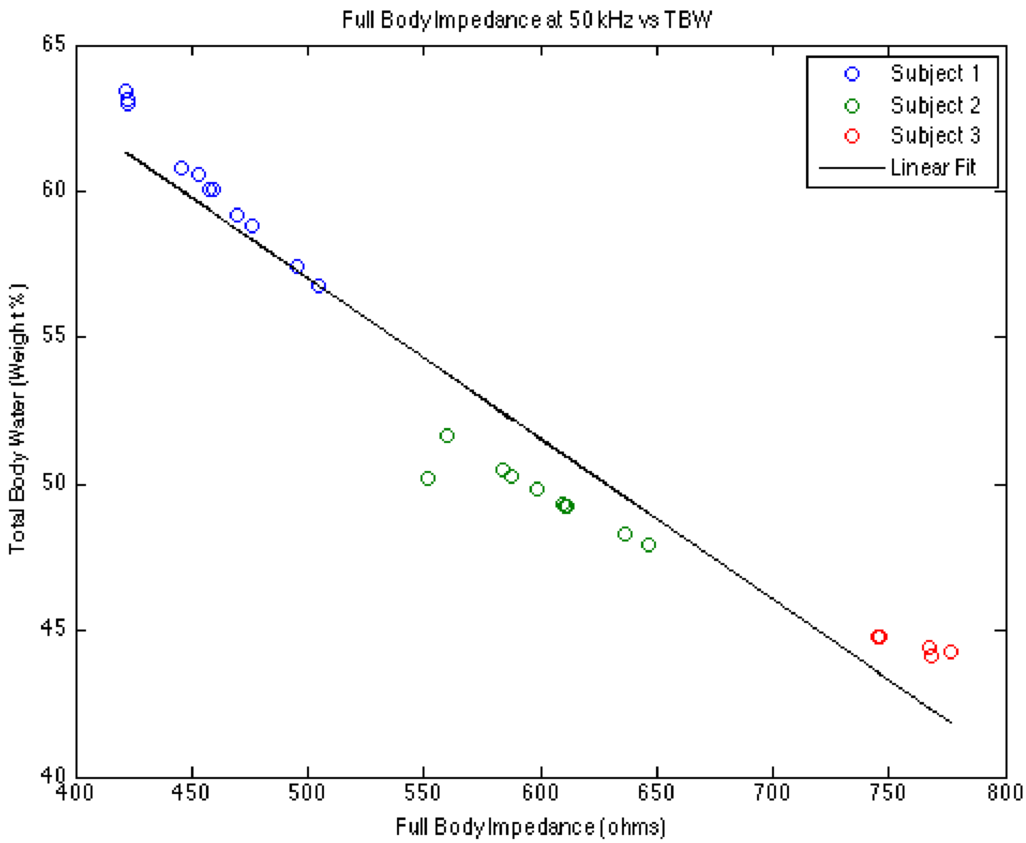


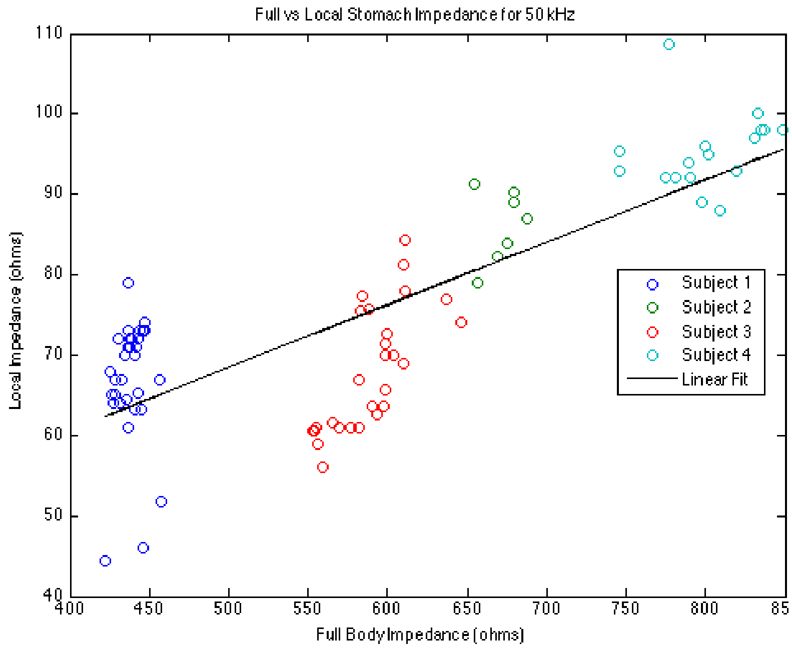
Figure 13: Total body water percentage plotted against full-body impedance at 50 kHz.

Correlations were calculated in MATLAB using the `corr` function, which computes Pearson's linear correlation coefficient. Each individual's data follows a line of its own slope. However, together the data exhibit a correlation of -0.9677. A correlation of -1 indicates that the two variables are inversely related. Through linear regression, the data fits to a line of $TBW = -0.0550 * Full\ Body\ Impedance + 84.53$. This line has an R^2 value of $R^2 = 0.9364$. Both the R^2 value and the correlation indicate that the total body water calculated by the Bodystat is well correlated with the measured full body impedance.

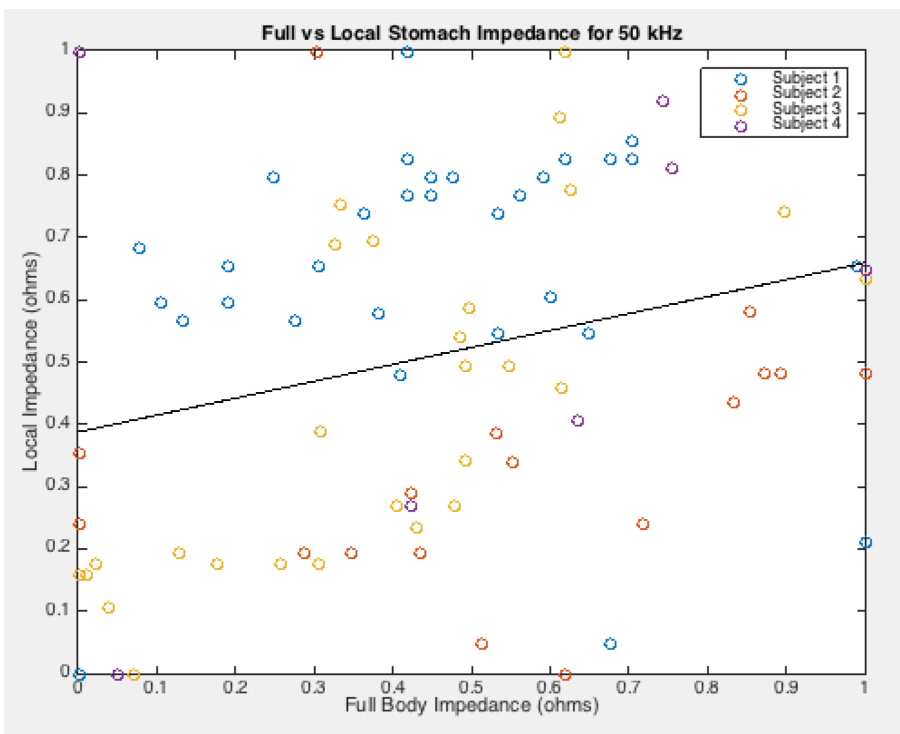
Imperfections in the correlation are due to the other parameters that the Bodystat uses to calculate total body water. This is seen by the offsets of the lines between each individual's data.

5.3. LOCAL AND FULL BODY IMPEDANCE

As described in Section 4.2, one important aspect to test is whether localized impedance can correlate to full body impedance. Because the Proteus patch is often worn on the torso, the team has so far collected the most data with electrodes on the stomach, centered around the belly button. Other wear locations will be discussed in Section 5.6. Figure 14 below contains the local, full-body data set for each of the four researchers.



(a)



(b)

Figure 14: (a) The collective data of all researchers for local impedance on the stomach plotted against full body impedance. (b) The same data normalized from 0-1 on both axes for each data set.

For the non-normalized data, the linear equation is $Local\ Impedance = 0.0781 * Full\ Body\ Impedance + 29.39$. This regression equation has an R^2 value of 0.6491. The two variables also have a correlation of 0.8057. For the normalized data, the correlation is 0.2686. Examining the data as a whole, a majority of the data from Subject 1 does not fit with the trend exhibited in Subjects 2, 3, and 4. However, Subject 1 also has the largest quantity of individual data points, causing a large influence on the overall linear fit of the plot. The set of data on Subject 1 was taken over more separate testing sessions compared to that of Subject 3 and Subject 4. This may contribute to the noisier behavior exhibited. The variation in the data may be due to inaccuracies in electrode placement between tests, as the team discovered through during initial testing that exact electrode placement has a significant effect on the repeatability of measurements.

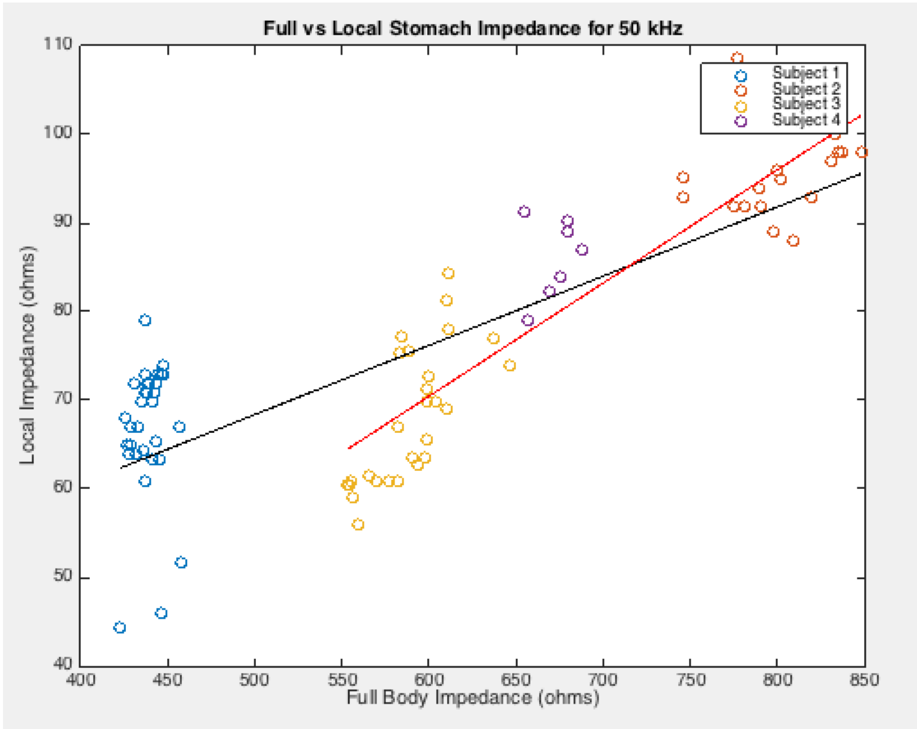
Examining each individual's set of data yields the correlations contained in Table 2 below.

Table 2: Correlations between each individual's full body and local impedance data, and the R^2 value generated from a linear fit.

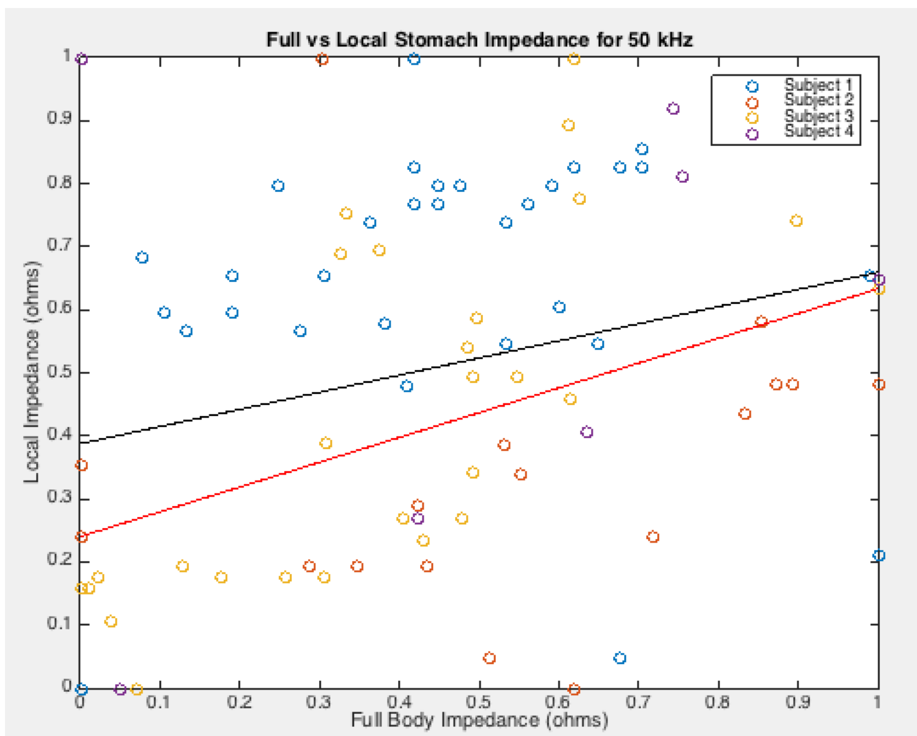
Subject	Linear Fit Equation	Correlation	R^2
1	Local = 0.0693*Full + 36.45	0.0802	0.0064
2	Local = 0.1022*Full + 17.51	0.2733	0.0747
3	Local = 0.2298*Full – 67.30	0.7140	0.5098
4	Local = 0.0293*Full + 71.76	0.1883	0.0355

The most promising correlation occurs in Subject 3's data with a correlation of 0.7140, and an R^2 value of 0.5098. The collective data from Subjects 1, 2, and 4 does not indicate a strong correlation. The current data indicates that local impedance measurements taken on the stomach could be used to distinguish between the test subjects, but it might not have great enough resolution to determine the relative hydration state for one subject.

Additionally, Subject 1 is the only male subject, and sex is a strong determining factor used in the typical regression equations for conventional BIA because the body compositions of the two sexes is typically different. In particular, the proportions of mass to body fat are often different, and muscle is more conductive than fat. Because of this, many studies only look for impedance-hydration correlations within sub-populations as specific as African-American men in a certain age range, and so it is appropriate to also examine the correlation of the female subjects in isolation. This exclusion is further justified by the normalized graph in Figure 14 (a). In this display, it is clear that Subject 1's data seems to by y-axis shifted upwards from the other subject's data. It is likely that this reflects a difference in body composition. This is shown in Figure 15 below.



(a)



(b)

Figure 15: The collective data of all researchers for local impedance on the stomach plotted against full body impedance. The black fit line is for all subjects. The red fit line is excluding the male subject, subject 1. The bottom graph, (b) shows the normalized data set.

Excluding subject 1 improves the combined correlation from 0.2686 to 0.4132 for the normalized set, and from 0.8057 to 0.9044 for the original set

.

Because the local measurements are so sensitive to electrode placement, it may be more useful to examine the data taken during continuous time periods when the electrodes were not removed. Individual testing sessions where electrodes were not moved and the subjects were inactive during testing. The results of these tests are given in Figure 16 - Figure 19.

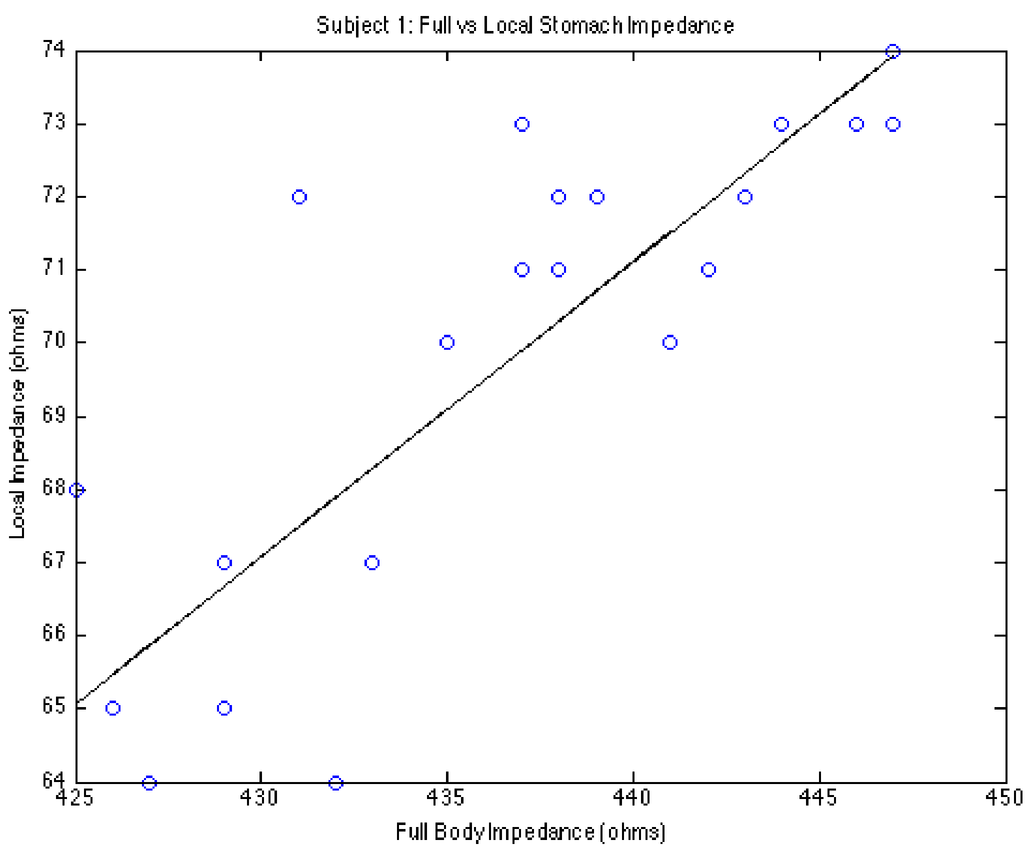


Figure 16: Data from Person 1 for full body versus local impedance for a single session of testing.

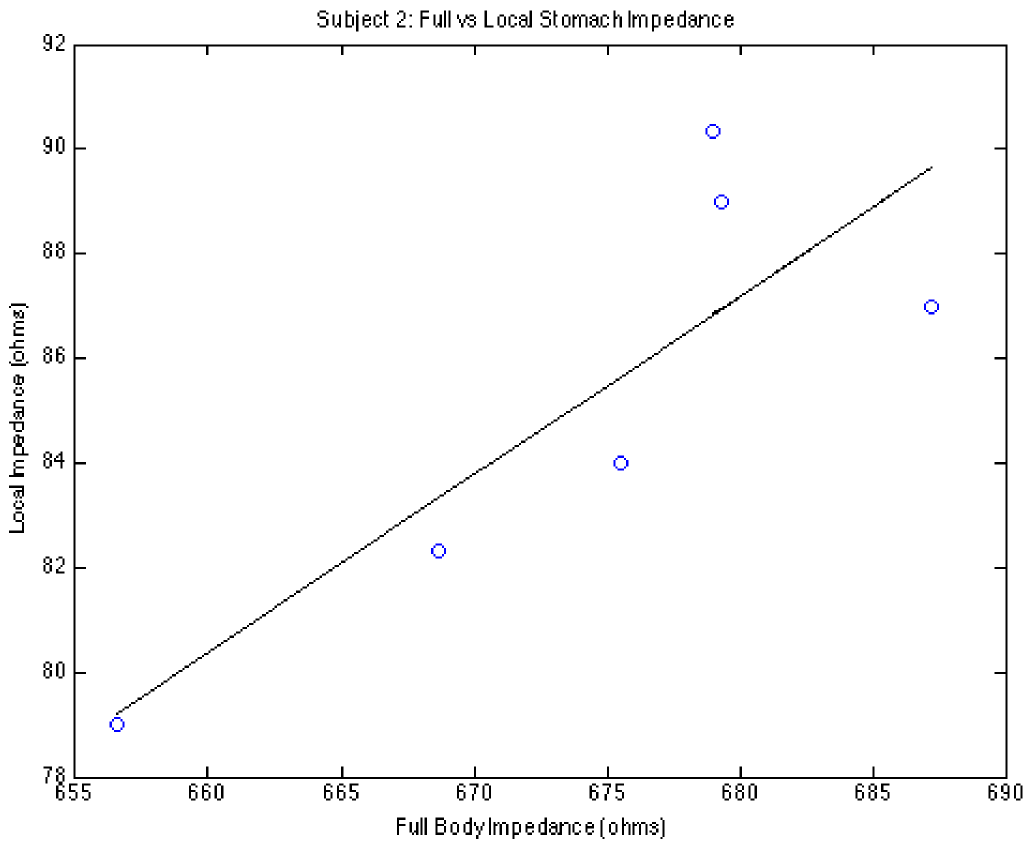


Figure 17: Subject 2’s local vs. full-body impedance data for a single testing session.

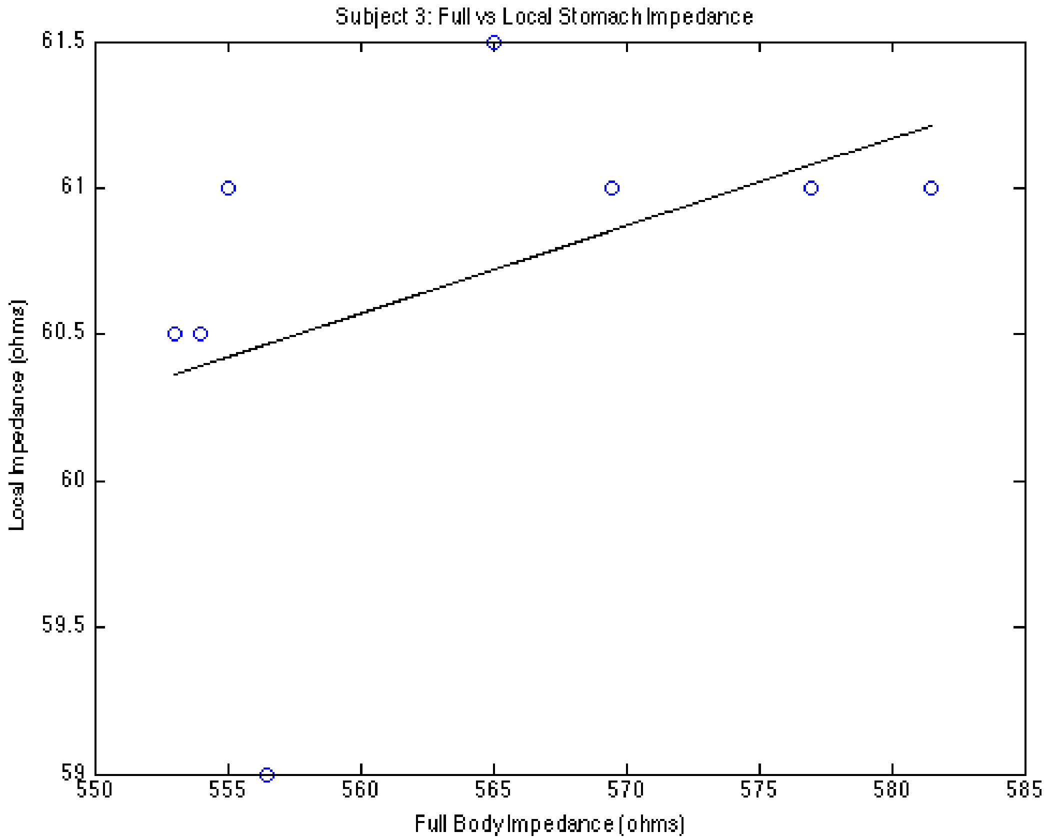


Figure 18: Subject 3's full-body versus local impedance for a single session of testing.

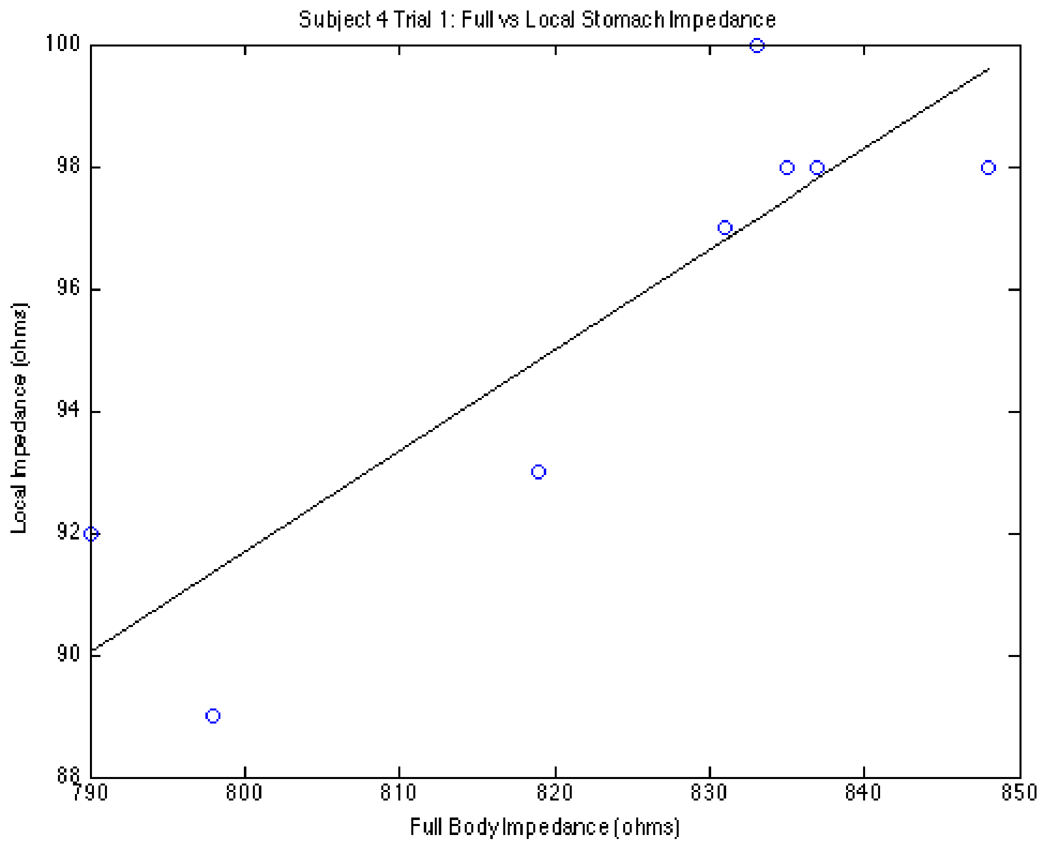


Figure 19: Data from Subject 4 for local versus full-body impedance during the first of two testing sessions.

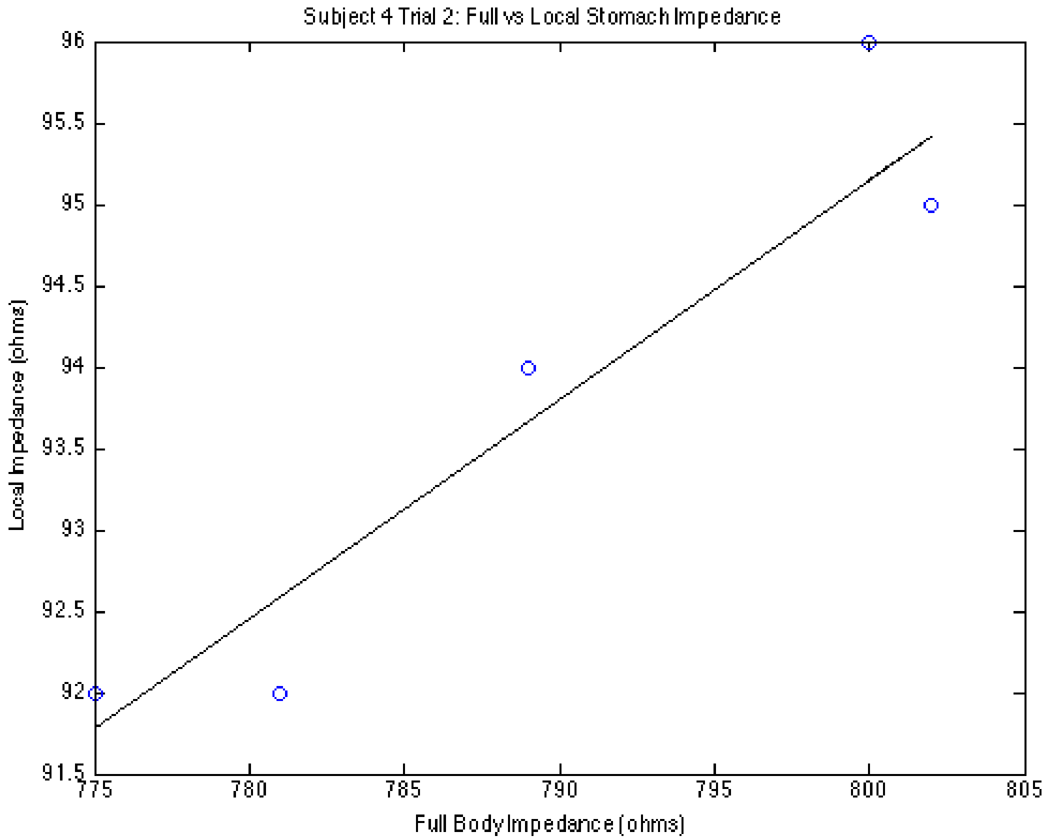


Figure 20. Local vs. full-body impedance data for Subject 4 during a second session of testing.

The data from Figure 16 spans approximately four hours with data taken from Subject 1 every 10 minutes. The samples from Figure 17 were collected about every 20 minutes over a period of about three hours. For Figure 18, samples were taken on Subject 3 approximately every 10 minutes for a total of one hour and 30 minutes. For Subject 4, two sessions were completed. The first is contained in Figure 19 where data was taken approximately every 20 minutes for about two hours and 30 minutes. The second set of data in Figure 20 was taken every 10 minutes for an hour. Table 3 contains a summary of the results from this testing.

Table 3: Correlations between the full body and local impedance data, and the R^2 value generated from a linear fit from the individual long tests.

Person	Correlation	R^2
1	0.8384	0.7029
3	0.4412	0.1946
4 – Trial 1	0.8748	0.7653
4 – Trial 2	0.9419	0.8871

As shown in Table 3, correlations are much higher for each person, except for Subject 3, than those found from the full set of data in Table 2. As seen in Figure 17, Subject 3 exhibited a single extreme outlier at a point where full body impedance equals 556.5 Ω, and local impedance equals 59 Ω, which likely accounts for the low correlation. For this dataset, besides the outlier, the full body impedance increases over time while the measurements on the stomach stay about the same. If these measurements are credible, this could indicate that there is a limit to the amount of dehydration that occurs in the abdominal region, as opposed to other parts of the body.

Despite the lower correlation from Subject 3, Subject 1, Subject 2, and Subject 4 exhibited a large increase in correlation when only considering a single test session. This also indicates that despite the researchers' attempts to control for all variables, the controls in testing approach may not be sufficient to obtain consistent data across multiple days. However, for the actual intended use of the patch for long term monitoring, tracking the change in impedance over time could provide useful data on the relative hydration of the user.

5.4 TWO- AND FOUR-ELECTRODE MEASUREMENTS

Two- and four-electrode local measurements were compared against each other and against the industry-standard full body four-electrode measurement. The Proteus patch is currently configured in hardware and software to conduct two-electrode measurements. Thus, these tests were necessary to determine whether such a configuration was sufficient, or if reconfiguration to the four-electrode measurement is necessary. The two configurations were compared using two metrics: variability of measurement, and correlation between each configuration and the industry standard.

5.4.1 Simulation of Two-Electrode Measurements

The current Proteus patch has two electrodes and can take impedance measurements to determine whether the patch is in contact with the skin. However, a two-electrode measurement can be shown analytically to be less accurate at measuring small impedances than a four-electrode setup, as described in Section 2.2.4, due to its measurement of the electrode-skin interface in addition to the measurement of tissue impedance. To quantify how much the tolerances on the electrode-skin interface affects the voltage measured across the resistance of interest, the team performed a simulation on the two-electrode setup.

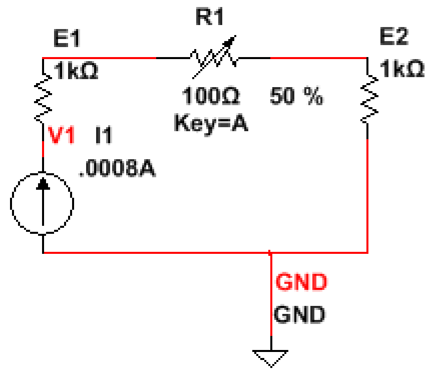


Figure 21: Schematic for two-electrode simulation.

The team created a schematic for a two-electrode measurement setup in Multisim, shown in Figure 21, with the resistances E1 and E2 representing the resistances of the electrodes. R1 is the resistance under test--this is the tissue impedance to be measured. The values of E1, E2, and R1 were chosen to conservatively represent the approximate range of expected impedance values for a local measurement, based on data from the Proteus patch. A Monte Carlo simulation was performed in which E1 and E2 were assigned normally-distributed tolerances for each run, while R1 was varied from 75 to 150Ω. Experimental tests later determined that the impedance range measured by two electrodes on the stomach is about 300-400Ω. This goal of this simulation was to determine the maximum tolerances on the electrode-skin interfaces, E1 and E2, such that the team could still determine that the tissue impedance, R1, had changed with 95% confidence. These maximum tolerances tell us the accepted maximum variability in the electrode-skin interface such that the two-electrode measurement can accurately measure tissue impedance.

From the 1000 runs of the Monte Carlo simulation, a 95% prediction interval was determined. First, the team simulated using the tolerance for the two-electrode measurement experimentally determined in Section 5.4.2, which was 5.56%, assuming a normal distribution. A tighter tolerance of 2% was then tried. The results are summarized in Table 4 below. The team sought a confidence interval of $\pm 5\Omega$ because this is the approximate resolution needed to determine a dehydration change over the course of a three hour test based on experimentally determined hydration changes for these tests using the full body four-electrode method. However, the results show that this cannot be achieved with a plausible electrode tolerance.

Table 4: Confidence intervals for two-electrode measurements with varying electrode tolerances.

Tolerance	Standard Deviation	95% Prediction Interval
2%	9.3Ω	±18.6Ω
5.56%	27.5Ω	±55Ω

These results tell us that the observed tolerance on the electrode-skin interface is too variable to determine hydration changes with statistical significance. That is, the a measured change in impedance between two time points is too uncertain due to variability of the electrode-skin interface to confidently determine if an impedance change is due to changing hydration. To eliminate this, the variability of the electrode-skin interface would have to be drastically reduced.

5.4.2 Variability of Two- vs. Four-Electrode Measurements

The variability of each configuration was measured by calculating the variance of repeated measurements. All variables were held as constant as possible—measurements were taken immediately after one another to hold the hydration state, and the patient remained still in order to keep electrodes connections as consistent as possible. The findings are shown in Table 5 below, in which the pooled variance has been computed for two different people over five tests.

Table 5: Standard deviations and impedance change magnitudes for a two-electrode configuration vs. a four-electrode configuration.

	Variance	Standard Deviation	% Variance	% Standard Deviation
Full	15.857	3.982	2.787	0.6999
Local 4E	2.267	1.506	3.418	2.270
Local 2E	155.992	12.490	34.696	2.778

The variance of the full body measurement is very small—the measurement is consistent. Similarly, the standard deviation for the local measurement is small, and the absolute standard deviation is actually smaller than the absolute standard deviation for the full body measurement. It is higher in percentage terms because the four-electrode local measurement is a magnitude smaller than the full body measurement.

However, the two-electrode local measurement is significantly more variable. This variability seems to be in large part caused by a drift that occurs over time for this measurement. The team is unsure of the exact cause of this drift. The full body and local four-electrode measurement sometimes drift as well. The team hypothesizes that this drift is caused by decaying electrode contact over time, which would increase the impedance of the electrode-skin interface. It is logical that this drift was only observed for the two-electrode measurement, and not the four-electrode measurement, because the four-electrode measurement is specifically designed to avoid measuring the skin-electrode interface. The team believes that this is a major, prohibitive problem with the two-electrode measurement. It is very likely that such drift would make changes in impedance caused by hydration indistinguishable from changes in impedance caused by the variability of this interface.

During a hydration change, the impedance for the local two-electrode method changes more significantly than that of the other two configurations. The change in impedance is so large for two-electrode measurements that it is likely affected by more than just hydration changes. This is because the magnitude of the two-electrode measurement is similar to the full body measurement, but changes five to ten times more than the full body measurement for small hydration changes.

5.5 PHASE ANGLE

After reviewing the literature, the team decided that it would be worthwhile to consider using BIVA techniques to qualify hydration, as in Section 4.1.1. This analysis requires constructing a vector plot from the measured impedance magnitude and phase angle. Phase angle is generally a measure of the integrity of the cell membranes, represented by the capacitor in the Cole model (described in Section 0). Although phase angle may not correlate directly with total body water, it is expected that local phase angle measurements should correlate in some meaningful way to full body phase measurements. The data showed virtually no correlation between local and full body phase, however. As shown in Figure 22, all of the local and full body phase angle measurements are within different ranges for different people, but among a single subject's data points, there is no discernable correlation between local and full body changes in phase angle. This supports the idea that one person's phase angle is unlikely to change much as long as their general health remains the same. It also indicates that BIVA is unlikely to be feasible for local body measurements.

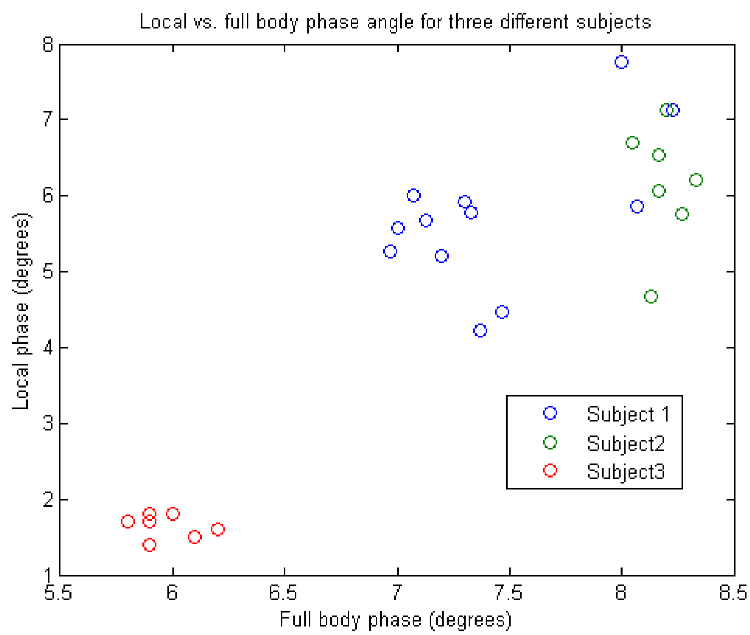


Figure 22: Local vs. full body phase angle measurements for three different subjects, all taken on the stomach with an outer electrode separation of 11 cm.

5.6 ELECTRODE PLACEMENT

From initial tests with the Bodystat, it became apparent that electrode placement has a large influence on the consistency of local impedance measurements, as predicted in Section 2.2.3. The team then carefully controlled the distance between electrodes during tests, and tried varying the distance between outer electrodes while keeping the distance between inner electrodes constant at 6 cm. This distance was chosen based on the geometry of the Proteus wearable patch. Figure 23 below shows an example of how the electrodes were placed on the stomach.

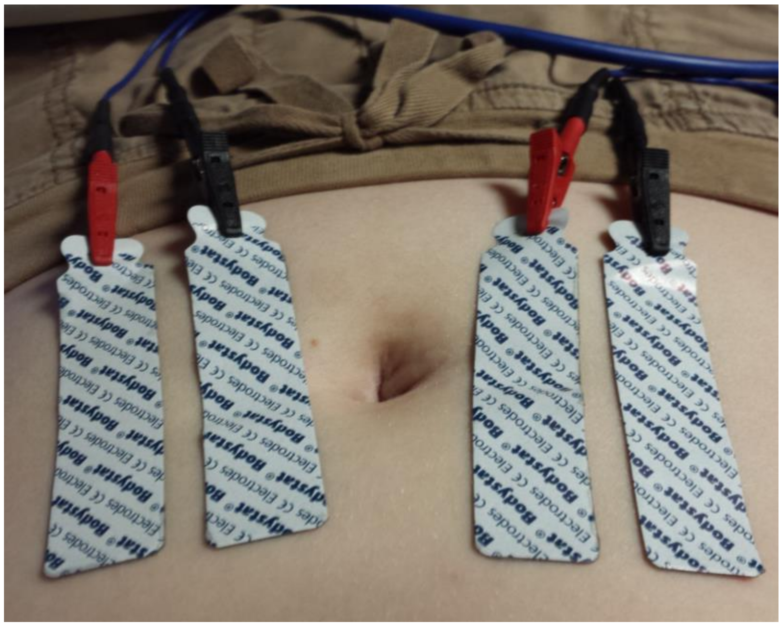


Figure 23: Example of the four-electrode local testing setup on the stomach. Here the inner electrodes were separated by 6 cm from their centers, and the outer electrodes are separated by about 11 cm from their centers. This electrode setup was used for a majority of tests on the stomach. Note that the colors of the alligator clips are incorrect in this photo.

These measurements were all taken with the electrodes centered on the navel. Figure 24 shows the local impedance measurements at 50 kHz with the outer electrodes location varied against total body water percentage for one subject. Table 6 gives the values for the calculated correlation between the impedance measurements and total body water percentage. These data show that stomach impedance measurements generally correlate with changes in total body water, but they can be noisy. We also see that this correlation declines as the electrode separation becomes too large (14 cm). Although the 11 cm measurements do not have the best calculated correlation, the team decided it would make the most sense to take measurements at this distance, since the patch is not very big and this was the smallest possible distance that could be used with the inner electrodes. Thus, all further measurements were taken with an inner electrode separation of 6 cm and an outer electrode separation of 11 cm.

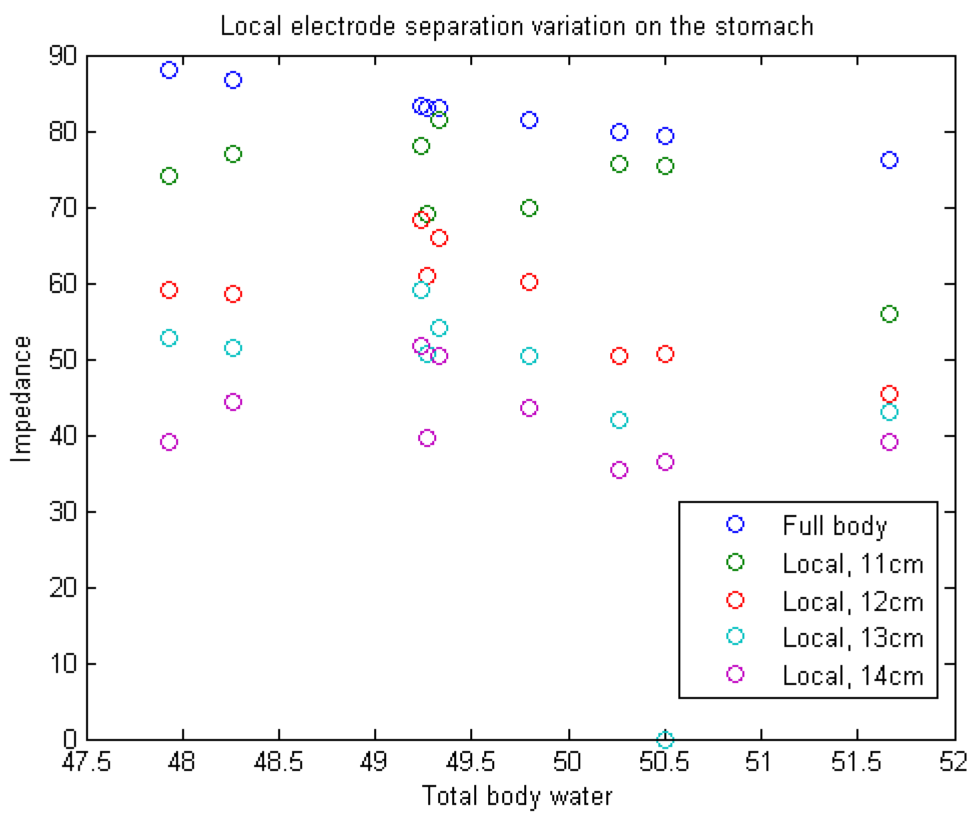


Figure 24: Local impedance measurements with inner electrodes 6 cm apart from their centers with outer electrode separation varied. The fully body impedance shown was normalized by 7.35 to be on the same scale as the other measurements.

Table 6: Correlations between impedance measurements and total body water percentage. This should ideally be -1.

	Correlation
Full body	-0.9981
11 cm	-0.6102
12 cm	-0.6753
13 cm	-0.6604
14 cm	-0.3448

To investigate whether the electrodes could be placed even closer, the team took the next set of measurements with full electrodes centered around the navel and compared those to measurements taken with electrodes cut in half lengthwise and placed in the same locations. For these tests, local impedance was compared to full body impedance rather than total body water because those two values correlate well (See Figure 13) and impedance is a more valuable measurement. . Figure 25 shows the results of these tests. Local measurements with both full and half electrodes are somewhat noisy but show a generally positive correlation with full body impedance.

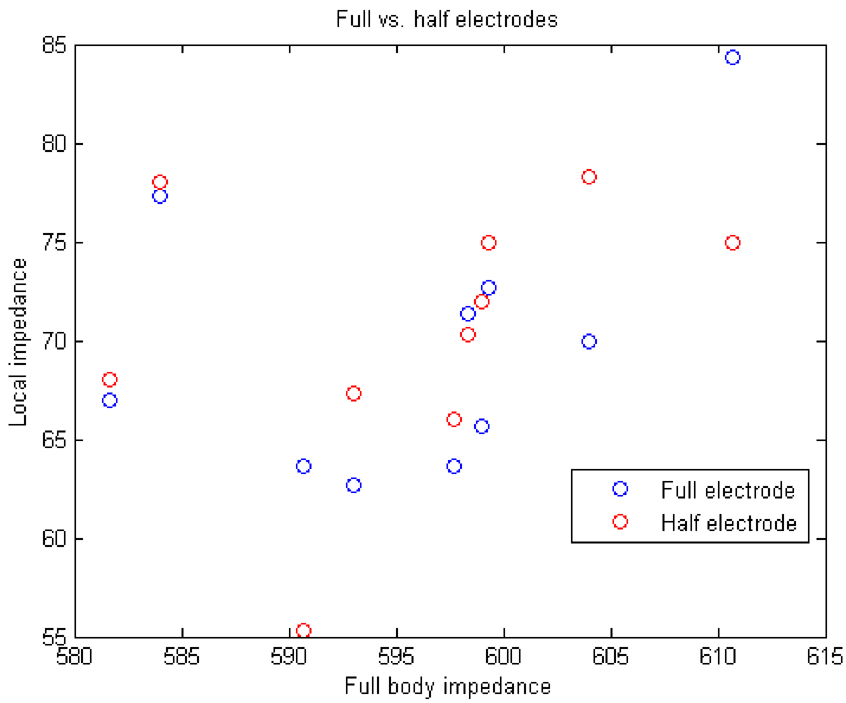


Figure 25: Local impedance vs. full body impedance for full electrodes and electrodes that have been cut in half lengthwise.

The more interesting result of these tests is that they produced two apparent outliers at 581.7 Ω and 584 Ω. These two data points could be strange because they were taken within an hour of eating. Suspecting that digestion could cause significant variation in local measurements taken on the stomach, the team next tested placing the electrodes on the ribs and chest and comparing those to measurements taken on the stomach. The results are shown in Figure 26. The correlation in this set of tests is not as strong as with other tests, but they do show that there is an outlier in the stomach measurements that is not seen in the rib and chest measurements, possibly due to digestion.

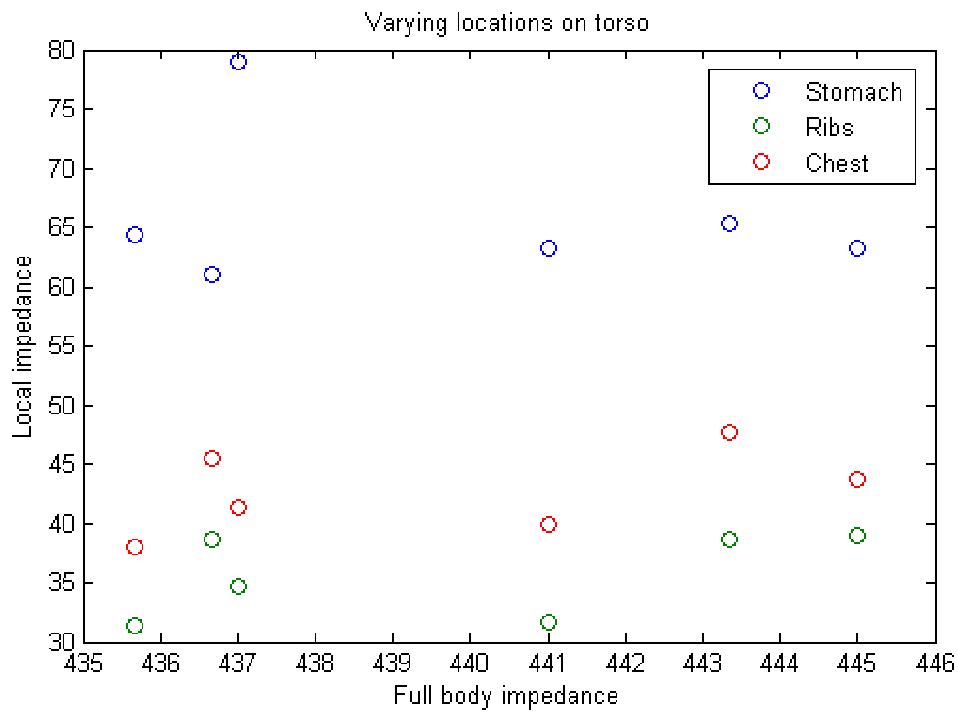


Figure 26: Local measurements taken at different locations on the torso.

In order to determine the correlation between local measurements and full body measurements under more standardized conditions, the team finally tried taking several measurements over the course of two to four hours without moving the electrodes or eating or drinking in between measurements. The results for tests taken on the full body, stomach, and chest for one test subject are shown in Figure 27 and Figure 28. Similar tests were taken on a second test subject, this time also including measurements taken on the ribs. These results are shown in Figure 29 and Figure 30. Table 7 gives the calculated values for the correlations between full body and local impedance measurements for these tests.

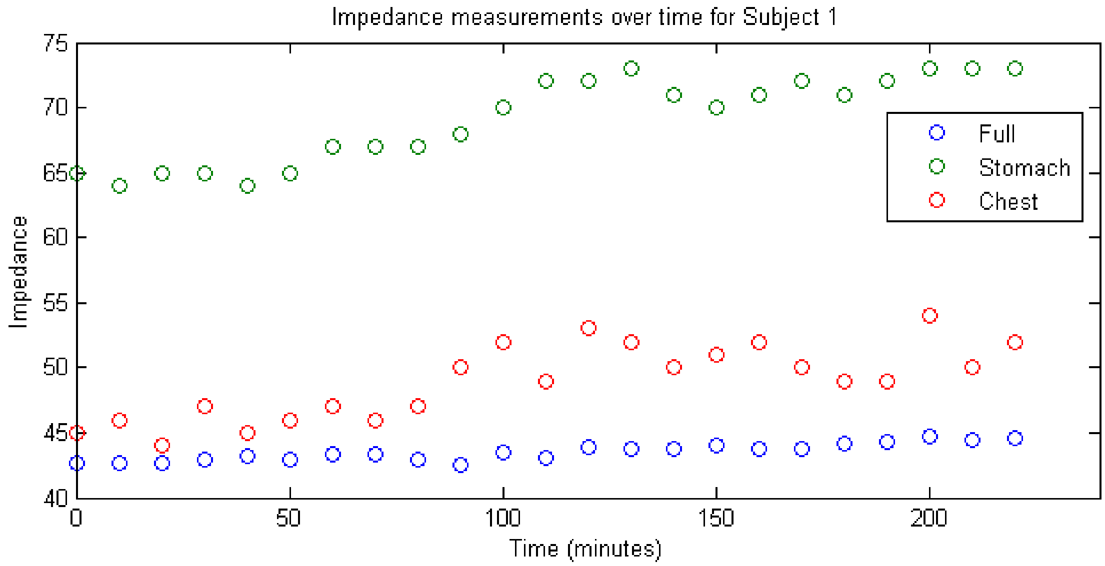


Figure 27: Full body and local impedance measurements for Subject 1 taken over a continuous time period for. The full body measurements have been normalized to be on a similar scale as the local measurements.

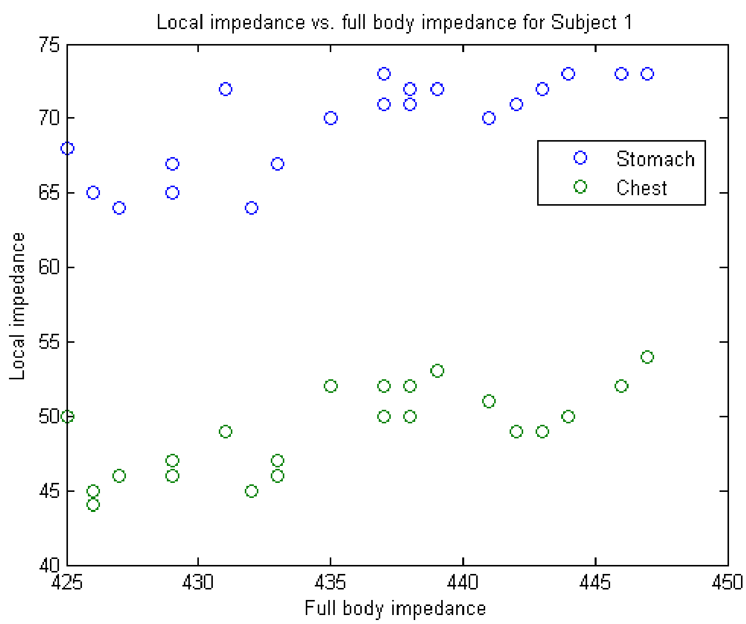


Figure 28: Local impedance measurements over a continuous time period plotted against full body impedance for Subject 1.

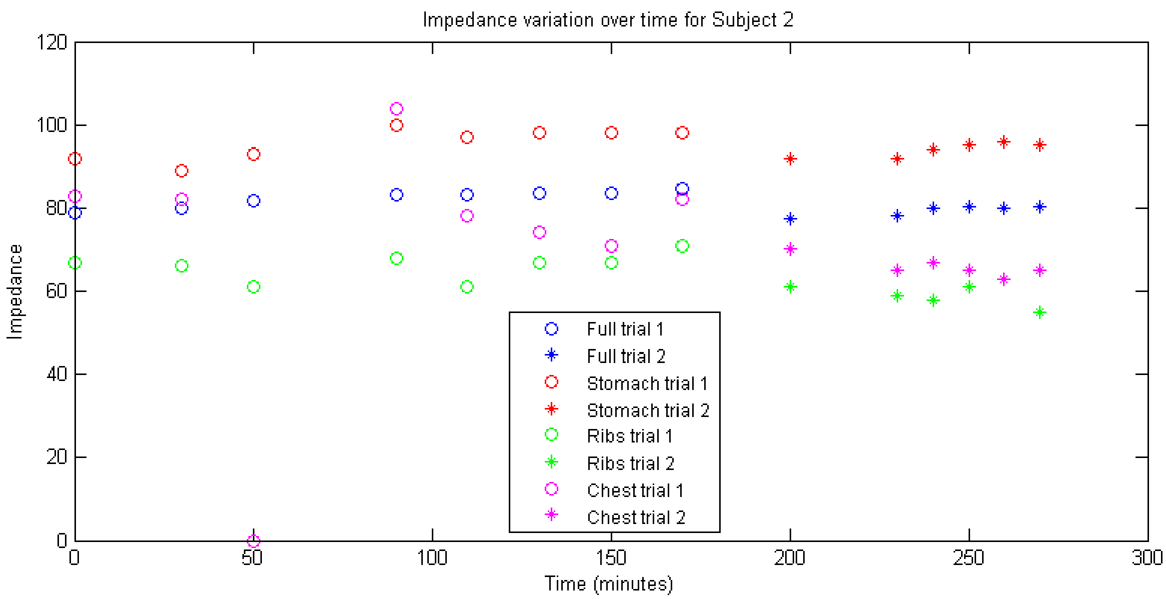


Figure 29: Full body and local impedance measurements for Subject 2, taken periodically over two separate time trials. The full body measurements have been divided by 10 to normalize them. One of the chest data points is missing, so it is set to 0.

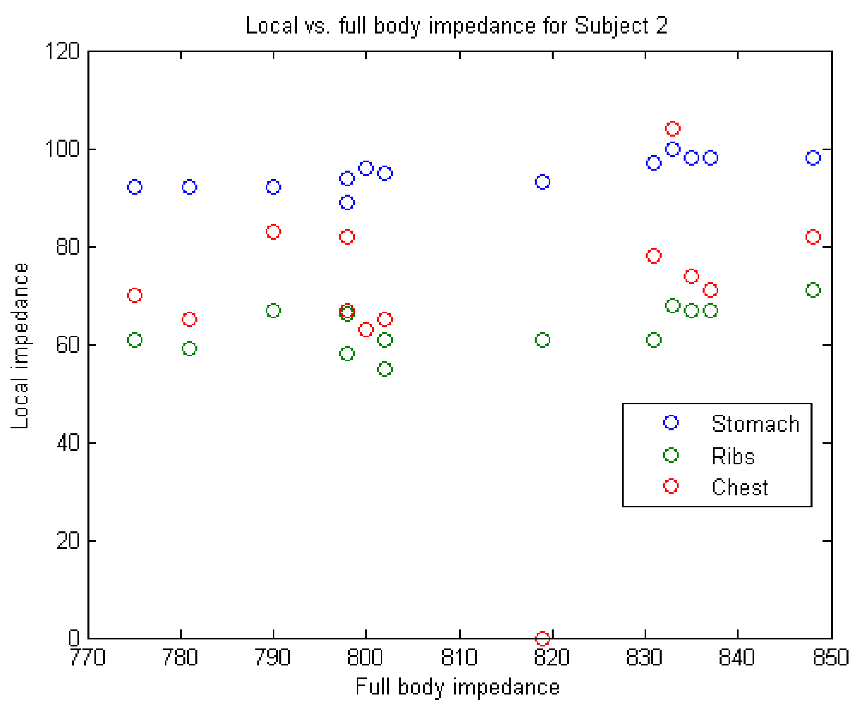


Figure 30: Local vs. full body impedance measurements for both time trials on Subject 2.

Table 7: Correlations between full body measurements and local measurements taken over continuous time periods.

	Correlation
Subject 1 Stomach	0.8215
Subject 2 Stomach	0.7967
Subject 1 Chest	0.7252
Subject 2 Chest	0.45
Subject 2 Ribs	0.5748

These data show a promising correlation for measurements taken at the stomach under a relatively controlled environment. The team plans to continue investigating local measurements taken on the stomach, including the effects of food and water consumption on the consistency of those measurements.

5.7 CONCLUSIONS AND RECOMMENDATIONS

The Proteus Clinic team determined from the existing bioimpedance research that BIA at 50 kHz and 400-800 μA would be the most successful impedance technique for measuring local hydration. A four-electrode configuration is necessary because of the high measurement variability caused by the electrode-skin interface in the two-electrode measurement.

Furthermore, experimental evidence suggests that the local measurement correlates best with the full body measurement when it is taken on the stomach, as opposed to the ribs or chest, and when the signal electrodes are as close to the sensing electrodes as possible. Additionally, measurement variability is lowest for the stomach measurement. Testing results indicate that this local measurement can track dehydration when not eating, drinking, or exercising. The indicated changes are significant for dehydrations occurring over time periods of at least three hours. That is, the testing results thus far show that dehydration changes are differentiable from measurement variability. However, the team needs to collect more data in order to confirm the statistical significance of their findings, and plans on continuing testing in the spring.

Given this dehydration resolution, the team expects that this local measurement could identify larger dehydrations that would pose a threat to geriatric patients. However, the evidence collected thus far only demonstrates feasibility for a relative comparison of body hydration. For example, a measurement at night could identify a hydration change relative to a measurement taken that morning. The team has not yet determined if a single measurement value itself can identify whether a patient is dehydrated. This has to do in part with the fact that the human body is very complex, and each individual looks very different in terms of their hydration. This was seen even for full-body Bodystat measurements, wherein two individuals had hydration percentages that could differ by 20%.

Because of this limitation, the team recommends that the next steps for this project are to determine the clinical applicability by answering the following questions:

- What is the required correlation between local and full-body impedance that can be useful in clinical applications?
- How many more data points need to be collected so that the team may be confident in their results?
- Does a single absolute impedance measurement have significance?
- How can one establish a baseline hydration level that can be used to interpret hydration status?
- Can the local measurement identify edema and other diseases that present fluid management issues?

Additionally, the team recommends that wear location and electrode geometry be further explored to optimize the measurement and determine which other locations are satisfactory. This exploration should include different parts of the stomach, distance between inner (sensing) electrodes, and development of a protocol to deal with flawed data caused by exercising, stomach contents, or electrode connection problems.

Moreover, the team advises further developing a prototype of a four-electrode impedance measuring device. The team will continue to work from the AD9533 evaluation board, and begin to consider design with regard to implementation into the current Proteus patch system. Developing this device will improve the team's understanding of the product, aid in Proteus' eventual four-electrode development, and allow the team to adjust additional parameters for testing of the four-electrode measurement, such as carrier frequency. This will allow better optimization of the four-electrode, local technique.

6 PROJECT MANAGEMENT

6.4 FALL PROGRESS

The team's goals for the fall included completing research on bioimpedance analysis and putting together a testing setup for local measurements. These goals were reached this semester. The team conducted a literature review and worked with Proteus Digital Health to choose a device that that could be used to perform hydration tests (the Bodystat Quadscan), and ran tests with the device to determine whether two-electrode and four-electrode local measurements could be used to measure systemic hydration.

A rough breakdown structure of the fall work is shown below in Figure 31. The breakdown mainly serves as a guide to evaluate how much time has been put towards certain aspects of the project. Most of the chart is consistent with the estimate for time consumption throughout the semester, however, the team spent more time on the IRB document and within meetings than anticipated.

Activity	Planned Time (hours)	Actual Time (hours)
Background research *(completed in September)*		
Hydration measurement techniques	10	10
Impedance-based methods	15	15
Existing devices	6	6
Budget & reimbursements	5	5
Testing design		
Research & preparation		
IRB application	10	15
Testing approach	12	12
Data processing techniques	10	4
Comparison of design alternatives	3 × 4 = 12	10
Testing & analysis *(Total)*	58	45
Evaluating showstoppers	5	
Preliminary electrode placement		
2 Electrodes	10	
4 Electrodes	10	

Evaluation of electrode quantity	2 × 4 = 8	
Electrode placement revisited	15	
Electrode size variation	10	
Circuitry simulation	8	7
Team meetings		
Teleconferences	15 × 4 × 1 = 60	10 × 4 × 1= 40
Internal team meetings	15 × 4 × 2 = 120	35.5 × 4 = 142
Tuesday presentations	6 × 4 × 1 = 24	6 × 4 × 1 = 24
Team leader meetings	4	4
Planning	15 × 1 = 15	27
Presentation and preparation		
Orientation Day	3 × 4 = 12	12
Design Review #1	2 × 4 = 8	8
Design Review #2	3 × 4 = 12	12
Design Review #3	3 × 4 = 12	12
Fall site visit	12 × 4 = 48	16 × 4 = 64
Reports		
Work Plan & Literature Review	6 × 4 = 24	50
Midyear Report	6 × 4 = 24	40
Total Time	509	564

Figure 31. Fall semester work breakdown structure.

6.5 SPRING OVERVIEW

In the spring, the team envisions completing more extensive tests based on the results this fall. While the data taken this semester has been useful in understanding different intricacies of the hydration measurements, it is also noisy, making it difficult to draw conclusions. Also, using the Bodystat as a measurement tool is quite time consuming. While the team plans on running more tests with the Bodystat in the spring to obtain a more complete data set, the team would also like to explore other options for data collection, such as building a bioimpedance-measuring system. This would help move towards a product that could be integrated into the Proteus Patch, and it could allow the team to take more data points without having to manually enter data.

A set of goals for the spring are outlined below.

Spring Semester Goals:

- Complete data collection with the Bodystat device
  - Perform statistical variance analysis
  - Test different electrode geometries (i.e. circular ECG electrodes)
  - Obtain more complete data on how impedance measurements are affected when participants eat, drink, and exercise
- Create a bioimpedance analysis system
  - Perform testing to check conformity to electrical safety standards before testing on humans
  - Test and analyze the system
  - Provide recommendations on how the system could be integrated into the Proteus patch
- Reports and Presentations
  - Spring presentation for Harvey Mudd audience
  - On-site presentation for Proteus
  - Projects Day presentation at Harvey Mudd
  - Final report

7 REFERENCES

[1] Adlago, KE, et al. “Development and validation of bioelectrical impedance analysis equations for predicting total body water and fat-free mass in North-African adults” in *European Journal of Clinical Nutrition*, vol. 67, pp. 1081-1086, 2013.

[2] Anderson, Lindsey J., David N. Erceg, and E. Todd Schroeder. "Utility of multi-frequency bioelectrical impedance compared to deuterium dilution for assessment of total body water." In *Nutrition & Dietetics*, 2014.

[3] B. G. Crum, J. B. Williams, and K. A. Nagy. “Can tritiated water-dilution space accurately predict total body water in chukar partridges?” in *Journal of Applied Physiology,* Bethesda, Md., vol. 59.5, pp. 1383, 1985.

[4] Bera, Tushar Kanti. “Bioelectrical Impedance Methods for Noninvasive Health Monitoring: A Review,” in *Journal of Medical Engineering*, 2014.

[5] *Bodystat.com*. Bodystat, 2015. Web. 27 Sept. 2015.

[6] Chen, Xiaohui, et al. “Multi-channel electrical impedance tomography for regional tissue hydration monitoring,” in *Physiological Measurement*, vol. 35, pp. 1137–1147, 2014.

[7] Codognotto, Marta, et al. “Influence of Localized Edema on Whole-body and Segmental Bioelectrical Impedance,” in *Nutrition*, vol. 24.6, pp. 569-74, 2008 [Online].

[8] Di Somma, S, et al. “Consensus paper on the use of BIVA (Bioelectrical Impedance Vector Analysis) in medicine for the management of body hydration,” in *Emergency Care Journal*, December 2011 [Online]. Available:

http://iopscience.iop.org/article/10.1088/0967-3334/35/6/1137/pdf. [Accessed September 2015]

[9] Gartner, A, et al. “Importance of Electrode Position In Bioelectrical Impedance Analysis,” in Letters to the Editor, *The American Journal of Clinical Nutrition*, pp. 1068-9, 1992 [Online].

[10] "Hydration Data." *Tanita.com*. Tanita, 2014. Web. 27 Sept. 2015.

[11] Gastelurrutia, Paloma, et al. “Bioelectrical Impedance Vector Analysis (BIVA) in Stable and Non-stable Heart Failure Patients: A Pilot Study,” in *International Journal of Cardiology*, vol 146.2, pp. 262-64, 2011 [Online].

[12] Heikenfeld, Jason. “Sweat Sensors Will Change How Wearables Track Your Health,” in *IEEE Spectrum*, Oct 2014 [Online]. Available: http://spectrum.ieee.org/biomedical/diagnostics/sweat-sensors-will-change-how-wearables-track-your-health. [Accessed Sept 2015]

[13] Jebb, Susan A., et al. "Evaluation of the novel Tanita body-fat analyser to measure body composition by comparison with a four-compartment model" in *British Journal of Nutrition*, vol. 83.02, pp. 115-122, 2000.

[14] Kyle, Ursula G., et al. "Bioelectrical impedance analysis—part I: review of principles and methods" in *Clinical nutrition*, vol. 23.5, pp. 1226-1243, 2004 [Online]. Available: http://www.sciencedirect.com/science/article/pii/S0261561404000937. [Accessed: September 2015]

[15] Lukaski, Henry C., and Piccoli, Antonio, “Bioelectrical Impedance Vector Analysis for Assessment of Hydration in Physiological States and Clinical Conditions,” in *Handbook of Anthropometry*, pp. 287-305, 2012 [Online].

[16] Maioli, et al. “Pre-Procedural Bioimpedance Vectorial Analysis of Fluid Status and Prediction of Contrast-Induced Acute Kidney Injury,” in *Journal of the American College of Cardiology*, vol. 63.14, 2014.

[17] Margutti, Ana et al. “Bioelectrical Impedance Vector Analysis (BIVA) in Stable Preterm Newborns,” in *J Pediatr (Rio J) Jornal De Pediatria*, vol. 88.3, pp. 253-8, 2012 [Online].

[18] Mulasi, Urvashi, et al. "Bioimpedance at the Bedside Current Applications, Limitations, and Opportunities" in *Nutrition in Clinical Practice*, vol. 30.2, pp. 180-193, 2015.

[19] Nebuya, S, et al. “Study of the optimum level of electrode placement for the evaluation of absolute lung resistivity within the Mk3.5 EIT system,” in *Physiological Measurement*, vol. 27, 2006 [Online]. Available: http://iopscience.iop.org/article/10.1088/0967-3334/27/5/S11/pdf. [Accessed: September 2015]

[20] Nescolarde L., et al. “Different Impedance Indexes in Segmental and Whole-body Measurements in Peritoneal Dialysis Patients,” in *IFMBE Proceedings 13th International Conference on Electrical Bioimpedance and the 8th Conference on Electrical Impedance Tomography*, pp. 779-82, 2007 [Online].

[21] Nescolarde L., et al. “Localized bioimpedance to assess muscle injury,” in *Physiological Measurement*, vol. 32.2, pp. 237-45, Feb 2013.

[22] Norman, K, et al. “Bioelectrical phase angle and impedance vector analysis--clinical relevance and applicability of impedance parameters,” in *Clinical Nutrition*, vol. 31.6, pp. 854-61, Dec 2012 [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/22698802. [Accessed: September 2015]

[23] Nuñez, C., et al. "Bioimpedance Analysis – A New Approach to Measuring Resistance” in *Faseb Journal*, Bethesda, Md., vol. 8, no. 4, 1994.

[24] Nuñez, C., et al. “Bioimpedance analysis of body composition: A new measurement approach." Poster presentation presented at the annual meeting of the North American Association for the Study of Obesity, Cancún, México, 1997.

[25] Nuñez, C., et al. "Bioimpedance analysis: evaluation of leg-to-leg system based on pressure contact footpad electrodes," in *Medicine and Science in Sports and Exercise*, vol. 29.4, pp. 524-531, 1994.

[26] Piccoli, Antonio. “Bioelectric impedance vector distribution in peritoneal dialysis patients with different hydration status,” in *Kidney International*, vol. 65, pp. 1050-1063, 2004 [Online]. Available: http://www.nature.com/ki/journal/v65/n3/full/4494355a.html. [Accessed: Sept 2015]

[27] Piccoli, A., et al. “Equivalence of information from single versus multiple frequency bioimpedance vector analysis in hemodialysis,” in *Kidney International*, vol. 67, pp. 301-313.

[28] Piccoli, Antonio, "Estimation of Fluid Volumes in Hemodialysis Patients: Comparing Bioimpedance with Isotopic and Dilution Methods," in *Kidney International*, vol. 85.4, 2014 [Online].

[29] Proteus Digital Health, 2015 [Online]. Available: http://www.proteus.com. [Accessed: Sept 19, 2015].

[30] Simpson, J. A. D., et al. "Body water compartment measurements: a comparison of bioelectrical impedance analysis with tritium and sodium bromide dilution techniques" in *Clinical Nutrition*, vol. 20.4 pp. 339-343, 2001.

[31] "Technical Note: An introduction to bioelectrical impedance analysis and BIVA," in *EFG Diagnostics* [Online]. Available: http://www.efgdiagnostics.com/intro.pdf. [Accessed: September 2015]

[32] Villa, F, et al. "Portable Body Impedance System for Long-Term Monitoring of Body Hydration," in *Ph.D. Research in Microelectronics and Electronics (PRIME)*, presented at the 2012 8th Conference, pp. 1-4, June 2012.

[33] Walter-Kroker, Anja, et al. "A practical guide to bioelectrical impedance analysis using the example of chronic obstructive pulmonary disease," in *Nutritional Journal 10.35*, 2011.

[34] Weyer, S, et al. "Evaluation of Bioimpedance Spectroscopy for the Monitoring of the Fluid Status in an Animal Model," in *Wearable and Implantable Body Sensor Networks (BSN)*, presented at the 2012 Ninth International Conference, pp. 22-27, May 2012.

[35] "1 MSPS, 12-Bit Impedance Converter, Network Analyzer," *Analog Devices*, data sheet [Online]. Available: http://www.analog.com/media/en/technical-documentation/data-sheets/AD5933.pdf. [Accessed: November 2015]

[36] "Evaluation Board for the 1 MSPS 12-Bit Impedance Converter Network Analyzer," *Analog Devices*, data sheet [Online]. Available: http://www.analog.com/media/en/technical-documentation/evaluation-documentation/537700023EVAL_AD5933EB.pdf. [Accessed: November 2015]

[37] Seone, Fernando, et al. "An analog front-end enables electrical impedance spectroscopy system on-chip for biomedical applications," in *Physiological Measurement* 29, pp. 273, 2008.

[38] Noveletto, F, et al. "Analog Front-End for the Integrated Circuit AD5933 Used in Electrical Bioimpedance Measurements," *II Latin American Conference on Bioimpedance: 2nd CLABIO, Montevideo*, September 30 – October 2, 2015.

APPENDIX A. IRB INFORMATION

Before beginning any empirical testing, the team was required to have their testing procedure approved by the Claremont Graduate University IRB. This application included procedures that the experimenters would take for testing on themselves, and allowed for the possibility of testing on additional participants. This IRB determined the experiment to be exempt from IRB approval. The materials that were created for this process are shown below.

A.1. RESEARCH DESCRIPTION

A.1.1. Research Summary

Proteus Digital Health is sponsoring a clinic project at Harvey Mudd College to determine how to best use impedance measurements to determine hydration. It is therefore critical to obtain impedance and hydration data on human participants.

Data is to be collected using a body measurement device that is on the market, Bodystat Ltd.'s QuadScan4000. This device uses four electrodes placed on the skin to measure impedance through the body, which is used to calculate metrics such as body composition and hydration. The device will be used normally, and again with the electrodes positioned on the torso to see if a local measurement of impedance can correlate with hydration.

The research for this project is to be conducted in two phases. The first phase will involve obtaining hydration data on the investigators themselves, without recruiting outside participants. If these measurements prove useful, the investigators would like to obtain hydration and impedance data from a wider diversity of participants (Phase Two). Participants will be fully informed of the risks of the procedure as well as what the purpose of the research is for. They will also be told that they can drop out of the experiment at any time.

A.1.2. Participants and Recruitment

Phase One of the study will test only the four researchers. No recruitment will be necessary for this phase. Phase Two of the study will test approximately 10-20 participants. The only inclusion criteria for these participants is that they must respond to our recruitment email (provided in Appendix C). If more participants respond, we will test all who respond with no exclusion criteria. The Harvey Mudd College population (students, faculty, and staff) will receive the email, but anyone will be allowed to participate if they sign up.

Phase One of the project will require no recruitment procedures, as only the investigators will be involved. Phase Two participants will be recruited via email, which is attached in Appendix C. Participants will be compensated with snacks and hydrating drinks, such as water and Gatorade. Another incentive is that participants' can learn more about their body compositions using this specialized medical device.

Participants will be given two copies of an informed consent form. A researcher will discuss the form with the participant, answer questions, and ask the participant to sign the document if they give their consent to participate. The researcher will keep the signed copy and the participant will keep the other copy. There will be no deception of participants.

A.1.3. Research Procedures and Methods

Most of the testing for this experiment will be done in November 2015. Some additional testing, especially for Phase Two, may occur in Spring 2016. All research will be done at Harvey Mudd College. For each phase, the participants will be asked to read and sign a consent form before testing. For Phase One, after the form is signed, the participants will be tested will the Bodystat Quadscan 4000 (Figure 1 in Appendix A). Before testing participant will be asked to roll up any sleeves or pant legs enough to expose the wrist and ankle so that electrodes can be applied by the researchers. The area will first be cleaned by with a clean gauze soaked in soap and water and then two adhesive electrode (seen in Figure 2 of Appendix A) will be affixed to the wrist area and two to the ankle area. If the participant is uncomfortable with this, they will be allowed to apply the electrodes themselves under guidance from the researcher and reminded that they can back out of the study at any time. Following these measurements, the same procedure will be followed to attach the electrodes to the torso of the participant. If the participant is uncomfortable slightly lifting or rolling up their top and allowing the researchers to apply the electrodes, they will be allowed to apply the electrodes to themselves and reminded that they can back out of the study at any time. The data for Phase One will be collected on the same four participants over the course of the month at varying levels of hydration. These measurements might be taken before and after the researcher goes to bed, or before and after the researcher does some physical exercise. The procedure for the exercising portion is outlined in the procedure for Phase Two below.

For Phase Two, after the consent form is signed, the participant will be asked to fill out a questionnaire providing the researchers with their height, weight, and demographic information. If they do not know their height and weight, a scale and measuring tape will be provided. A draft of the questionnaire is provided in Appendix D. The researchers will verbally inform the participants that they do not have to fill out any information they are uncomfortable with on the questionnaire. They will then be tested with the Bodystat Quadscan 4000 to obtain a hydration baseline. The same test procedure from Phase One will be followed for measurement. The participants will then be asked to lower their hydration level through exercise. This exercise will be for 5-10 minutes, unless the participant is interested in exercising longer. The participants will be asked to wear their normal workout clothes. The participant may choose whichever form of exercise they like, which will most likely take place in Harvey Mudd's Linde Activities Center (LAC) gym. This gym has equipment that includes a treadmill, elliptical, bike, row machine, and free weights and machines. There is also space for participants to practice dance, basketball, or other activities. The LAC employees are trained in CPR, AED, and in exercise form and safety.This activity serves only to produce some sort of change in the participant's hydration. There is no target threshold for dehydration that should be reached. Although the detached electrodes may still be attached to their body, no measurements will be taken on the participant during exercise. After the exercise activity, the measurement procedure will then be repeated with the Bodystat Quadscan 4000 to obtain data on the less hydrated participant. The participants will be reminded while exercising that if they are feeling tired or dehydrated that they may drink water and opt out of this portion of the study. If participants do opt out of this portion, they will still be asked if another measurement can be taken after they have been allowed to rehydrate.

It is important to protect the confidentiality of the data, as some detailed personal information about an individual's body is being collected. Therefore, the team will use an encryption software in order to store the data safely. No names or individual sets of data will be used in the final presentation and report. Instead, trends will be used among groups.

No information will be withheld in order to mislead or deceive the participant. Therefore, no debriefing procedure will be required.

A.1.4. Potential Risks and Benefits

In both phases, because the test involves applying electrodes to the skin of the participant, the participant may feel uncomfortable having said skin exposed or in contact with the electrodes. There is minimal risk of developing slight skin irritation as a result of the adhesive electrodes. If the electrodes are irritating the participant, researchers will be there to help the participant remove the electrodes and will provide lotion if the participant desires.

In Phase Two, there is an additional minimal risk of dehydration while exercising. This is mitigated by the researchers reminding participants that if they are feeling dehydrated that they should stop and rehydrate. The researchers are providing water and gatorade to help the participants rehydrate.

In Phase One, as participants and researchers, the project team members will benefit from the data collected in the study that will assist with the project. The data collected will help the researchers determine whether local impedance measurements can be correlated with hydration, and how to best measure hydration.

In Phase Two, the participants may benefit from the study by learning their body composition from the testing data. There are no other direct benefits to the participant. However, the information collected by the researchers will be beneficial to increasing knowledge on a topic not thoroughly researched.

A.2. BODYSTAT QUADSCAN4000

Figure 32. Bodystat Quadscan 4000 device. This device takes non-invasive physical measurements using electrodes placed on the body. Such measurements include body mass and

hydration levels. Image obtained from http://www.smartmedical.co.uk/news-exhibitions/bodystat-quadscan-4000**.**

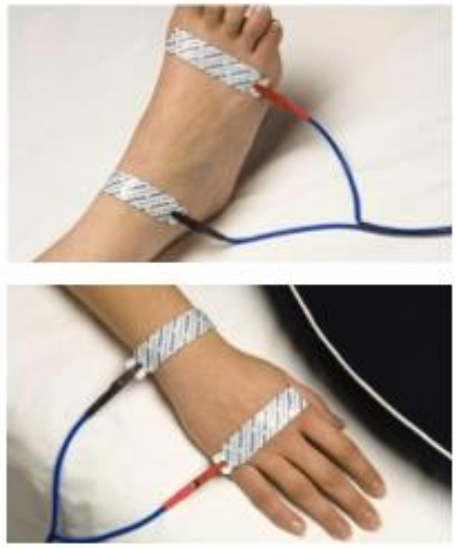


Figure 33. Electrodes from the Bodystat are placed onto parts of the body such as the ankle or wrist. Image obtained from http://www.donovanrussell.com/body-fat--body-composition-testing.html**.**

A.3. TRAINING CERTIFICATES

Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Nicole Kyle** successfully completed the NIH Web-based training course "Protecting Human Research Participants".

Date of completion: 10/06/2015

Certification Number: 1880043

Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Kaitlin Kimberling** successfully completed the NIH Web-based training course “Protecting Human Research Participants”.

Date of completion: 10/03/2015

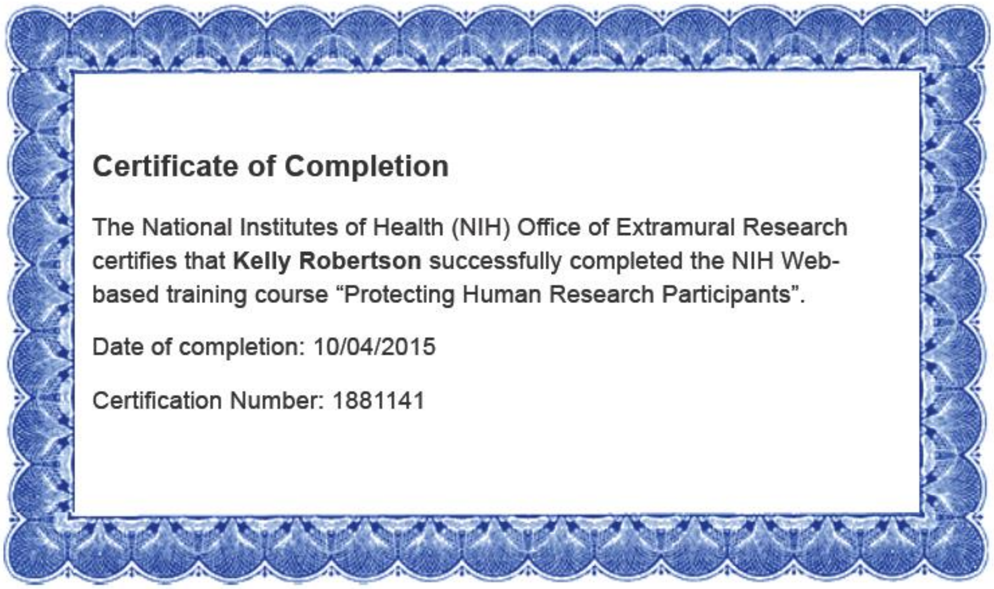
Certification Number: 1880095

Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Nathan Miller** successfully completed the NIH Web-based training course “Protecting Human Research Participants”.

Date of completion: 10/03/2015

Certification Number: 1880474



Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Kelly Robertson** successfully completed the NIH Web-based training course “Protecting Human Research Participants”.

Date of completion: 10/04/2015

Certification Number: 1881141

A.4. EMAIL FOR PARTICIPANT RECRUITMENT

SHORT VERSION:

Sign up <here> if you want to be part of a clinic project that will help us understand more about the body's hydration levels. As part of the study, you will have the option to learn different metrics about your body, such as your body fat percentage and hydration levels.

LONG VERSION:

Hello everyone,

The Proteus Clinic Team is in need of participants for their hydration research.

We are collecting non-invasive hydration measurements using a Bodystat device, and we need a diverse pool of participants. You will be compensated with snacks and gatorade (so you can stay hydrated, of course!). Also, you get to find out information about your body using a special medical device (such as your body composition, body fat percentage, and hydration level) FOR FREE! There is an optional exercising component to this research, so if you are going to work out anyways, please consider signing up.

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So sign up <here> if you want to contribute to a one-of-a-kind
hydration research project.

Please email me if you have any questions. :)

-Nicole and the 2015-2016 Proteus Clinic team
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A.5. PARTICIPATION FORMS

Harvey Mudd College
2015-2016 Proteus Clinic Team

Informed Consent form for Harvey Mudd College faculty, staff, and students

Principal Investigator: Nicole Kyle
Co-Investigators: Kaitlin Kimberling, Nathan Miller, Kelly Robertson
Organization & Sponsor: Harvey Mudd College
Proposal: Hydration & Impedance Bodystat Data Collection, v2

Introduction:

The Proteus Clinic Team is collecting data for their project which involves on measuring the body's hydration levels using impedance-measuring devices. This research will involve collecting certain non-invasive measurements using the Bodystat Quadscan 4000 device. This device calculates a variety of measurements using four electrodes placed on the body. The QuadScan4000 measures resistance, reactance, and phase angle of the signal it sends through the body. The device uses this data to calculate the impedance of the body as well as hydration and mass levels.

Purpose of the Research:

The purpose of this study is to collect hydration data so that the team can better understand the precision of the Bodystat device as well as how hydration levels vary from person to person. A big part of this research is in understanding how measurements vary with electrode placement. The data from this research will inform the clinic team of whether it is possible to take hydration measurements with electrodes localized over a small region.

Type of Research Intervention:

If you agree to take part in this study, you will first complete a questionnaire that gives information about your height, weight, age, sex, and ethnicity. Then, preliminary measurements will be taken by placing the four Bodystat electrodes on your body. The investigators may ask you to roll up a sleeve, pant leg, or shirt so that the electrodes can be placed. The investigators may need to wipe the area with soap and water if the skin isn't dry enough. If you are uncomfortable with the investigators placing these electrodes or wiping the skin, you will have the option to place them yourself. Then, you may be asked to lower your hydration levels through an exercise of your choice, and measurements will be taken with the Bodystat once more. This will give the researchers data on the comparison between a hydrated and a less-hydrated status.

Participant Selection & Participation:

Participants were recruited through an email that was open to the entire Harvey Mudd College population. Your participation in this research is completely voluntary. You may choose to leave the research study at any time.

Duration:

This study shouldn't take more than an hour of your time.

Risks:

Participants in this study may risk dehydrating themselves. However, the investigators plan to alleviate this risk by providing water and Gatorade after measurements are completed. There is also a possibility of the electrodes causing skin irritation. If the skin is irritated when the electrodes are removed, lotion will be offered to the participant.

Contact Information:

You may contact the principal investigator anytime if you have questions, before or after the study.

Name: Nicole Kyle
Email: nkyle@hmc.edu
Mobile Phone: 650 619-4730

Investigator questions:
Do you understand that you may withdraw from this research study at any point?
You can ask us questions anytime during the study. Do you have any questions for us now?

Certificate of Participant Consent:

I have read the information provided to me about the Proteus Clinic Team's research study. I have been given the opportunity to ask the researchers questions, and I understand that I can withdraw from this research at any time. I voluntarily consent to participate in this research.

Print Name of Participant: ____

Signature of Participant: ____

Date: ____

Statement by the researcher:

I have provided the participant with all the information they need to know about the study.

I confirm that the participant has consented to take part in this research study voluntarily and freely, and that I have answered all of their questions to the best of my ability.

A copy of the consent form has been given to the participant.

Print Name of Researcher: ____

Signature of Researcher: ____

Date: ____

APPENDIX D. Draft of Questionnaire for Participants

Name: __________________

Height: ___________________

Weight: ___________________

Age: ______________________

Sex: _______________________

Ethnicity: ___________________

Did you eat or drink within the last two hours? _______________________________

Have you worked out in the last few hours? __________________________________

Do you think you might already be dehydrated? _______________________________