Ambulatory Vascular Monitoring for Quantification of Lower Leg Hemodynamics and Severity of Venous Disease

Christian Redd

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Reading Committee:

Joan Sanders, Chair

Brian Hafner

R. Eugene Zierler

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Abstract

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Christian Redd

Chair of the Supervisory Committee:
Professor Joan Sanders
Bioengineering

Persons with chronic venous disease experience progressive deterioration of their lower extremity hemodynamics. Different factors are thought to contribute to this hemodynamic decline, including incompetent venous valves, thrombotic blockage, and reduced efficiency of extremity muscle pumps. Left untreated, venous disease and resultant extremity hypertension can progress to the point of functional tissue changes, skin breakdown, and even amputation. Early clinical intervention directly increases positive outcomes; however, diagnostic efficiency in early or mild venous disease is low. This is due to a variety of reasons, one of which is the lack of a consensus gold-standard diagnostic method to quantify the global extent of extremity venous disease. The aims of this dissertation were to (1) design and validate tools for measurement of physiological and behavioral characteristics of persons with venous disease, (2) assess the utility of hemodynamic parameters derived from impedance plethysmography to quantify differences between diseased and healthy individuals, and (3) extend vascular monitoring beyond the clinic to assess how venous behavior changes over time during normal daily activity.

A noninvasive behavioral datalogger was developed to utilize a variety of small noninvasive sensors to measure activity and behavioral characteristics of persons both in and out of the lab. This platform, named the ECHO system, is mounted at the ankle or on a prosthesis and

can record continuous data from different sensors for up to 4 days before being recharged. Data from this system can be evaluated to gain insight into daily activities and behaviors to provide context and enhance analysis of physiological or clinical data. A portable multi-channel spectroscopic bioimpedance device was developed for the continuous measurement of lower extremity extracellular fluid volume changes. This device was shown to accurately quantify changes in blood volume in persons with and without vascular disease towards the aim of continuous noninvasive hemodynamic monitoring.

Twenty healthy and vascular compromised individuals participated in a study protocol to measure their venous flow parameters using the impedance plethysmograph developed in Aim 1. Results showed differences in the amount of blood ejected during calf muscle pump activation and blood reflux during subsequent relaxation These hemodynamic differences suggest that individuals with a prior diagnosis of venous insufficiency may have lower calf muscle pump function and higher levels of retrograde flow. While this study reported physiological differences in venous flow parameters between groups, measurements were limited to a short temporal snapshot similar to current diagnostic techniques.

Expanding from laboratory studies, extraclinical hemodynamic measurements were studied in a series of individuals with increasing level of previously diagnosed venous disease. All participants performed a structured series of activities including sitting, standing, walking, and ascending and descending stairs. This protocol was designed to emulate normal daily activities to evaluate their differing effects on hemodynamic parameters. Results showed increasing lower extremity blood retention correlating to greater severity of venous disease. Further evaluations showed an improvement in venous function when external compression stockings were worn across all subjects in the study. In a single participant comparison, old compression stockings were less effective at reducing venous blood pooling compared to a new pair.

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GLOSSARY

CVD: Chronic Venous Disease. A disorder affecting the veins of the legs, where venous valves become incompetent allowing blood to flow backwards and pool in the legs. This leads to increased pressure in the veins, and a multitude of different symptoms.

DVT: Deep Vein Thrombosis. Obstruction of one or more veins in the body by formation of a blood clot (thrombus)

AVP: Ambulatory Venous Pressure. A "gold standard" test of the efficiency of the calf musculovenous pump, performed by placing a needle into a posterior distal vein and then measuring pressure during different postural or exertional exercises.

BIS: Bioimpedance Spectroscopy. Also known as Multifrequency Bioimpedance Analysis (MFBIA). A method for noninvasively measuring the extracellular and intracellular fluids within the body, or segmentally within a single extremity.

EMG: Electromyography. A measurement of the electrical activity of muscle tissue using dermal or intramuscular electrodes.

GSV: Great Saphenous Vein. A large superficial vein of the lower leg that is commonly the site of development of early venous disease.

ABI: Ankle brachial index, a non-invasive method for testing for peripheral arterial disease through the use of a Doppler probe and a blood pressure cuff on both the arms and ankles.

CEAP: A comprehensive classification system developed to allow uniform diagnosis of chronic venous disorders and comparison of patient populations CEAP stands for **C**linical, **E**tiology, **A**natomy, **P**athophysiology.

IPG: Impedance Plethysmography - Measurement of impedance changes in the extremity to quantify the underlying hemodynamics in the limb.

MIZ: Portable bioimpedance device developed in the Sanders Lab for use in long-term out of lab studies in amputees and able bodied persons.

OZONE: Third generation portable multi-channel bioimpedance monitor developed for long-term limb fluid volume studies in amputees and able bodied persons.

ECHO: Bioinstrumentation datalogger developed in the Sanders Lab for use in measuring activity, posture, and physiological conditions of subjects during long-term out of lab studies.

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DEDICATION

To the 3 M's in my life

Chapter 1

INTRODUCTION

The venous system of the lower extremity is a complex network of valves, veins, and muscular pumps, tasked with returning blood to the heart against the force of gravity. Accordingly, anatomical or physiological alterations to parts or the whole of the system can have a significant influence on an individual's health and quality of life [7]. This impact includes chronic or acute pain, decreased mobility, skin changes, and psychosocial impacts [8]. Venous obstruction, and valvular insufficiency are the primary contributors to chronic venous disease, a disorder that is estimated to account for 1-3 percent of total health care expenditures worldwide [9]. In the United States, lower extremity venous disease is the seventh leading cause of chronic debilitating illness [10]. In developed countries overall, chronic venous insufficiency affects approximately 5% of the adult population [11]. The overall seriousness of venous disease is reflected by the fact that there are 10 times the number of people suffering from venous disease of the extremities as there are from symptomatic arterial disease of the same area [12, 13].

Development of valvular insufficiency often occurs on a congenital basis, the underlying etiological factors of which are still poorly understood [9, 14]. While the progression of chronic valvular insufficiency is slow, more serious problems are often found in patients whose valves have been acutely altered as a result of thrombotic disease [12]. Whether the etiology is chronic (primary venous disease) or post thrombotic (secondary venous disease) the signs and symptoms often change dynamically within the adult population, presenting difficulty in timely and accurate diagnosis [15]. The transient nature of these symptoms is not yet well understood, and prior long-term studies have been hampered by a lack of monitoring methods that can be used outside of the the traditional vascular clinic.

Following diagnosis of venous disease, pharmaceutic, external compression, or surgical intervention may delay disease progression [7], however many individuals with CVD are referred to medical treatment too late, only after tissue changes have started [14]. In such cases, intervention becomes more difficult, and is frequently unsuccessful [11]. If CVD progresses to the point of ulceration (Approximately 1% of the US population [16]), the health care costs further increase to approximately $19,000-$24,000 USD yearly, with poor prognosis for complete healing and 48% likelihood of recurrence within 5 years [17].

Timely diagnosis and subsequent treatment of chronic venous disease is of paramount importance to ensure a positive outcome for the patient. Earlier definitive treatment is directly linked with better long-term health outcomes, especially in cases of venous blockage due to DVT [14]. Diagnosis at or close to the onset of venous disease is difficult however for those who do not have access to a vascular clinic and venous specialists [18]. Even patients with access to specialized vascular care would benefit from faster diagnosis and intervention, which is directly linked to better long-term health outcomes [14]. Non-invasive diagnostic tests to determine the global severity of arterial disease, such as the Ankle-Brachial index have made progress toward this goal, but as yet, there is not a gold-standard method to reliably and universally measure and report the severity of global venous disease [19, 7, 20].

Ambulatory venous pressure (AVP) measurements have shown strong correlation to the severity of venous insufficiency, but are not regularly prescribed due to the invasive and consuming nature of the procedure [20, 21]. This is especially true in cases of mild venous disease where less invasive diagnostic tests are preferred. AVP is generally only recommended for patients who show outward clinical evidence of venous insufficiency (CEAP 3+) [22], or in studies testing the efficacy of other non-invasive diagnostic methods. There remains a deficit in accepted and reliable methods by which vascular specialists, or even primary care doctors can assess the extent and pathophysiology of mild venous disease (i.e. CEAP 1-2) outside of specialized (e.g. duplex ultrasound) or invasive (e.g. AVP) measures.

1.1 Problem statement

Today, chronic venous disease is not diagnosed early enough. The ability to accurately measure and diagnose early stage venous disease would significantly lower lifetime health care costs and fundamentally improve patient outcomes. The purpose of this dissertation work is to develop instrumentation and study methods by which researchers and clinicians can better understand the hemodynamics of their patients during normal everyday activity outside the laboratory. The development of diagnostic tools capable of assessing mild venous insufficiency will increase the patient standard of care by enabling earlier detection and identification of venous disease, especially for patients in lower resource settings without access to specialized vascular care. Measurement and evaluation of extremity vascular parameters during normal daily activities over long periods of time may provide a more accurate understanding of the extent and location of venous disease and better measure its progression. Extra clinical vascular monitoring will increase the understanding of how individual factors may contribute to the progression of venous disease, which in turn will allow for greater efficiency in planning patient treatment and facilitate better long-term outcomes for sufferers of CVD.

1.2 Dissertation Goals & Specific Aims

It is the goal of this dissertation work to develop and validate novel imaging modalities for the measurement of vascular hemodynamics and then study their clinical performance through a series of participant studies. The focus of this research effort is on measuring the characteristics and extent of lower extremity venous insufficiency, and the novel implementation of long-term monitoring through the use of bioimpedance spectroscopic plethysmography. This effort is divided into 3 Aims, summarized in subsections 1.2.1 through 1.2.3.

1.2.1 Aim1 - Instrumentation and Validation

Contemporary vascular diagnostic equipment is limited to large hospitals and specialized labs and can not measure the the functionality of the venous system during normal every-

day activities. The purpose of this aim is to develop instruments for long-term vascular studies, and assess their operation in bench-top tests and participant trials. The vascular bioinstrumentation described in this aim include:

1. **ECHO Datalogger** - A lightweight and unobtrusive custom microcontroller board for extra-clinical monitoring of activity, compliance, and other patient characteristics.
2. **Portable Impedance Plethysmograph** - A 3rd generation portable bioimpedance monitor for noninvasive monitoring of extremity fluid volume change. Development efforts in this aim involved validation and implementation of bioimpedance measurements for vascular subject testing, expanding from previous amputee clinical studies.

The ECHO and Impedance Plethysmograph [IPG] systems implemented individually have been designed to provide an accurate measure of different subject characteristics both within the laboratory/clinical setting, as well as without. Implemented together, they provide a means by which hemodynamic, physiological, and behavioral characteristics can be measured during controlled study protocols or within natural environments.

The performance of the ECHO system and peripheral sensors was measured through an initial series of bench tests. Tests were performed to characterize short and long term drift, temperature dependence, mechanical isolation of system and peripheral components, and battery lifetime. Individual peripheral sensors were calibrated against established standards. These sensors include force sensing resistors, infrared proximity sensors, inductive proximity sensors, thermistors, and electromyographic muscle sensors. Following the conclusion of bench testing, the ECHO system was used in a variety of participant trials to measure characteristics of their prosthesis use, limb volume change accommodation strategies, and activity both during in-lab testing as well as outside of the laboratory setting.

The application of bioimpedance spectroscopy for quantification of venous hemodynamics in this research effort is a novel application of an accurate, long-term, and non-invasive sensing modality. Previous efforts to measure venous insufficiency or blockage using IPG were limited in scope, and suffered from instrumentation limitations. The development and

implementation of a portable bioimpedance monitor for use in clinical and extra-clinical studies provides a powerful approach to measure flow parameters in persons with venous disease.

1.2.2 ***Aim2 - Vascular Laboratory Studies***

The purpose of this aim was to measure and report on the clinical performance of the instruments developed during Aim 1 in controlled human subject trials. A matched subjects study was designed to asses the ability of IPG to measure hemodynamic parameters indicative of healthy or compromised venous function. Different study parameters were evaluated to determine which were most relevant to hemodynamic deterioration and had the potential to be clinically useful towards increased diagnostic efficiency. Study participants with a previous diagnosis of mild venous disease were recruited through the University of Washington Vascular Clinic and underwent a short IPG test to measure blood volume changes in their leg during a controlled protocol. Control group participants were recruited from the general population and matched to the vascular compromised group by age and sex. Study results indicated that measures of calf muscle pump function [EP] and venous reflux [VRP] can be quantified using IPG and statistically significant differences were observed between the vascular compromised and control groups. Increasing clinical severity of vascular disease was also shown to correlate with lower blood ejection during calf muscle pump exercises in the vascular compromised group. Increasing age in the control group also correlated with a decrease in venous blood ejection during calf muscle pump activation. This study expanded from previously validated plethysmographic measures by differentiating hemodynamic response by anatomical location. Differences in blood volume changes between the anterior and posterior muscle compartments were reported, a result that addresses a fundamental limitation of previous plethysmographic devices: The ability to provide hemodynamic and anatomic information at the same time using a single diagnostic instrument. Hemodynamic Parameters examined in this study, such as Ejection Percent [EP] and Venous Reflux Percent [VRP] were most promising for further use in venous studies using IPG.

1.2.3 ***Aim3 - Extraclinical Studies***

The purpose of this aim was to assess the ability of a portable IPG device to measure clinically relevant hemodynamic parameters during a scripted set of activities outside of the traditional laboratory setting. Drawing from the outcome of the studies in the previous aim, an activity protocol was designed to represent normal daily activities over a two hour period around noon. Participants completed the study protocol three times on three different days, in an A-B-A format. During the first and third test (A), participants were asked not to wear external graduated compressions stockings. During the second test (B), the participant was asked to wear their prescribed compression stockings, a commonly prescribed intervention for persons diagnosed with venous insufficiency. This study was designed to measure the repeatability of extra clinical hemodynamic measurements between the two A sessions, and assess the impact on lower extremity venous hemodynamics when compression stockings were used (session B).

Four participants were included in this study, with levels of diagnosed venous disease from C2 to C4. One of the participants had no previous diagnosis of venous disease and was included in the study as a healthy comparison against the other participants. From the study IPG data, long term hemodynamic trends were calculated and measures of calf muscle pump function and reflux were extracted. Study results indicated that compression stockings attenuated or reversed venous blood volume rise associated with venous insufficiency in all subjects. As clinical severity of venous disease increased, the number of steps required to reduce venous pressure and achieve steady state venous volume also increased, irrespective of whether compression stockings were worn. One subject reported using compression stockings that were approximately 9 months past their ideal retirement date for the B session. Post-hoc data analysis indicated that use of those stockings did not effectively slow venous blood volume rise. The subject repeated the protocol a fourth time using new compression stockings with hemodynamic data showing better venous function as compared with the old stockings. This outcome is important as it is the first time that the efficacy of external compression

stockings has been measured under conditions approximating normal daily behavior.

1.3 Future Work

This dissertation effort has established the foundation for the use of portable IPG in the measurement and diagnosis of lower extremity venous disease. It was assessed for its potential use under clinical conditions as well as for use in the measurement of hemodynamic behavior under real world test conditions. Future studies should involve the concurrent use of IPG and a previously validated gold standard diagnostic device, such as AVP or duplex ultrasound to compare measurements and better refine test protocols. The extraclinical test sessions in this research effort were carefully controlled over a several hour period, and should be expanded in future research efforts to non-structured activity and longer measurement periods. This will provide insight into individual and population-wide hemodynamic influences and better inform clinicians on the efficacy of prescribed interventions. Multi-channel IPG as was demonstrated in this effort should also be expanded to better localize the incidence of venous insufficiency and enhance the diagnostic efficiency of followup studies by other imaging methods.

Chapter 2

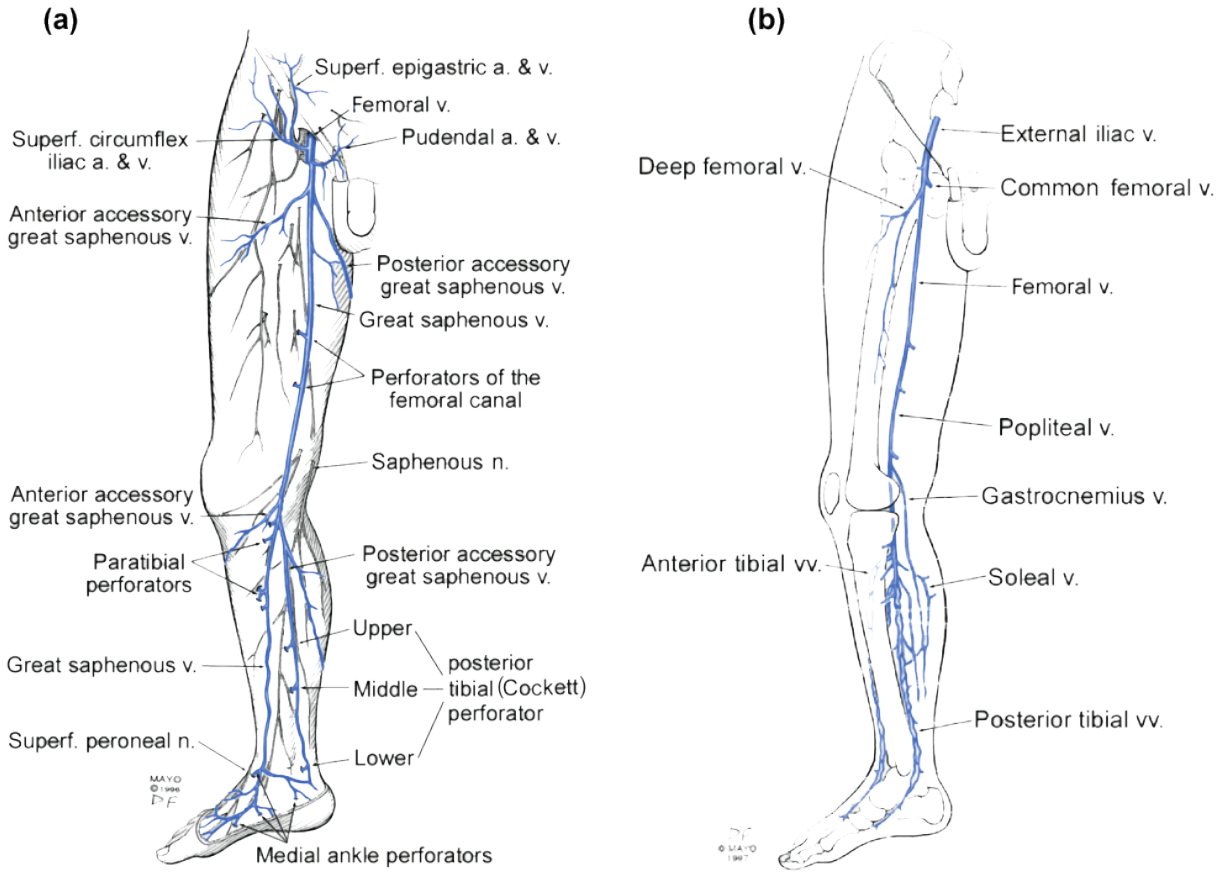
BACKGROUND

The venous system of the lower extremity is a complex network of valves, vessels, and muscular pumps, tasked with returning blood to the heart against the force of gravity [12]. Thinner walled than arteries, veins are composed of three primary layers (intimal, medial, and adventitial), and in the lower extremities, have an elliptical cross section [19]. This elliptical cross section allows volume to increase within without an increase in circumference or pressure, and as a result, the venous system has a high capacitance for blood storage [23]. Bicuspid valves within the veins serve to divide the hydrostatic column of blood in the legs into segments and ensure one-way flow from distal to proximal against the force of gravity [19]. Muscular pumps (Foot, Calf, and Thigh) generate high pressures within the muscle compartments, which then compress the vein walls and drive venous flow proximally. Approximately 90% of the venous return in the lower extremities take place in the deep veins through the action of the muscle pumps [19, 24]. After cessation of muscular compression, retrograde flow causes the venous veins to quickly close, preventing reflux. Reflux can occur when valves are either absent, or incompetent. This can be caused by progressive degeneration over time, or due to acute damage by an episode of deep vein thrombosis (DVT) [25].

The lower leg venous system is further anatomically divided into superficial, deep, and perforating veins, classified according to their relationship to the muscle fascia, and location within the muscle compartment. Deep veins lie beneath the muscular fascia and drain blood from the lower extremity muscles, while superficial veins lie above the fascia and drain the cutaneous microcirculation [19]. Perforating veins connect superficial and deep veins through the fascia. Blood flow in healthy individuals occurs from superficial veins to deep, distal to

proximal. The primary anatomy of the lower extremity venous system is illustrated in figure 2.1, however substantial anatomic variability exists from person to person. This anatomical variation is especially apparent in the distribution and number of valves and can significantly influence the efficacy of diagnosis when venous disease is present [26, 27].

Figure 2.1: Anatomy of the lower extremity venous system (a) Superficial Veins (b) Deep Veins. Image adapted from:[1]



2.1 Venous Disease

Venous disease can be segmented into two different classifications, Primary and Secondary. Primary, or chronic venous disease occurs when venous valves are rendered incompetent due

to a degenerative process [19, 28]. Primary venous disorders are often not associated with identifiable etiologic mechanisms of venous dysfunction [29]. Secondary Venous disease is defined as abnormalities in the venous system caused by an episode of deep vein thrombosis [29]. Under both classifications, retrograde flow during muscle pump relaxation prevents normal reduction in pressure and rapid refilling of the deep veins [19]. In addition to valvular incompetency, research has suggested that patients with chronic venous disease often suffer from reduced efficacy of their calf muscle pump, potentially due to limitations in their range of motion [30, 31, 32]. Decreased levels of activity can compound disease progression in patient populations already considered vulnerable [33].

2.1.1 Primary Venous Disease

Lower extremity venous disease is the seventh leading cause of chronic debilitating disease in the United States [10]. 10-35% of the US adult population has some form of chronic venous insufficiency (CVI) [9]. Due to the prevalence of CVI, as well as the diverse manifestations, a comprehensive classification system (CEAP) was developed to facilitate uniformity in diagnosis and reporting [28, 6]. The CEAP classification was created by an international ad hoc committee of the American Venous Forum in 1994, and is the accepted standard for classifying chronic venous disorders [34]. The CEAP classification is summarized in Table 2.1. Following an evaluation and diagnostic testing, the clinician classifies the patients venous disease according to their [C]linical manifestation, [E]tiological cause, [A]natomical location, and underlying [P]athophysiological modality.

The two most common manifestations of primary venous disease are varicose veins and chronic venous insufficiency [10]. Varicose veins are characterized by aching, heaviness, cramps, tingling, and pruritis, as well as visual changes to the overlying skin [35]. Visual changes can include enlargement and twisting of the veins due to venous hypertension and are easily identified during a clinical evaluation. No single symptom is pathognomonic for varicose veins, however quality of life among afflicted patients is significantly lower than population norms [35, 36]. Early theories presumed that varicose veins were caused by

Clinical Classification
C_0: no visible or palpable signs of venous disease
C_1: telangiectasias or reticular veins
C_2: varicose veins
C_3: edema
C_{4a}: pigmentation or eczema
C_{4b}: lipodermatosclerosis or atrophic blanche
C_5: healed venous ulcer
C_6: active venous ulcer
S: symptomatic, including ache, pain, tightness,skin irritation, etc.
A: asymptomatic
Etiologic classification
Ec: congenital
Ep: primary
Es: secondary (post thrombotic)
En: no venous cause identified
Anatomic classification
AS: superficial veins
Ap: perforator veins
Ad: deep veins
An: no venous location identified
Pathophysiologic
Pr: reflux
Po: obstruction
Pr,o: reflux and obstruction
Pn: no venous pathophysiology identifiable

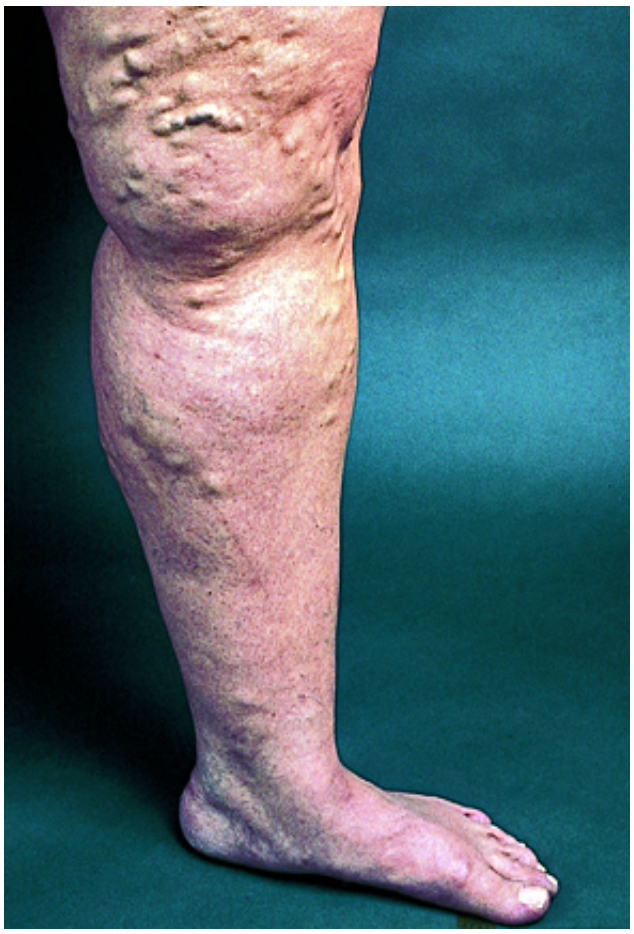
Table 2.1: CEAP classification of chronic venous disease, adapted from [6]

underlying venous valvular incompetence [10], however recent studies have shown to suggest that varicose veins and underlying valvular incompetence are not necessarily linked and can often develop spontaneously in discontinuous vein segments [37, 38].

Chronic venous insufficiency is primarily characterized by measured hemodynamic irregularity caused by valvular incompetence and subsequent reflux. While there is no evidence to support the development of varicose veins from valve failure [19], it appears that varicose changes precede the development of overt valvular incompetence, especially in superficial veins [39, 40, 41]. Left untreated, venous valvular reflux causes progressively higher levels of chronic venous hypertension and associated skin breakdown. Ultimately, venous stasis will progress to the point of open ulceration, most commonly around the medial distal leg

[16, 42, 29]. Venous stasis ulcers are slow to heal and have a high rate of recurrence, so intervention before venous disease progresses to the point of open ulceration is critical.

Figure 2.2: Varicose veins in the lower extremity. Image Credit: [2]



2.1.2 Secondary Venous Disease

Secondary chronic venous disorders result from an acute venous event that damages the walls or valves within the vein. This is most commonly an episode of acute deep vein thrombosis (DVT) [29]. In secondary venous disease, insufficiency most often results from a combination of both valvular reflux and obstruction. When this condition develops following DVT, it is referred to as post thrombotic syndrome, and can be characterized by pain,

edema, skin changes, and ultimately ulceration [29, 42]. Post thrombotic disease is caused by the destruction of deep venous valves during recanalization after an episode of acute DVT [43]. Similar to primary venous disease, if left undiagnosed and untreated, post thrombotic disease can lead to open ulceration and its associated health consequences, even to the point of amputation [44, 29, 45].

2.2 Venous Diagnostics

The challenges in diagnosing various forms of venous disease can be grouped into the recognition of venous obstruction, and determining venous valvular incompetence [12]. While varicosities can be identified through a physical examination by a vascular specialist, the functional status and patency of the underlying veins is important to quantify to help recommend appropriate treatment. Current diagnostic tools and their associated strengths and weaknesses are summarized in Sections 2.2.1 through 2.2.6.

2.2.1 Positional and Tourniquet Tests

Early vascular diagnostics were based on altering the physiology of the venous flow through positional changes and/or strategically placed tourniquets, and then measuring the hemodynamics of the patient through observation and palpation. These tests include the Brodie-Trendelenburg Test, Perthes test, and Saphenous Impulse tests[46, 12]. These tests have historically shown strength in being able to quantify individual patient hemodynamics, but their results are largely qualitative and rely on a skilled clinician to be considered reliable [46]. Advances in imaging technologies for the measurement of hemodynamic parameters have largely rendered these diagnostic procedures redundant.

2.2.2 Plethysmographic Tests

Plethysmography is defined as the measurement of volume changes within an organ or whole body, generally due to fluctuations in blood or air. In the diagnosis of lower extremity venous

disease, plethysmographic tests measure changes in blood volume of the leg, either at rest, or due to external influence [12]. Plethysmographic techniques include those listed in Table 2.2. In practice, all plethysmographic tests measure the volume change of the limb, either directly

Name	Method	References
Water Volume Pleth.	Changes in lower extremity volume measured by water displacement	[47, 48]
Air Celluloid Pleth.	Sleeve placed over the entire leg and pressure changes recorded	[49, 50, 51]
Pneumatic Cuff	Cuffs placed strategically along the leg and pressure changes recorded	[52, 49]
Strain Gauge Pleth.	Strain gauge placed around calf muscle, and length changes measured	[53, 54]
Photoplethysmography	Measure changes in light absorption and perfusion of subcutaeous blood	[55]
Impedance Pleth.	Resistance changes due to fluid flow measured and volume calculated	[56, 57, 58, 59]

Table 2.2: Different plethysmography modalities used in venous diagnostics

or indirectly. Standard measures of interest that can be quantified through plethysmography include [12]:

1. *Venous Volume* - Increase in leg volume in response to a standardized congesting pressure
2. *Maximum Venous Outflow* - Rate at which blood flows out of the leg after a congesting cuff is released
3. *Postural Venous Change* - Rate at which venous volume changes due to a shift in posture
4. *Inspiration Venous Change* - Increase in leg volume occurring when a deep breath is taken.
5. *Venous Reflux* - Measurement of the rate at which blood flows back into the leg after calf muscle pump is relaxed

2.2.3 Past Efforts in Impedance Plethysmography

Impedance plethysmography (IPG) was originally applied to the field of lower extremity venous diagnostics in the 1960s by two different research groups [58, 57, 60]. Originally, impedance plethysmography was implemented by placing electrodes on leg of the patient, and having them take a relaxed deep breath, holding at peak inspiration, and then exhaling passively [12, 60]. This maneuver causes an increase in intra-abdominal pressure, thereby obstructing venous outflow from the lower extremities. Accordant increase in leg impedance

was measured, and analyzed on a percent basis, with results similar to those obtained using a strain gage [59, 61, 54, 62]. Additionally, impedance plethysmography was used in studies to test its efficacy in measuring the presence of DVT [57]. Though initial results were promising, other efforts to implement impedance measurements in CVD were not as successful. Dmochowski et al. found that the overall accuracy of IPG at diagnosing the presence of obstruction was only 53.5% [63]. Steer et al. reported a false negative indication for the presence of DVT at 38% [64]. One of the primary reasons that impedance plethysmography was not pursued further was that the results of the test were determined to be inconclusive, or technically inadequate in many cases. One study reported difficulties in obtaining IPG results in 19 to 33 percent of patients [60]. Due to inconsistent results and technical limitations with the instrumentation, research efforts into the implementation of impedance plethysmography have dwindled in recent years.

2.2.4 Ultrasound

Ultrasound, and more specifically, duplex ultrasonography for the diagnosis and measurement of venous disease was first implemented by Strandness et al. [65] and Sigel et al. [66]. Subsequently, duplex ultrasound has become the most widely used diagnostic tool for localized vascular measurements to determine valvular patency and areas of potential obstruction [12, 25, 29, 10]. Duplex ultrasound requires different examination protocols to assess the existence of chronic venous disorders as opposed to DVT [10]. When measuring venous characteristics, a duplex examination is optimally performed in the standing position and includes a full assessment of both reflux and obstruction in the deep, superficial, and perforating veins [10]. This extensive evaluation can therefore be taxing on the sonographer due to poor ergonomic support and the amount of time required for a thourough examination. Often the extent of the ultrasound examination is based upon prior a clinical evaluation, and is used to accurately quantify the etiological, anatomic, and pathophysiologic state of the patient for accurate CEAP classification [10, 6].

2.2.5 Venous Pressure Measurements

Venous pressure measurements have been considered the most accurate and repeatable test for efficiency of the calf musculovenous pump [67]. Clinical evaluation involves placing a small needle in the back of the foot and measuring pressure during postural changes or walking (ambulatory venous pressure, or AVP) [20, 68]. AVP measurements directly measure the pressure in the lower extremity veins, and can provide a sensitive measure of venous obstructive or valvular disease. If the calf muscle pump is functioning normally, exercise will produce a marked drop in venous pressure at the foot level [12, 10]. Additionally, AVP has been shown to differentiate between venous disease and other conditions that produce edema, ulceration, and swelling [69]. Despite its strengths, ambulatory pressure measurements are considered invasive and are generally only prescribed in limited or difficult cases where other diagnostic methods have failed [7].

2.2.6 Venography

Venography (also called contrast phlebography) is a radiographic imaging method used to diagnose the status of a vein and determine the location and size of obstructions [7, 70]. It is performed by injecting a contrast agent into a vein and then collecting sequential x-ray images to assess hemodynamics in the patient. Venography can be performed either as ascending or descending, to measure obstruction or reflux respectively [71]. Although it is considered a reference method upon which other tests are based [72], it is an imperfect diagnostic standard because it is often painful for the subject, and induces deep vein thrombosis in 3-4% of patients [73]. Venography can also be used in concert with plethysmographic methods to assess the location and flow characteristics of the lower leg [70, 74]. Similar to venous pressure measurements, the invasive nature and potential for side effects limits the application of venography to cases where the clinician feels that it is warranted [75].

2.3 Problem Statement

As described above, there is no shortage of diagnostic tools for clinical evaluation of the venous insufficiency or blockage. Methods for extraclinical measurement are still extremely limited however, and if achieved, would significantly impact patient quality of life and clinical outcomes. The purpose of this dissertation work is to develop instrumentation and study methods by which clinicians can better understand the hemodynamics of their patients during normal everyday activity outside the laboratory. The development of accurate diagnostic tools for assessing mild venous insufficiency will increase the standard of care of patients by enabling earlier detection and identification of mild venous disease, especially for patients in lower resource settings. Quantification of extremity vascular parameters during normal daily activities over long periods of time is a novel application, and will serve to better the understanding of the presence and progression of venous disease. This will allow for greater efficiency in planning patient treatment, and promote better long-term outcomes for sufferers of CVD.

Chapter 3

AIM 1 - INSTRUMENTATION AND VALIDATION

3.1 Background

In modern vascular clinics, there exist many different diagnostic methods and tools to monitor and diagnose the vascular state of the individual patient. These diagnostic procedures have different degrees of invasiveness, depending on their measurement modality and how they are used to measure individual disease characteristics in the patient. The ideal diagnostic tool permits a quantitative assessment of blood flow through any given vessel, at any instant, without disturbing the flow itself [12]. According to Yanof and summarized by Strandness, ideal system characteristics are as follows [76].

1. A measurement range between 0 ml/min and 10 l/min
2. A linear response across the measurement
3. A signal-to-noise ratio of at least 50;
4. Capability and sensitivity to measure both forward and reverse flows
5. Temperature stability
6. A frequency response that is flat out to 50 cps

Strandness further states that when flow techniques are to be applied to patients in the lab, further requirements include [12]:

1. The technique must be safe
2. It should be capable of being used on a repetitive basis to obtain sequential data from the same subject
3. It should employ an externally placed sensing device.

While diagnostic procedures in the vascular clinic generally meet these criteria, there still exists a deficit in repeatable noninvasive measurement modalities used for the measurement

of the progression of venous disease. Current imaging modalities include but are not limited to plethysmography, ultrasonic velocity detection, and direct venous pressure measurement, among others [55]. While powerful, these tools are limited to use during visits to a vascular clinic [77]. The clinical setting, while controlled, is not representative of environment that patients live in and can potentially introduce some level of unknown bias to the vascular study results. Additionally, a significant portion of the developing world does not have access to a clinic staffed with vascular specialists, and their diagnostic options are limited [18].

There has been a recent push in medical research to develop diagnostic methods that can be used outside of the hospital or clinic, to better measure variables indicative of disease progression during the daily life of the patient. One example is long-term EKG monitoring for detection of arrhythmia, now considered standard in many practices [78]. Development of wearable medical devices have been made possible primarily by recent advancements in portable computing [79], increases in battery power density [80], and miniaturization of electronic components driven by the mobile communication industry [81]. As the population ages and prevalence of chronic disease increases, wearable technology for remote patient-monitoring and diagnosis will become increasingly ubiquitous.

3.2 Motivation

Lower extremity venous disease affects approximately 2.5 million people in the United States alone [82]. Diagnostic tools and trained staff are mostly limited to specialty vascular clinics however, which can cause delays in the identification of symptoms of venous disease and subsequent referral to a specialist. Ambulatory vascular monitoring has been proposed as a method by which specialists can collect hemodynamic data from the patient in a variety of out-of-clinic settings, but previously has not gained traction due to limitations in the instrumentation required [83]. The need for out-of-clinic monitoring is especially significant for patients suffering from early stage venous disease, when mild and transient symptoms can cause delays in proper diagnosis [7]. To provide a comprehensive suite of diagnostic

data in these patients, the information collected out of lab should not be limited to just hemodynamic characteristics. Increasingly, studies are showing that an understanding of behavioral characteristics during daily activities can aid in diagnostic efficiency when out-of-clinic [84]. To fully understand the overall venous health of the patient, it is important that a combination of objective behavioral data and accurate physiologic information is used.

3.3 Behavioral Characteristic Monitoring

The need for a noninvasive behavioral monitoring device has led to the development of the ECHO, a portable, lightweight system that can be used to monitor physiological and behavioral characteristics of patients during their normal daily activity. The ECHO can be implemented in custom configurations to measure variables of interest in individual patients. It implements a variety of sensors to monitor the activity levels and posture of the subject, as well as compliance with prescribed therapies (such as compression socks). The development, characterization, and exemplary implementations of the ECHO system are outlined in the following sections.

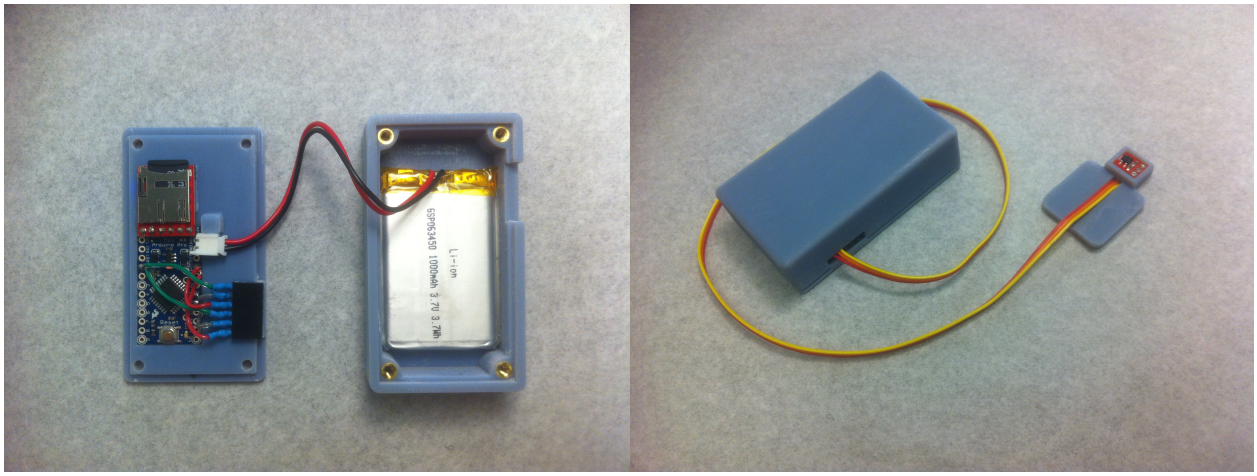
3.3.1 Version 1 - Custom Datalogger

A low profile datalogger was developed for use in out-of-lab studies on amputee subjects. It was designed to log data from force sensitive resistors and infrared proximity sensors, to determine how study participants used their prosthesis, and how force distribution changed within the socket or underfoot, over the course of a day. This datalogger was primarily designed for use with amputee subjects to measure prosthesis use, and was designed to be retrofitted to any socket and used over a period of up to 24 hours.

Hardware The Version 1 datalogger was designed using an Arduino Pro microcontroller with embedded ATmega328 microprocessor and integrated 10-bit ADC. Data was sampled from the sensors at a configurable sampling rate from 1-80 Hz, and stored to an SDHC card. Additionally, the signal was able to be transmitted via Bluetooth to a cellular

phone or computer for real-time analysis by a researcher or clinician. System power was provided by a 1000mAh Lithium Polymer battery, for up to 24 hours of continuous sampling. Peripheral sensors were connected using 0.1" header pins and variable length wires. The version 1 system is shown in Figure 3.1.

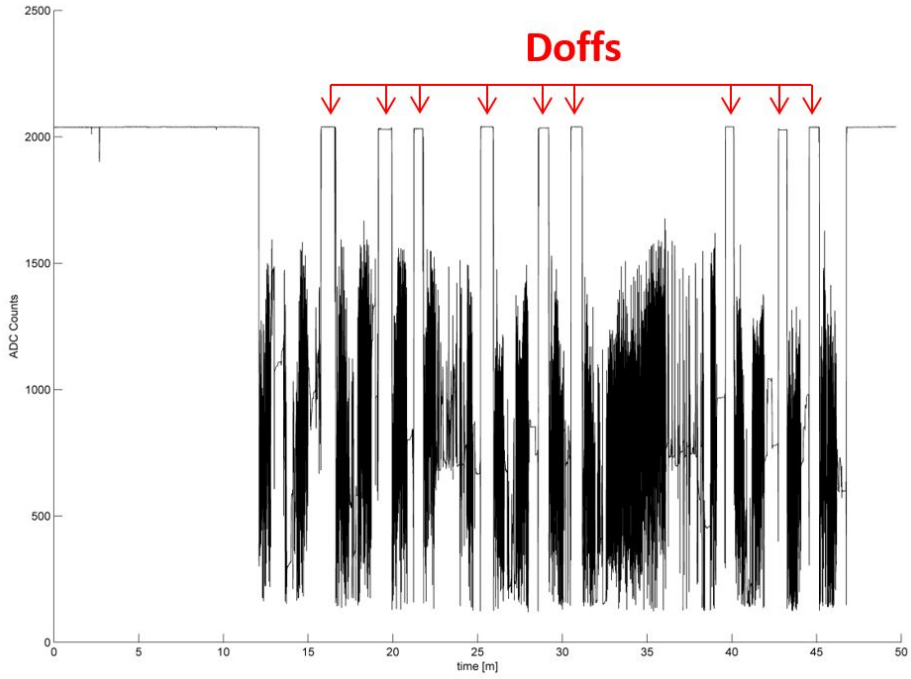
Figure 3.1: Version 1 datalogger and infrared proximity sensor



Performance Characteristics The Version 1 datalogger was initially used in a study of 11 amputee subjects to determine its ability to accurately measure whether or not the user was wearing their prosthetic leg. The datalogger implemented in this configuration was called the "Socket Proximity Sensor" (SPS) and used two infrared proximity sensors to determine if their prosthetic leg was donned or doffed. Subjects performed at 45 minute walkaround on the University of Washington campus, where they underwent periods of sitting, standing, walking, and sitting with prosthesis removed. Subjects were accompanied by a researcher, and their activities were videotaped to provide a ground truth. In all subjects, the SPS correctly identified all periods of doffing, with no false positives. Example prosthesis doffing data from the SPS can be seen in Figure 3.2 showing periods of high activity, and sitting while doffing the prosthesis. These proximity sensors, combined with accelerometry was shown to

accurately classify activity, posture, and prosthesis use 23 unmonitored participants during unrestricted out-of-lab activities (Figure 3.3) [85].

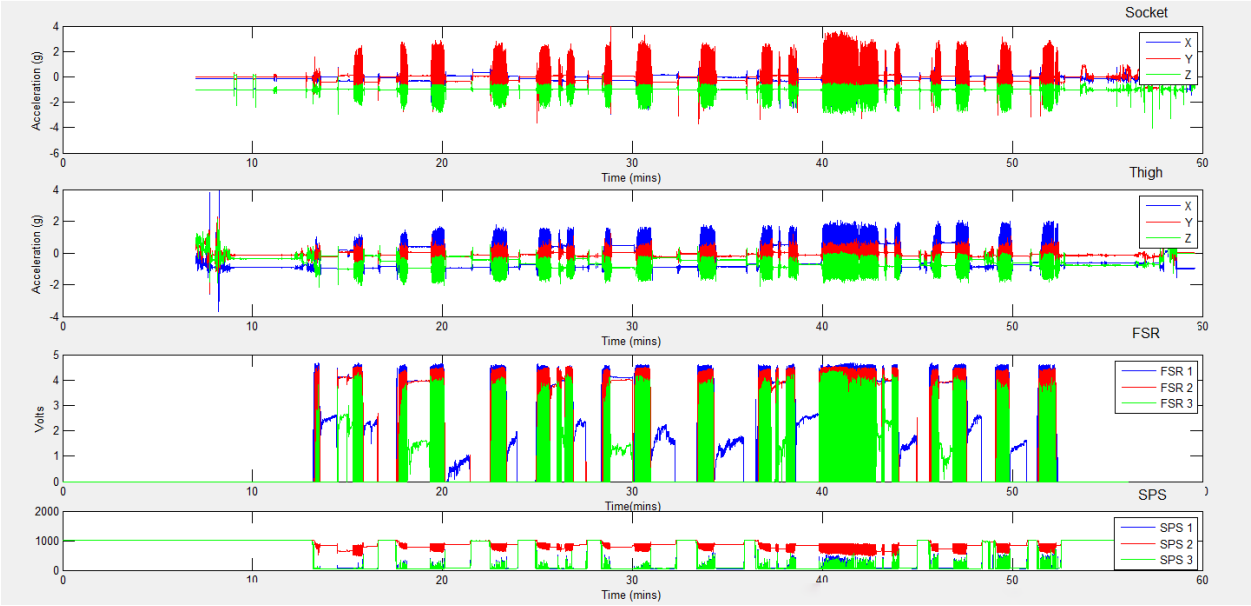
Figure 3.2: Exemplary SPS data during controlled subject activity showing periods of prosthesis doffing



Limitations The Version 1 datalogger was capable of accurately characterizing specific aspects of the individual's behavior, however it was limited by its power consumption to only 24 hours of data logging before the battery needed to be recharged. It was also limited to the use of a single sensing modality at any given time; if two sensor types (i.e., infrared proximity sensors and force sensitive resistors) were to be used at the same time, it required two discrete dataloggers. This dependency increased the bulk of the sensing package and limited the ability for longer term out-of-lab studies without needing to visit the clinic to charge the battery and download the data. Additionally, while it showed good resolution in specialized applications, it lacked additional sensors and implementation schema to be used

in more general amputee studies or vascular practice. These limitations were the reason for modifying the system architecture and switching to a custom board design.

Figure 3.3: Clinical GUI displaying accelerometer, FSR, and SPS data during 60 minute test protocol

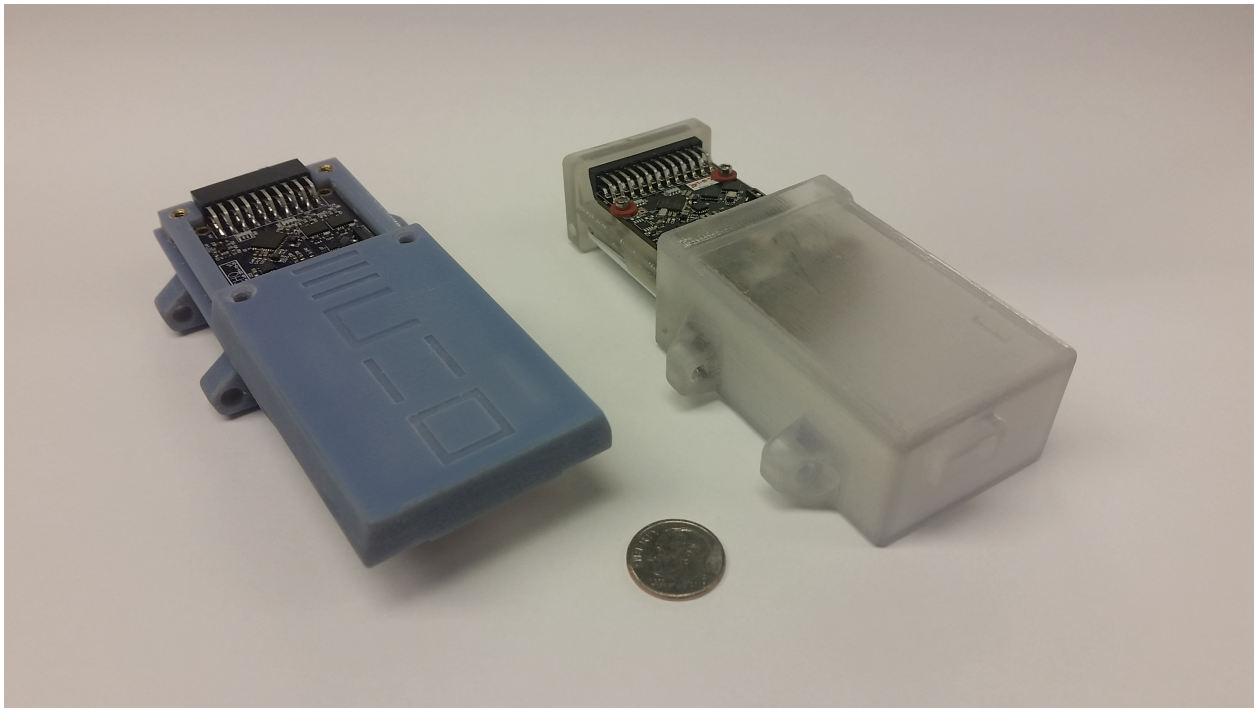


3.3.2 Version 2 - ECHO

Version 2 of the behavioral monitoring system, known as the ECHO system, has been developed as a general-purpose bioinstrumentation platform for use in studies on the use of lower extremity prostheses. It features a low-profile hardware installation, and the ability to implement a variety of different integrated and peripheral sensors to measure different aspects of the subject's health and activity. These sensors include infrared proximity sensors, force sensing resistors, inductive proximity sensors, and a 6-axis inertial measurement unit (IMU). It also has general purpose analog inputs for future integration of additional sensors or system modules. It can be used with any type of prosthetic socket or on an able-bodied patient, and has the flexibility to be used with a variety of different sensor combinations

depending on the study being performed. During extraclinical use, the ECHO system can be used for up to 96 hours at a time without the need for recharging. This system provides a powerful tool to assist researchers and clinicians in measuring user's behavior and the efficacy of different clinical interventions both in and out of the laboratory or hospital setting.

Figure 3.4: Two versions of the ECHO system with SLA fabricated enclosure boxes



Hardware Overview The ECHO system is a versatile hardware platform designed to provide the user with the ability to use flexible sensor combinations for long-term unobtrusive monitoring. The ECHO system is custom designed PCB based on a 32-bit ARM Cortex-M0+ microcontroller. It also utilizes a 16-channel 24-bit ADC to sample general analog sensors, and has integrated chips and conditioning circuits for specific peripheral sensing systems. The system architecture of the ECHO box is shown in Figure 3.5. The peripheral sensors designed and integrated into the ECHO system are outlined in Table 3.1. The ECHO

system has a configurable sampling rate from 1Hz to 40Hz and can record data from any combination of sensors simultaneously. For low-power applications where high or continuous sampling resolution is not required, the ECHO system can record data at an asynchronous duty cycle from 10-100%. When recording at low duty cycles (i.e., sampling for 1 second every 10 seconds = 10% duty cycle) the ECHO automatically goes into low-power mode, extending the battery life between charge cycles. The ECHO can also enter a low-power state in response to a detected user action, such as prosthesis doffing. These power management strategies enhance the usability of the ECHO by extending the amount of study time out of lab before the system must be recharged.

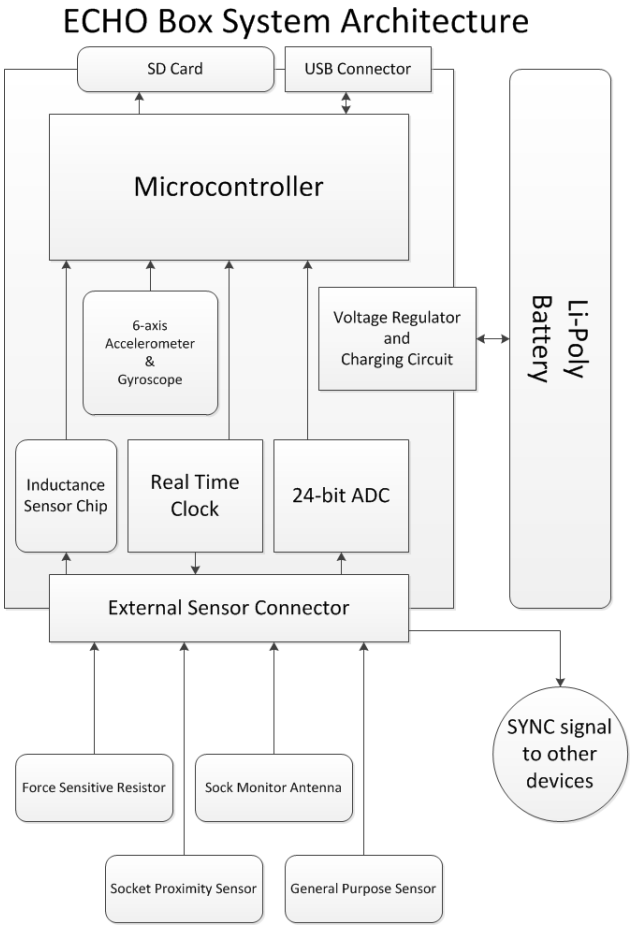
QTY	Name	Component	Description or Typical Use
8	Inductance Sensor	TI LDC1614	Used for inductive proximity sensing
4	Proximity Sensor	Fairchild QRE1113	Detecting socket Donning or Doffing
4	Force Sensitive Resistor	Interlink FSR400	Measure activity and force distribution
4	General Purpose ADC	GPADC	Ports for integration of additional sensors
1	Heart Rate Monitor	Polar T31	Connet to Polar T31 Coded Transmitter
1	Muscle Activation Sensor	EMG Amplifier	Measure electrical muscle activity and exertion
1	SPI	SPI	Can drive external sensors using SPI protocol
1	I2C	I2C	Can drive external sensors using I2C protocol
1	SYNC	SYNC	Sync time between ECHO and other devices

Table 3.1: Peripheral Sensors used for extra clinical patient monitoring in the ECHO system

In addition to the peripheral sensors listed in Table 3.1, the ECHO system has an integrated 6-axis IMU (3-axis accelerometer and 3-axis gyroscope)that can be used to monitor the subject's activity and posture throughout the day. This functionality is especially important for determining compliance with clinical recommendations or evaluating the efficacy of a prescribed treatment.

The ECHO system can be programmed using USB, and stores all collected data to an onboard microSDHC card. The system is capable of writing to microSDHC cards up to 32GB for extended monitoring periods. If the expected participant monitoring period is expected to be longer than 96 hours, the system Lithium-Polymer battery can be charged via a micro USB cable by the subject using a standard cellular phone charger. The ECHO

Figure 3.5: ECHO system architecture



system has an integrated real time clock (RTC) and backup battery that provides absolute time-stamped data during collection, even if power is lost while the subject is out of the laboratory. This RTC can also be used as an external square wave sync signal to align time-series data collection between multiple ECHO boxes, or other data collection system such as bioimpedance.

Applications The ECHO system was designed as a highly versatile data collection system for use either as a standalone monitoring device, or as part of a larger study involving additional sensors and protocols. Examples of applications for the ECHO system include:

- Socket Proximity Sensor - Use with Infrared Proximity Sensors to determine if a subject has donned/doffed their socket (Table 3.1)
- Sock Thickness Monitor - Use a single proximity sensing external antenna to reliably measure prosthetic sock usage out of the laboratory and quantify donning and doffing
- Socket Fit Sensor - Use with multiple proximity sensing antennas at strategic locations in the socket to measure prosthetic socket fit. Sensing antennas can be placed in 3D printed inserts or integrated directly into the socket wall during fabrication.
- Compression Stocking Monitor - Use with a single proximity sensor and shoe insole-mounted antenna to reliably measure compression stocking use out of the laboratory (As in Figure 3.6)
- Activity Monitor - Use the integrated or peripheral IMU units to measure subject activity, prosthesis orientation, and posture.
- Calf Muscle Pump Monitor - use dermal EMG electrodes to monitor amplitude and frequency of calf muscle pump firing during venous function studies.

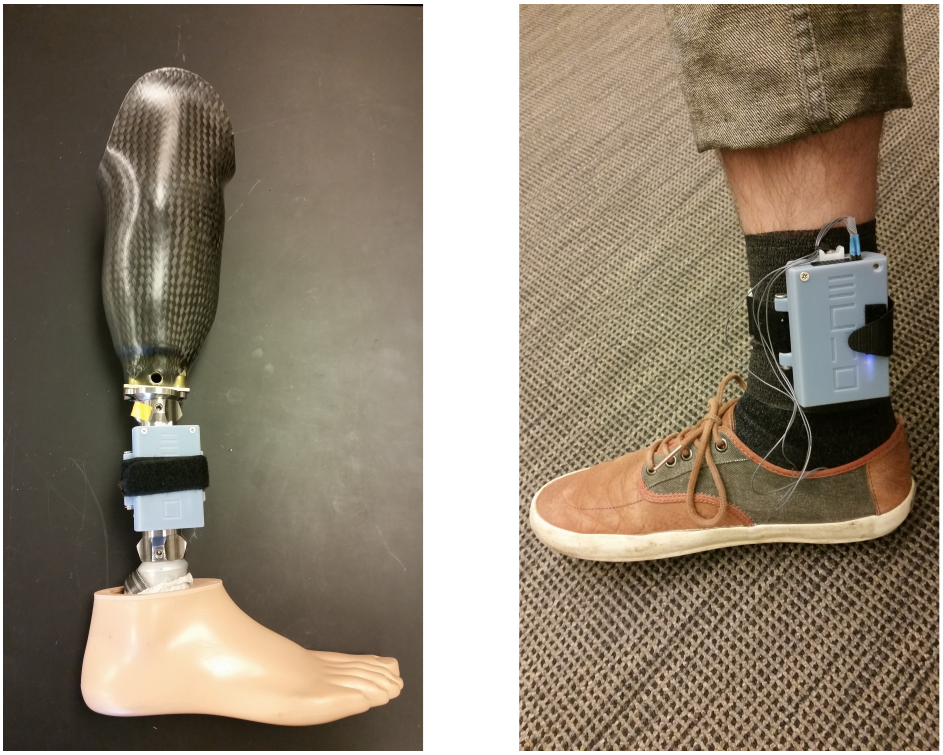
These individual applications can be combined with custom power-management profiles and sampling rates to form standardized testing schema. Examples of sensor combinations previously validated or proposed for future use are listed in Table 3.2.

Name	Sensors Used	Sampling Rate	Duration
Amputee Socket Fit	Inductance [x8], IMU	40Hz, 100% Duty Cycle	8 Hours
Amputee Sock Use	Inductance , IMU, SPS	40Hz, 10% Duty Cycle	2 Weeks
Vascular Compression Stocking Use	Inductance Sensor, IMU	1Hz, 10% Duty Cycle	4 Weeks
Vascular Subject Activity Monitor	IMU	40Hz, 100% Duty Cycle	4 Weeks
Calf Muscle Pump Monitor	External EMG, IMU	100% Duty Cycle	4 Weeks

Table 3.2: Peripheral Sensors used in the ECHO system

Extra-Clinical Monitoring The primary use of the ECHO system has focused on long-term out-of-lab monitoring of amputee subjects to characterize their use of prosthetic socks. Prosthetic sock use is one of the primary methods of accommodating for diurnal residual limb volume change in below-knee amputees [86]. Self-reporting of sock use has

Figure 3.6: Example implementation on able-bodied subject for compression stocking monitoring, and on amputee prosthesis for prosthetic sock wear monitoring

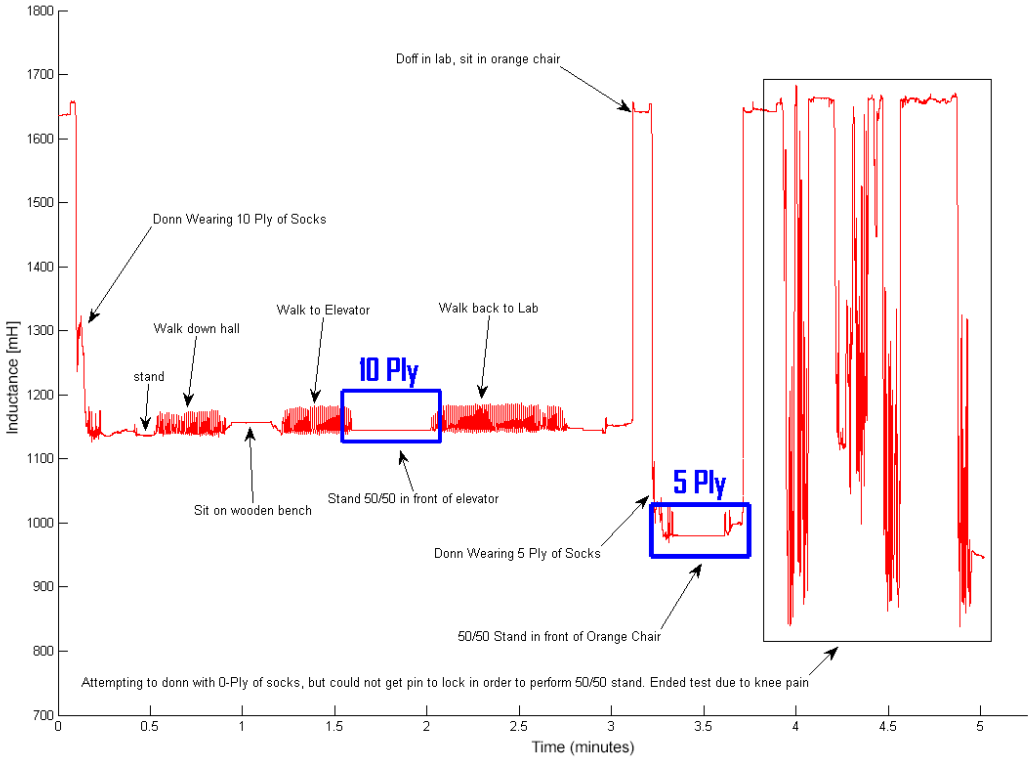


previously been shown to be a clinically useful tool, and studies about use characteristics are ongoing [86]. Efforts to build a device to measure sock use by radio frequency identification (RFID) in the socket have yielded promising results, but they have not been validated in large-cohort studies [87]. The ECHO box builds on previous engineering efforts to provide a means by which amputee sock use can be measured over several weeks, to better inform the practitioner of patient behavior and compliance.

The ECHO box can measure sock thickness data in amputee subjects through the use of a novel implementation of an inductance sensor, with a sensing antenna placed inside the prosthetic socket of the amputee subject. The subject uses a modified off-the-shelf elastomeric liner, with a silver-lined conductive fabric adhered to the exterior. When the socket is donned, the conductive target on the prosthetic liner alters the inductive field inside

the prosthetic socket, measured using the ECHO. This change in inductive field can be used to calculate the relative position of the liner with respect to the antenna on the socket wall. From this measurement the absolute thickness of the prosthetic socks that the subject is wearing. Exemplary sensor data from an in-lab study session are shown in Figure 3.7.

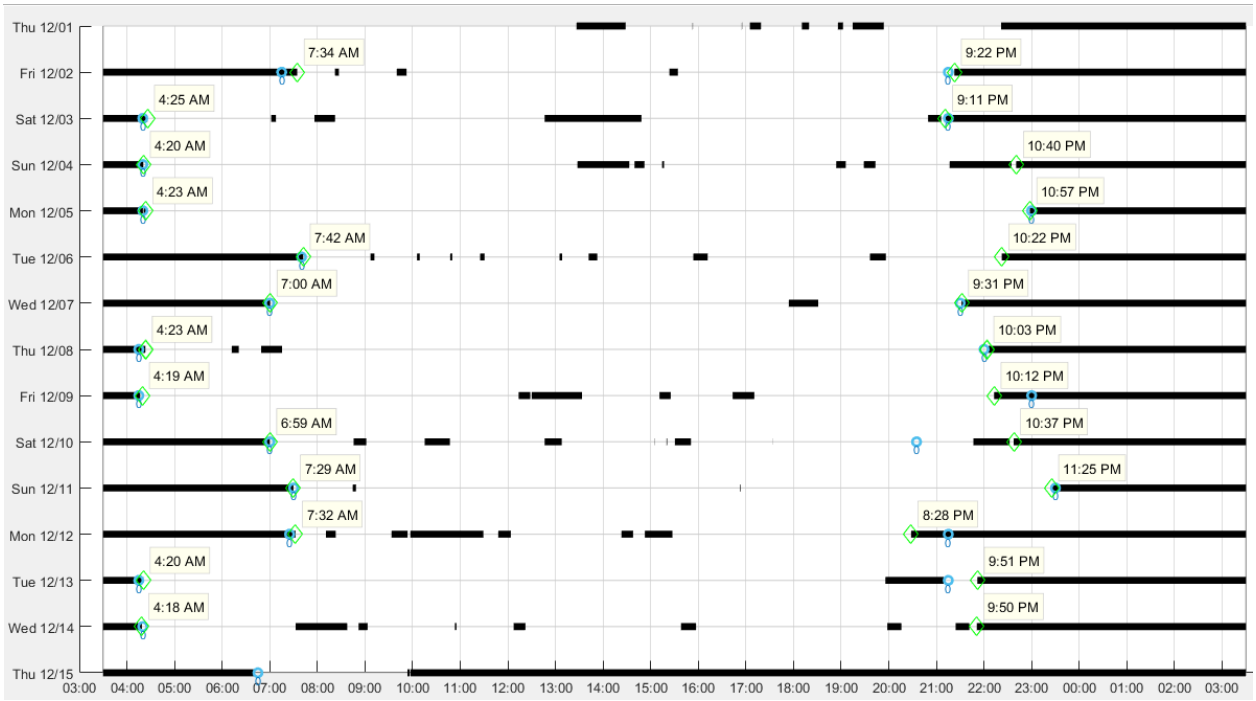
Figure 3.7: Example inductance data during amputee sock thickness testing



Using the ECHO system, data can be collected over a multi-day period to observe sock-change and prosthesis use trends during normal daily activities. An example of a two-week data collection period is shown in Figure 3.8 with prosthesis doffing periods indicated by black bars for each day. This data provides a new method for clinicians to observe how a commonly prescribed residual limb volume accommodation strategy is used on an individ-

ual basis. Clinical insights from quantitative data may facilitate further discussions with their patients or help prosthetists modify recommendations to better reflect the individual's measured behavior.

Figure 3.8: Socket use characteristics over a two-week period with doffs indicated by black bars



3.3.3 Future Work

The ECHO box has shown the ability to measure important behavioral characteristics of participants during initial in-lab and out-of-lab studies. While larger cohort studies are ongoing, the true strength of the ECHO system is the ease by which different sensor combinations can be used during prospective study investigations. Hypotheses can be quickly tested without a large investment in custom hardware, and overall study progression is accelerated as a result.

Further engineering efforts to refine the ECHO system hardware are also ongoing, based

on the experience gained during bench and user testing. The system will go through a planned board revision, to further miniaturize the components for ease of implementation on the subject's prosthesis or lower limb. ECHO v2 will be optimized for attachment to the shoe of vascular patients, with a minimal increase in weight, to minimize encumbrance.

3.4 Physiological Monitoring

Methods for out-of-clinic physiological monitoring are relatively nascent, brought about primarily through recent advances in microcontroller computing and battery technology [81]. The Holter monitor and subsequent iterations of long-term cardiac event recorders have demonstrated the value to clinicians of monitoring cardiac patients during normal daily activities [88]. Similarly, long term measurement of ambulatory and home monitoring of blood pressure is becoming increasingly common in diagnosis and management of hypertension [89]. In a meta analysis evaluating the effectiveness of home monitoring technology on outcomes in patients with high blood pressure, all patients who had remote home monitoring of blood pressure had lower readings at 6 months [90]. However, those patients who had home monitoring as well as additional support from their physicians office had greater long term benefits. This result suggests that while measuring physiological parameters of a patient can bring about significant benefit, such technology is best used through the prescription and supervision of medical professionals.

There does not currently exist a method by which the hemodynamics of lower extremity blood flow can be measured for extended periods either within or outside the clinic. As discussed in Section 2.2.2, different methods of plethysmography have been developed to quantify blood flow during short ambulatory bouts, but none have been validated for long-term use [83]. Impedance plethysmography has traditionally been evaluated as a method by which venous circulation could be evaluated, but the devices used suffered from low resolution, and a high number of false positives [91, 92, 63, 93, 58, 64, 57, 60]. The primary deficit in previous efforts at measuring venous circulation through impedance changes is difficulty in standardizing the test procedures from trial to trial [12, 91, 64]. Tests based

on inspiration or postural changes by the patient suffer from low repeatability, and even those using a proximal pneumatic tourniquet suffer from measurement error if not carefully controlled by the investigator. The use of a tilt-table to control gravitational influence on venous flow during APG measurements has been reported to reduce test-retest variability but is still limited to a short investigational session in the vascular lab [94]. Long term monitoring of patient hemodynamics is therefore an alternative option to reduce the reliance on short patient trials, and their accordant difficulties. Time-series data recorded throughout hours or days, can offer insight into the blood transport in the lower extremities as the patient goes about their normal daily routine.

3.4.1 Portable Bioimpedance

Recent advancements in portable bioimpedance spectroscopy have resulted a new and novel device that can noninvasively measure the extracellular (blood/lymph) and intracellular fluid flow in extremities of patients [95, 96]. The theory and practice of bioimpedance spectroscopy for quantification of fluid volume changes in portable out-of-clinic monitoring is described in the following section.

3.4.2 Review of Bioimpedance Spectroscopy

Introduction The non-invasive use of electrical current to determine the composition and function of tissues has been widely researched and applied to different areas of biomedical imaging. These different research and development efforts are generally categorized as falling under the umbrella term of bioimpedance analysis, though their exact methods of instrumentation, implementation, and analysis vary widely. Past bioimpedance research has involved but is not limited to assessment of whole body composition [97], estimation of pulsatile blood flow [98], and development of models for segmental body measurements [99]. More recently, the Sanders Lab at the University of Washington has developed and published methods for in-socket measurement of residual limb volume in trans-tibial amputees using bioimpedance [100, 101, 86, 3, 95]. These research efforts are ongoing, and represent a novel

method to assess the utility of different prosthetic suspension systems, efficacy of clinical prescription practices, and long-term tracking of residual limb health. This review describes the general theory of bioimpedance measurements, the strengths and limitations of the different methods, and recent advances in technology and research for clinical implementation.

Bioimpedance Theory Bioimpedance measurements are based on the flow of electrical current described by Ohm's Law [4]. The flow of an electrical current (I) passing through two points of a conductor is equal to the voltage drop (V) divided by the electrical resistance (R) between those points [4]:

$$I = \frac{V}{R} \tag{3.1}$$

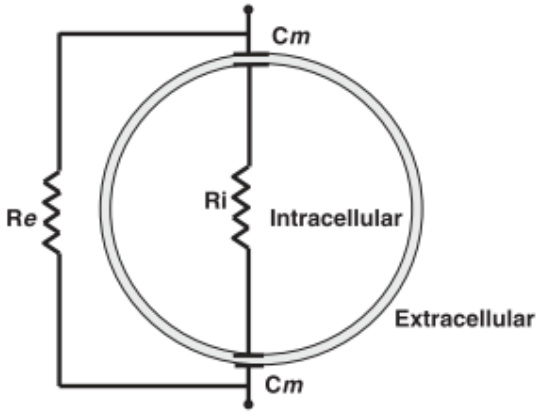
This law is based on direct current flowing through a simple conductor, such as a wire or similar homogeneous electrical conductor. Generalizing Ohm's law for use with alternating current, the resistance (R) is replaced with the impedance (Z). Electrical impedance is a complex number formed from two parameters: Resistance (R), and Reactance (Xc). The complex impedance can be represented according to:

$$Z = (R^2 + Xc^2)^2 \tag{3.2}$$

Resistance (R) is the opposition of a circuit element to the flow of electrons, or current, whereas reactance (Xc) is the opposition of a circuit element to a change in current or voltage, due to the element's inductance or capacitance [102]. In Bioimpedance analysis, the concept of impedance is important, because variations in the resistance and reactance within different areas of the body can be measured to quantify changes in biological variables [103].

The human body is comprised of conductive and non-conductive components. Fat cells are generally non-conductive, so the electrically active constituents are grouped together as the Fat Free Mass (FFM). These electrically active elements include tissue, fluid volumes, and cell membranes [4]. Current is conducted well by water and electrolyte-rich tissue (eg. Blood/muscle) and is poorly conducted by fat, bone, and air filled spaces [104]. Previous

Figure 3.9: Equivalent electrical circuit representing the bioelectric components of a living cell, adapted from [3]



research efforts have explored the theory that tissue conductivity is directly proportional to the amount of electrolyte-containing fluid present [4, 105]. These electrical components can be modeled as a controlled electrical circuit (Figure 3.9), where the measured voltage drop during the application of a small amplitude alternating current (AC) yields bioelectric measurements relating to changes in their structure and function [106].

In bioimpedance measurements resistance is the opposition to AC current flow through intracellular and extracellular ionic solutions. Reactance is the delay in the conduction of the current across cell membranes and tissue interfaces. Reactance in the body is due to the capacitance of the membrane structure and function, and causes the sensed voltage waveform to lag behind the input waveform.

There are several bioimpedance approaches that have been previously applied to the clinical measurement of tissue properties within the human body [107]. Single frequency bioelectrical impedance analysis (SF-BIA) is the most widely used bioimpedance methodology, having been developed and used primarily for the estimation and quantification of total body water (TBW). SF-BIA involves the application of a single current (commonly 50kHz), with the impedance data subsequently entered into predictive equations to deter-

mine TBW [107]. The use of multiple AC frequencies in bioelectrical impedance analysis is termed Bioimpedance Spectroscopy (BIS) and leverages the frequency-dependent response of the cell membrane to differentiate between extracellular and intracellular fluid [97, 107, 108].

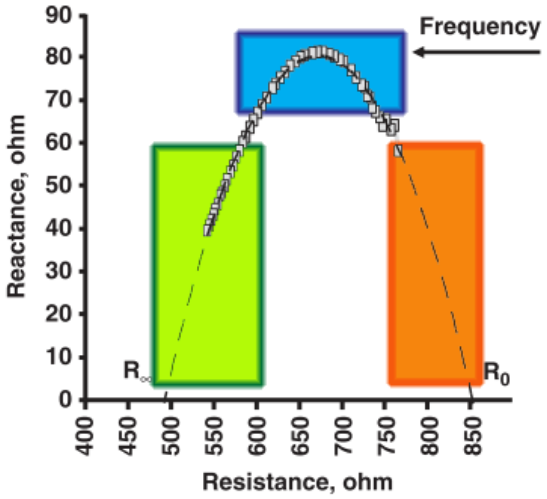
Bioimpedance Spectroscopy Bioimpedance spectroscopy is an approach to non-invasively assess biological parameters in vivo by measuring the impedance in the body while using a range of injected current frequencies [109]. This frequency spectrum typically spans from 5 to 1000 kHz, though the exact implementation varies between different commercial and research devices [105]. The current injected is of constant amplitude, the maximum level being limited and tested according to the IEC 60601-1 standards [110], which, depending on the method of implementation is approximately 800μA.

To record a spectroscopic bioimpedance measurement, the spectral data (resistance vs. reactance) are sampled at each frequency, and assembled into a locus plot (Figure 3.9). The behavior of the impedance locus can be described by an equivalent electrical circuit, as shown in Figure 1 [4]. Using Kirchoff's current law, the impedance of the circuit through the circuit can be derived according to Equation 3.3

$$\frac{1}{Z_{tot}} = \frac{1}{Z_{ECF}} + \frac{1}{Z_{ICF}} \tag{3.3}$$

At low frequencies, the electrical current travels exclusively through the extracellular fluid, as the capacitance of the cell membrane acts as a barrier to low frequency current. The impedance at this point is primarily resistive, and falls in the lower right corner of the locus (orange area of Figure 3.10). As the frequency increases, more of the electrical current begins to travel across the cell membrane and through the intracellular fluid. The reactance of the circuit increases, and the resistance decreases, represented by the values in the blue area of Figure 3.10. At very high frequencies, the capacitance of the cell membrane is negligible, and current flows through the extracellular and intracellular fluid equally. The impedance at this point is again primarily resistive, represented by the green area of Figure 3.10 The impedance

Figure 3.10: Impedance locus of the lower extremity. Semicircle distribution of the impedance values with extrapolation to calculate R_0 and R_∞, from [4]



locus across the frequency spectrum is then fitted using a least-squares nonlinear curve fit algorithm to a model developed by Cole-Cole (Equation 3.4), and revised by DeLorenzo et. Al [97, 111] (Equation 3.5). The De Lorenzo correction to the Cole Model includes two additional parameters, α and T_d which are meant to account for phase delay within the electrical system and instrumentation components.

$$Z = R_\infty + \frac{R_0 - R_\infty}{1 + (j\omega\tau_Z)^\alpha} \tag{3.4}$$

$$Z = (\frac{R_0}{R_e + R_\infty})(R_\infty + \frac{R_e}{1 + (j\omega C_m(R_e + R_\infty))^\alpha}(e^{-j\omega\tau_d}) \tag{3.5}$$

In practice, iterative nonlinear curve-fitting software is required to optimize and estimate R_0, R_∞, and C_m for observed values of Z, T_d and α. This curve fitting produces Cole model terms to describe the resistance of the individual components, including the resistance of the extracellular fluid (R_0), resistance of intracellular fluid (R_∞), and the cell membrane capacitance (C_m). The Cole model terms are used in equations derived from the Hanai mixture theory [97, 4, 99, 107, 112, 113] to calculate the volume of the extracellular and

intracellular water.

Hanai mixture theory is based on a generalization of the body's constituent parts into conducting (water, electrolytes, lean tissue) and non-conducting (fat, bone) mediums. The application of mixture theory for volumetric calculations has been subsequently revised from Hanai's original equations, with more accurate constants (Equation 3.6) derived for men and women using dilution data [114] by Jaffrin et, al [99, 112].

$$V_{ECF} = (\frac{\rho ECF * C}{R_0})^{2/3} * \frac{L^{5/3}}{(4\pi)^{1/3}} \tag{3.6}$$

This volume conduction model to relate impedance to volume in a uniform conductor is related to the product of specific resistivity (ρ) and length (L) of the conductor, and indirectly to the conductor cross sectional area [4] .This model is based on the assumption that the conductor has homogeneous composition and constant geometry. Divergence from these assumptions (as in the case of amputees) potentially affects the validity of the model [104].

Segmental Bioimpedance More recent advancements in the field of bioimpedance measurements have required the addition of models and measurement techniques to refine the accuracy of the method beyond whole-body composition measurements. Originally proposed by Organ et al., segmental bioelectrical impedance analysis described a new technique whereby individual segments of the body can be instrumented and analyzed including the trunk, lower, and upper extremities [115]. This technique introduced a more accurate model for volumetric calculation based on electrical resistivity, as well as a now widely-adopted six electrode instrumentation technique. The electrode placement called for 4 voltage sensing electrodes (Right Ankle, Left Ankle, Right Wrist, Left Wrist) and two current injecting electrodes.

In segmental bioimpedance analysis, the constant current is injected through the two current injecting electrodes, and the voltage is sensed at the 4 voltage sensing electrodes. The measured voltage at each electrode can then be used to determine the voltage drop

within that limb segment. The voltage drop across the limb segment is then used to calculate the impedance according to Ohm's law, as discussed previously. By measuring the voltage drop across the segment, and measuring the geometrical anthropometry of the segment, the Extracellular Water (ECW) and Intracellular Water (ICW) volume can be calculated. Time series analysis of this volumetric data forms the basis of clinical bioimpedance measurements [4, 107, 109].

Bioimpedance Measurements in Transtibial Amputee Subjects Residual limb volume change in people with lower limb amputations is a significant challenge to safe and comfortable ambulation using prosthetic sockets [116, 117, 118]. Short term (diurnal) and longer term (∼3-6 months) changes in the residual limb volume changes the fit of the prosthetic socket, leading to discomfort, skin breakdown, and inability to ambulate [118]. Bioimpedance measurements present a novel option for assessing residual limb volume change while the residual limb is within the socket [95].

Preliminary efforts to measure residual limb fluid volume change used a commercially available system (Xitron Hydra 4200, Xitron Technologies, San Diego, California). This system measured the impedance of the limb at 50 frequencies between 5 kHz and 1000 kHz. The system sampled at approximately 1 Hz. In these preliminary studies using the Xitron instrument, it was determined that the limitations in sampling rate, frequency profile control, and single-channel measurement were significant and justified the development of a custom bioimpedance instrument for use in studies of the residual limb volume of amputees. During this development process, the significant challenges to implementation were addressed through bench and clinical testing with able-bodied and amputee subjects [95].

Using the commercial Xitron system, a bench test was performed to assess instrumentation error. This test involved measuring the impedance of a static 50 circuit over 1 hour, repeated 5 times. During this test the variability (SD/mean) in the resistance measurement was calculated to be 0.005 percent. Instrument drift was 0.012 Ω/hour (0.012 percent/hour). Variability in the repeatability tests was 0.011 percent, leading to a calculation of RMS error

over the 1 hour interval of <0.014 percent.

Able-bodied subject tests were initially performed with two subjects to assess the change in measured impedance due to electrode removal and reapplication. The results of this test showed a change in R_{ECF} of 0.05Ω for subject N1, and 0.12Ω for subject N2. These changes in resistance correspond to volume differences of 0.025% and 0.075% respectively.

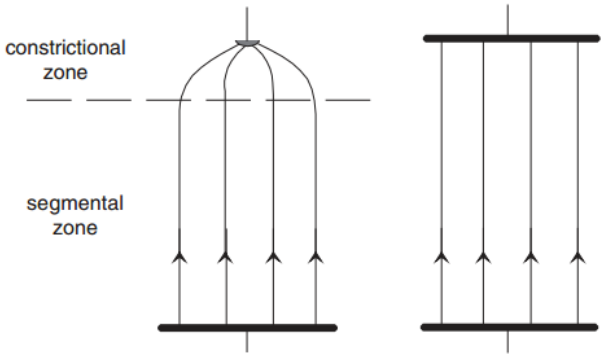
A significant consideration for bioimpedance measurements of the residual limb of amputee subjects is the electrode spacing. Variability in the length of the residual limb prohibits population standard measurement protocols, so experiments were performed to determine the dependence of the bioimpedance measurements on distance between voltage sensing electrodes, as well as the current injecting electrodes. Two different segment lengths were investigated, with spacing of 10.0 and 5.5 cms respectively. R_{ECF} measurements were shown to be lower for shorter distances between voltage sensing electrodes, as expected. R_{ECF} decreased by 47.7 percent for subject N1 and 36.6 percent for subject N2. Cole-Cole modeling still performed well for the short segment length, which is required for use on amputees with short residual limbs [95].

The distance between the distal current-injecting electrode and the distal voltage sensing electrode was also shown to have an effect on the measured R_{ECF}. At very short distances (<2 cm), data was noisy and did not fit the Cole model well. At distances from 3-5 cm and greater, the data conformed well to the model. Further testing on additional subjects [96], has verified a linear decrease in measured resistance with decreasing distance from the distal current injecting electrode to the distal voltage sensing electrode. This relationship holds for able-bodied subjects as well.

This result can be better understood by the concept of current density at the site of the impedance measurement [5]. In a tetrapolar electrode system (two current injecting electrodes and two voltage sensing electrodes), there exists a threshold distance away from the current injecting electrode, where the measured current is equally distributed throughout the muscular compartment. At distances closer than this threshold, known as the constrictional zone (Figure 3.11) [5], voltage sensing electrodes will sense a higher electrical density, and

thus a higher measured impedance. This result is significant due to the inherent geometrical limitations in placing electrodes on the residual limb of amputee subjects. Such geometrical limitations are lessened in able-bodied subjects, but are still a consideration for repeatable bioimpedance measurements. This finding has influenced our clinical protocols for consistent

Figure 3.11: Theoretical current distribution within the limb. Increased resistance is shown in the constrictional zone close to the current injecting electrode, from [5]



electrode placement. Experimental findings have shown that placing the proximal current injecting electrode at any distance greater than 25cm away from the closest voltage sensing electrode results in no change in the measured voltage for that limb segment. We have therefore adopted this threshold as the minimum distance when applying electrodes to the limb.

Our experimental findings with the distal current injecting electrode have shown that the inherent limitations in space on the residual limb will cause the most distal voltage sensing electrode to be in the constrictional zone. We have controlled for this limitation by standardizing the size and placement of the distal current injecting electrode, and specifying a consistent distance of 5cm from the distal current injecting electrode to the distal voltage sensing electrode. Repeatability studies have shown that this electrode placement protocol minimizes the bias introduced and produces repeatable measurements in subsequent bioimpedance sessions in both amputee and able-bodied subjects [95]. In able-bodied sub-

jects, the distal current injecting electrodes is placed at the distal termination of the anterior and posterior muscle compartments, and the voltage sensing electrodes placed exactly 5 cms proximal.

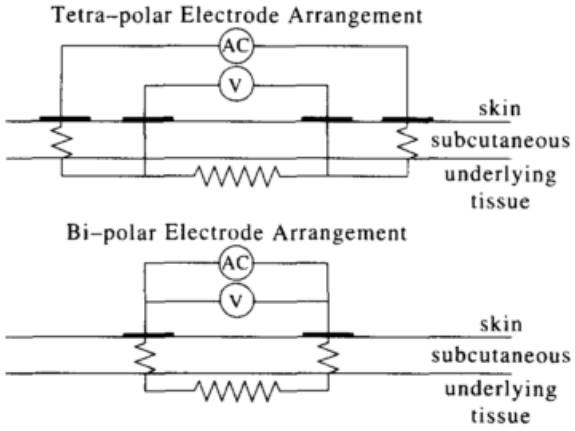
The size and placement of the voltage sensing electrodes was also investigated, to determine how changing the size of the voltage sensing electrodes influences the measured impedances. This experiment involved placing long (8cm) voltage sensing electrodes (VSE) on an able bodied subject, and then subsequently cutting them down, thereby reducing their side-to-side length. The rationale for this experiment is the existence of isopotential lines within the subject's leg [5, 119]. Isopotential (also termed equipotential) lines represent regions in a charged medium where all points exist at the same voltage potential. Long conductive voltage sensing electrodes overlap multiple isopotential lines, and therefore care must be taken to ensure that electrode size and placement does not influence the measured impedance. Experimental results showed that the measured impedance was reduced as the VSE became shorter, the magnitude of each change decreasing asymptotically to almost no change at VSE lengths 5cm and shorter. From these experimental results, we standardized the length of voltage sensing electrodes at 5cm for anterior and posterior bioimpedance measurements.

The depth to which tissue can be measured is also important to ensure that bioimpedance measurements recorded are representative of changes in fluid volume of the entire residual limb, and not unduly biased by local fluctuations [5]. Previous studies have been performed to optimize the electrode arrangement, with the tetra-polar arrangement (using surface electrodes) having been shown to eliminate the 'skin impedance' from the measurement circuit (Figure 3.12); therefore the measured impedance is that of the underlying deep tissue [114, 120, 121, 122].

The electrode wiring harness, and electrodes themselves have been chosen and tested after extensive review of available electrode designs. Electrode material is composed of a highly conductive tape (ARcare conductive adhesive), and a hydrogel (Katecho KM-10B, Later 3M) to eliminate effects of stray impedance on the measurements being collected. Additionally,

the entire wiring harness and electrodes are calibrated prior to each test to account for the impedance of the wires and connections [123].

Figure 3.12: Bi-polar and tetra-polar electrode arrangement, showing the influence of skin and subcutaneous tissue. From [5]



Summary of Bioimpedance Theory Measurements of the bioelectric properties of the human body have been researched extensively as a non-invasive in vivo imaging method. Previous research efforts have expanded from measurements of whole body water, to segmental measurements of individual appendages. Spectroscopic methods have been developed to leverage the frequency dependent nature of tissues, and differentiate between intracellular and extracellular fluid. Volumetric models have also been developed and refined to accurately characterize the fluid volumes of the measured muscular compartments, based on the measured impedances. Electrode schemes have been developed and validated to minimize measurement error due to electrode polarization, and variability in the geometric placement.

While the field of bioimpedance measurements is relatively well established, the application of bioimpedance spectroscopy to amputee subjects is nascent, and is the subject of continuing research initiatives. This research is ongoing, with future initiatives in instrumentation development, volumetric modeling, measurement accuracy and repeatability.

Additionally, absolute volumetric measurements require the assumption of certain parameter values, such as subject-specific fluid resistivity and anthropometric orientations. These assumptions have been previously studied, but could be refined to more accurately represent the population being studied.

Lastly, the Cole-Cole model has been shown to accurately represent data in ideal cases, but the model fit rapidly degrades in non-ideal implementations, such as a study subject who has implanted orthopedic hardware. Future work should therefore be undertaken to develop more robust and accurate models, to take advantage of advances in instrumentation hardware and computing power.

3.4.3 Portable Bioimpedance in Practice

Preliminary Bioimpedance studies within the Sanders Lab used a commercially available system (Xitron Hydra 4200, Xitron Technologies, San Diego, California) to characterize changes in residual limb volume in amputee subjects. The limitations in sampling rate, frequency profile control, and single channel measurement in the Xitron instrument were significant and justified the development of a custom bioimpedance instrument for use in studies of the residual limb of amputees [96, 124], and further investigations in to vascular hemodynamics. Subsequent to the development and validation of the in-laboratory bioimpedance monitor, engineering efforts were focused on portable bioimpedance solutions. The result of these engineering developments are the multiple generations of portable, battery-powered, bioimpedance devices optimized for use in out-of-lab studies on amputees and vascular patients. These portable bioimpedance devices are and an example of their use is shown in Figures 3.13 and 3.14.

OZONE Portable Bioimpedance System The current generation bioimpedance instrument (named the OZONE) was developed for unobtrusive fluid volume monitoring during long-term out-of-clinic studies on amputee and able-bodied subjects. Low-profile electrodes can be placed on the limb underneath a prosthetic liner or vascular compression stocking.

Figure 3.13: Two Portable bioimpedance instruments developed in the Sanders Lab for use on amputee and able-bodied subjects

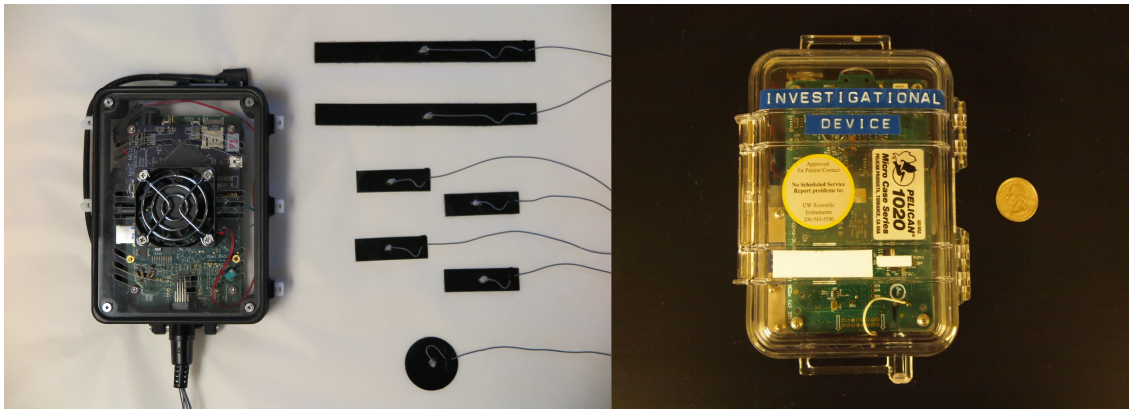
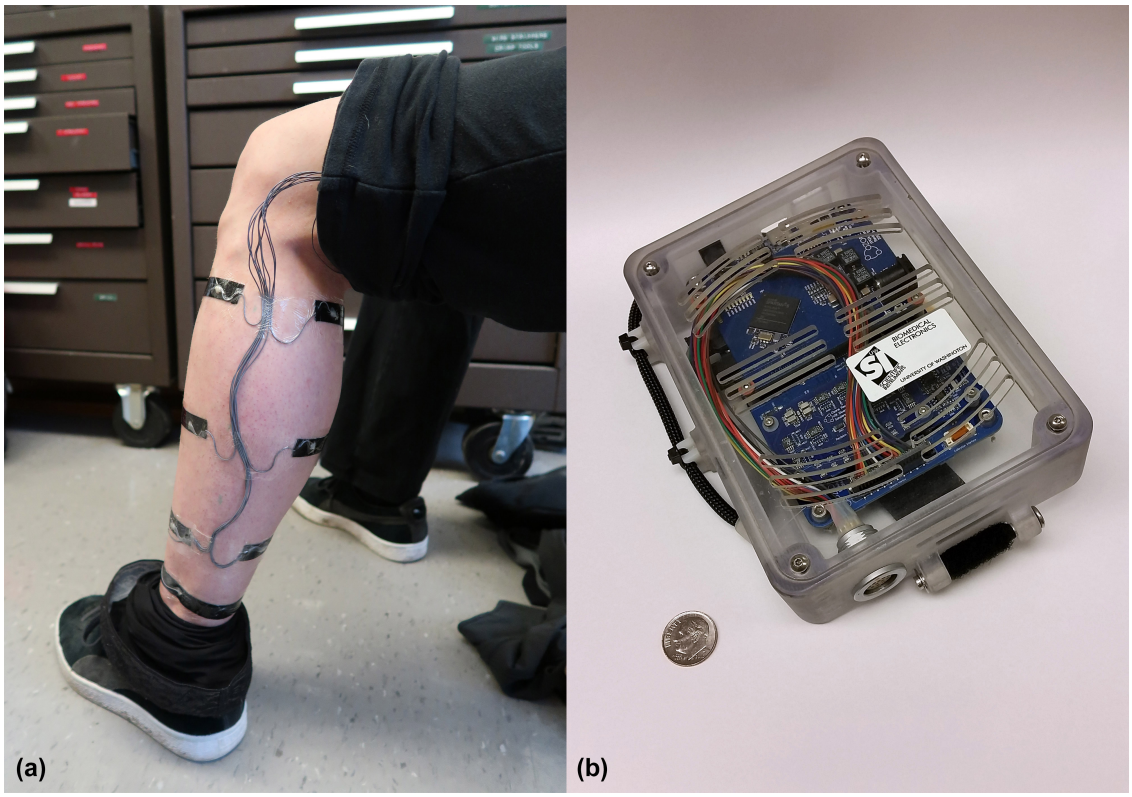


Figure 3.14: An example of sensing electrode placement on a vascular subject (a) and the OZONE portable bioimpedance instrument used for long term hemodynamic studies(b)



The hardware is battery powered and can be mounted to a waist belt, with electrode wires unobtrusively and discreetly routed underneath clothing. Performance characteristics of the OZONE during normal use conditions are included in Table 3.3 below.

Name	Parameter	Description
Sampling Rate	1Hz - 40Hz	Configurable sampling rate depending on implementation
Sweep Content	1 - 28 Freqs	Single sample can contain between 1 and 28 frequecies
Frequencies	3kHz - 1MHz	Sweep can include frequencies between 3kHz and 1MHz in any order
Channels	4 Channels	4 Channel system samples multiple regions of anterior and posterior
Communication	USB, 802.11g WIFI	Wired and wireless communication available
Battery Life	6600mAh, 8 Hours	Battery can be exchanged during data collection
Resolution	0.1mL	Minimum detectable fluid volume change in area of measurement

Table 3.3: Performance characteristics of the OZONE portable bioimpedance instrument

During a bioimpedance study session, the OZONE is connected to the lower limb of the participant through a set of low-profile custom electrodes (3M Hydrogel, ArCare8881 Conductive Substrate, 7 strand 32AWG wire). Electrodes are placed using anatomical landmarks to ensure consistency between testing sessions and validity of bioimpedance measurements. Bioimpedance study protocols are designed to measure the lower limb fluid volume characteristics in response to controlled or uncontrolled behavior in and around the laboratory setting. Further information on how bioimpedance measurements are collected and venous blood volume parameters are extracted and interpreted is included in Chapters 4 and 5 for both in-lab and out-of-lab participant studies.

3.5 Future Work

While limited forms of bioimpedance spectroscopy were previously investigated for use in the quantification and diagnosis of venous disease, they failed to gain traction for widespread diagnostic use. Early results indicated that blood volume changes can be accurately measured, however limitations in hardware and instrument resolution prevented accurate and reliable implementation by vascular specialists. Recent advances in bioimpedance technology however, both in accuracy and sampling duration, have allowed for non-invasive measurements of fluid volume changes in both lower extremity amputee subjects as well as in persons with

lower extremity venous disease. Portable bioimpedance monitoring both in and out of the clinical setting may provide insight into lower extremity hemodynamics not available through the use of current imaging modalities. This potential application of portable bioimpedance and supporting participant studies are presented in this dissertation in Chapters 4 and 5.

Chapter 4

AIM 2 - USING MULTI-CHANNEL IMPEDANCE PLETHYSMOGRAPHY TO MEASURE LOWER LIMB VENOUS HEMODYNAMICS

4.1 Abstract

Background

Plethysmography is an accepted method for measuring hemodynamic changes in the lower limb. However, the use of plethysmographic techniques for standalone diagnosis of venous disease is generally limited due to their inability to provide anatomic information about the location and extent of venous insufficiency.

Objective

This study evaluated several quantitative measures of venous blood flow using a novel impedance plethysmograph to determine which parameters provided the greatest ability to differentiate between hemodynamic features of healthy individuals and those with mild venous disease. Venous flow characteristics in the anterior and posterior region of the leg were measured simultaneously to evaluate if venous return differed between anatomical regions of the leg.

Study Design

Matched participants

Methods

Two groups of participants (vascular-compromised and healthy control) underwent hemodynamic testing using a multi-channel impedance plethysmograph. Test sessions involved inflation and deflation of a proximal pressure cuff while lying supine and a subsequent series of standing tiptoe maneuvers. Participant's hemodynamic characteristics were measured and differences between the groups were reported.

Results

Participants with prior diagnosis of venous insufficiency showed lower blood ejection capacity in a single tiptoe test and greater amounts of reflux during a ten tiptoe maneuver. Posterior regions showed statistically significant differences between subject groups, while anterior regions did not. This finding correlated to the previous vascular diagnosis of deep or perforator vein insufficiency of the vascular-compromised individuals.

Conclusions

Impedance plethysmography has the potential to measure directly blood volume changes in targeted regions of the limb. Results indicate that impedance plethysmography can be used to differentiate between characteristics of healthy and impaired venous return. The anatomical localization of hemodynamic trends in this study represents a step forward in the capability of plethysmographic measurements and may increase efficiency in follow up diagnostic tests.

4.2 Background

Persons with chronic venous disease undergo progressive hemodynamic deterioration. This deterioration can be caused by incompetent venous valves, thrombotic blockage, and inefficient extremity muscular pumps [125]. Early clinical intervention has been shown to address these issues and promote positive clinical outcomes. However, diagnostic accuracy

in early-stage or "mild" venous disease is often low [14]. The cause of this low accuracy is multifactorial, but is in part due to a delay in seeking diagnosis and treatment until venous disease has progressed to the point symptoms in the lower limb are perceived by the patient or present visually to a clinician. Better noninvasive diagnostic tools would directly address this issue by facilitating easier and more accurate measurement of lower extremity hemodynamics before significant disease progression has occurred.

Various imaging modalities are used to diagnose venous insufficiency, including duplex ultrasound, catheter contrast venography (Magnetic Resonance Venography or Computed Tomographic Venography), and direct venous pressure measurements. [126, 127, 128]. These methods, while accurate for measuring venous characteristics, are expensive and invasive and there is no consensus on indications for their diagnostic use [129, 127]. Duplex ultrasound is the most commonly used diagnostic test for detecting the location and severity of venous obstruction and valvular incompetence [7], though it is a localized measure and cannot measure the global extent of venous insufficiency in an individual. This often requires that the entire lower leg be studied to pinpoint the location and extent of disease, increasing the time and cost of diagnosis.

Plethysmography is the only existing noninvasive measurement method capable of assessing and quantifying the global extent of venous disease in the extremities [7]. Current plethysmographic methods, including air-plethysmography (APG) and photo-plethysmography (PPG) provide valuable insight into the hemodynamics of the lower limb; however they provide only indirect or superficial measurements and are not useful in the localization of the anatomic location of incompetent valves [130, 131].

Impedance plethysmography (IPG) provides a direct, but noninvasive, measurement of extremity blood volume change in response to postural or muscular perturbations to the venous system [60, 132, 58, 57, 133]. Previous IPG instruments used a single frequency measurement to quantify blood volume changes in response to direct perturbations of the venous system [134]. More recently, advances in multiple frequency impedance spectroscopy have shown the ability to differentiate between the different fluid components of the area

under measurement, such as interstitial fluid, blood plasma, and intracellular fluid [135]. These instrumentation advances, coupled with study protocols designed specifically to elicit and isolate venous blood volume changes provide a unique nonvasive method to measure hemodynamics of the lower limb [134]. The aim of this study was to assess the clinical utility of a novel impedance plethysmograph for differentiating healthy individuals from individuals previously diagnosed with venous insufficiency during a short in-clinic evaluation. This study also evaluated which parameters calculated from hemodynamic data were useful in differentiating between normal and impaired venous return. We hypothesized that healthy individuals would show greater calf muscle pump function and lower levels or venous reflux than diseased individuals during a short in-clinic IPG protocol using postural changes and tiptoe maneuvers.

4.3 Methods

Participants

Institutional review board approval and informed consent were obtained before study procedures were initiated. Volunteers were considered for the study if they had a prior diagnosis of lower extremity chronic venous disease that fell within the scale of C0-C3 according to the Clinical Class, Etiology, Anatomy, and Pathophysiology (CEAP) classification [6]. Participants in this group were assigned to the "Vascular Compromised Group" [VCG]. Individuals were selected for this study if they exhibited superficial or deep venous insufficiency in the lower limb, as measured during an ultrasound examination. Exclusion criteria were history of or suspected current venous obstruction, venous disease that had progressed to the point of pigmentation changes in the lower extremity, or the presence of metal orthopedic implants within the limb that could distort IPG measurements. VCG participants were recruited from the University of Washington Vascular Clinic from January 2015 through July 2015. Individuals who had received a varicose vein examination were contacted by clinic staff, informed of the study, and invited to participate. Informed consent was obtained and a detailed vas-

cular history including location, extent, and duration of venous disease was collected from individuals interested in participating in the study.

Matched control participants were recruited from the general population and assigned to the study "Control Group" [CG]. Participants were included in the control group if they had no history of vascular disease. Control group participants were excluded if, upon visual inspection of their legs, they showed outward signs of venous disease (e.g., telangiectasia, varicose veins, or unilateral edema). CG participants were matched with VCG participants by sex and age (±8 years) to minimize bias in the matched-group design.

Instrumentation

Plethysmographic data were obtained using a custom designed low-profile, battery powered bioimpedance device (Figure 4.1). This device was previously developed for measurement of residual limb volume change in people with lower limb amputation, and adapted for measurement of changes in blood volume in this study [96]. IPG measurements were recorded continuously at a rate of 30 samples per second through two measurement channels. Low profile measurement electrodes were placed on the anterior and posterior surfaces of the calf using anatomical markers to ensure consistent placement from subject to subject (Figure 4.2). Electrode wires were fixed in place using Tegaderm for the duration of the test.

Protocol

A research scientist followed a standardized investigational IPG protocol to evaluate each subject. The IPG protocol was performed following a standard plethysmographic procedure for diagnosis of occlusion, standing reflux, and calf muscle pump function [136, 137]. The subject was first asked to lie in the supine position. The subject's leg was elevated by 15 degrees so that the calf was above the level of the heart, and then rested in position on a foam block. A pneumatic cuff (D.E. Hokanson, CC17, Bellevue, WA, USA) was placed proximal to the knee and connected to a rapid deflator system (D.E. Hokanson, RD2, Bellevue, WA, USA). The cuff was inflated to 60mmHg to occlude venous outflow. Cuff pressure was held

Figure 4.1: Portable Impedance Plethysmograph and low profile measurement electrodes

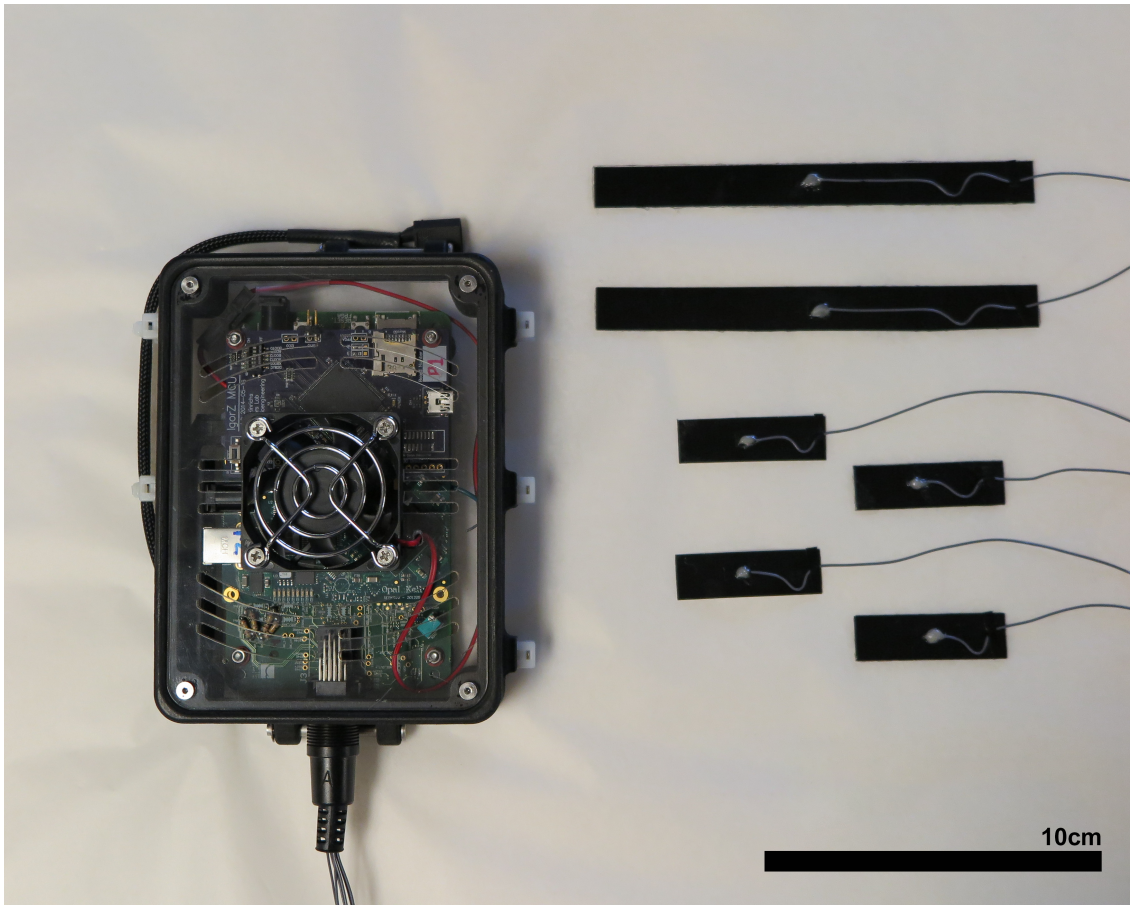
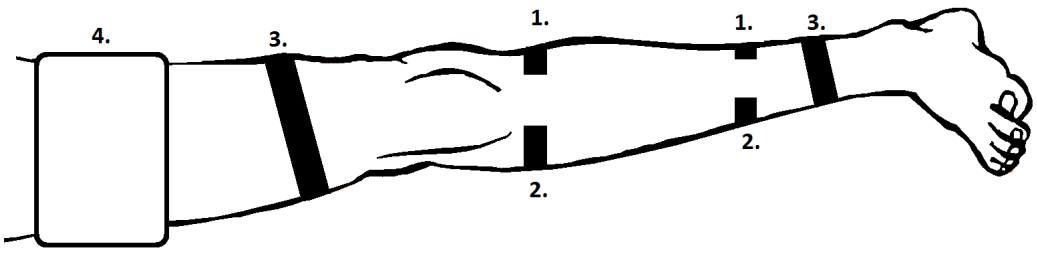


Figure 4.2: Electrode placement schema. Posterior sensing electrodes (1), Anterior sensing electrodes (2), Current injecting electrodes (3), proximal pressure cuff (4)



for two minutes or until the measured limb blood volume stopped rising, whichever came first. The cuff was then rapidly deflated and the blood volume outflow measured.

Standing venous reflux was measured by having the subject move from his or her supine position to a standing position while weight bearing on the non-instrumented limb. Once the blood volume plateaued, the subject performed a single tiptoe maneuver to measure their single ejection capacity. The blood volume was allowed to rise to a plateau again before the subject performed ten sequential tiptoe maneuvers to assess calf muscle pump function and valvular reflux. The subject then returned to the supine position for three minutes. If the difference in blood volume measured during the supine position at the beginning and end of the test was greater than 10% it was likely that an uncontrolled vascular event (e.g. pressure cuff leak, inconsistent leg position) had influenced the test and the entire protocol was repeated [138].

Analysis and Definitions

Following the test session, data were post-processed using custom software written in Python (Python Software Foundation, Delaware, MD, USA). Impedance data for each channel were derived using de Lorenzo's version of the Cole-Cole Bioimpedance modeling strategy [111, 97]. Calculated impedance data were then converted to blood volume using a limb segment model and the measured limb circumferences at and spacing in between the sensing electrodes on each subject's leg [99, 139].

Variables of Interest

It is important to note that IPG is a direct fluid volume measurement modality. This differs from indirect plethysmographic measurement techniques that record calibrated volumetric changes of an air cuff or strain gage. Because of the intrinsic differences in how these instruments measure venous volume change, disease thresholds derived from other modalities and reported in the literature are not valid for interpretation of the results of this study [130] . To mitigate confusion between the present method and previously reported results,

we have named our variables of interest using "percent" nomenclature, rather than the more commonly used "fraction" (e.g., "outflow percent" instead of "outflow fraction"). Blood volume data from anterior and posterior channels were measured and evaluated separately to differentiate hemodynamic parameters by anatomic location.

Outflow Percent [OP] Outflow percent is defined as the blood volume after 1 second of outflow (Figure 4.3, [C])divided by the total venous volume (Figure 4.3, [B]), measured during the supine cuff inflation portion of the test protocol.

$$OutflowPercent[OP] = \frac{Volume_{1second}}{Volume_{total}} * 100\% \tag{4.1}$$

Outflow percent is primarily a measure of whether or not there is impaired venous outflow in the extremity, and previously studied for use as a method to diagnose venous obstruction in the lower limb [138]. We hypothesized that we would not find a significant difference in OP between the CG and VCG in this study because history of venous obstruction was an exclusion criterion for participants in both groups. In the future, calculation of outflow percent may provide insight into the venous state of persons with undiagnosed venous obstruction or insufficiency associated with secondary venous disease.

Ejection Percent [EP] Ejection percent is defined as the blood volume expelled from the calf during a single tiptoe exercise (Figure 4.4, [B]), expressed as a percent of the total limb blood volume (Figure 4.4, [A]) (Equation 4.2).

$$EjectionPercent[EP] = \frac{Volume_{ejection}}{Volume_{total}} * 100\% \tag{4.2}$$

We hypothesized that participants with diagnosed venous insufficiency would have lower EP than participants in the control group due to impaired calf muscle pump function. The single tiptoe maneuver has been used previously in other plethysmographic studies to measure calf muscle pump function [136].

Venous Reflux Percent [VRP] Venous Reflux Percent is defined as the average venous

volume between tiptoe maneuvers (Figure 4.4, [D]) divided by the steady state standing venous volume (Figure 4.4, [C]), as measured over 10 tiptoes (Equation 4.3).

$$VenousRefluxPercent[VRP] = \frac{Volume_{relaxed}}{StandingVenousVolume} * 100\% \tag{4.3}$$

It was hypothesized that VRP will be more negative as the blood volume decreased in the leg over the 10 tiptoes in participants with patent venous valves and a functional calf muscle pump (i.e., control participants). In participants with venous insufficiency, we hypothesized that VRP would be less negative, or even positive, as the blood refluxes into the lower limb during calf muscle pump relaxation and no meaningful decrease in venous blood volume occurs.

Inflow Percent [IP] Inflow Percent is defined as the blood volume increase over one second following the completion of the 10 tiptoes. The blood volume increase is reported as a percent of the total blood volume (Figure 4.4, [E]) according to Equation 4.4.

$$InflowPercent[IP] = \frac{Volume_{1second}}{Volume_{total}} * 100\% \tag{4.4}$$

IP was hypothesized to quantify the rate of venous refill after activity (tiptoe) cessation. In healthy people, the patent calf muscle pump removes blood from the extremity during tiptoe maneuvers, reducing the overall blood volume and venous pressure. Upon cessation of activity, blood volume filling begins quickly, followed more slowly by an increase in venous pressure [7]. In people with venous disease, the calf muscle pump does not meaningfully reduce pressure and volume. After cessation of activity, volume increases slowly, if at all.

Analysis

A one-tailed paired-samples t-test was performed to assess whether there were differences in all variables between groups. This analysis was performed with the null hypothesis that there was no difference between the control and vascular compromised groups for each of

Figure 4.3: Outflow Percent [OP] data example, showing proximal cuff inflation at (A), and then deflation at (B).

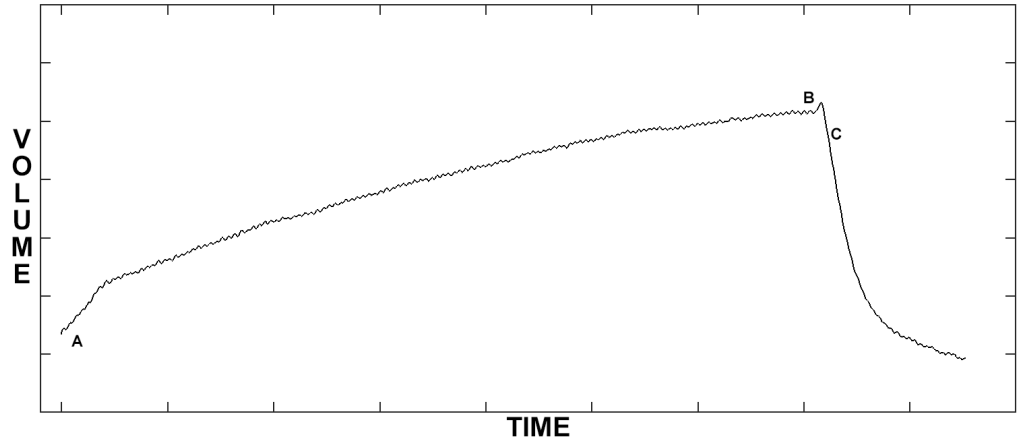
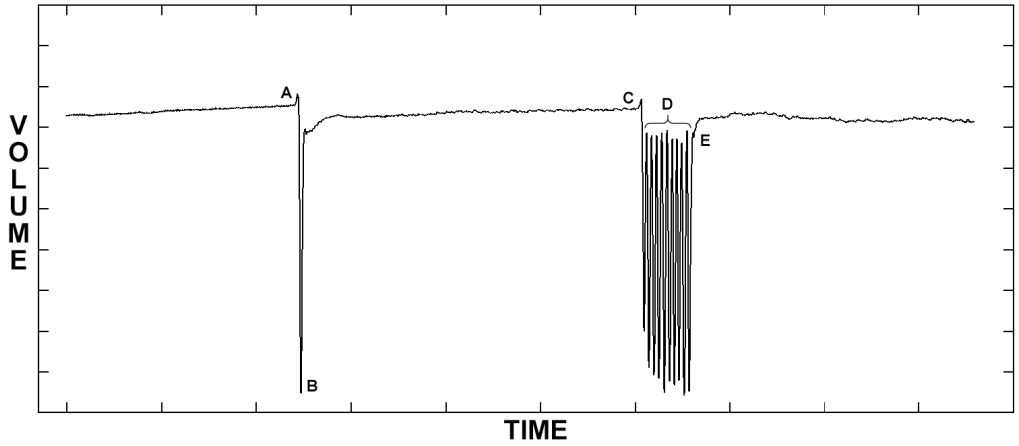


Figure 4.4: Ejection Percent [EP] from a single tiptoe (A-B) and Venous Reflux Percent [VRP] from 10 tiptoes (C-D). Inflow Percent [IP] is calculated beginning at (E) following the last tiptoe.



the variables measured. The threshold for statistical significance was set at α=0.05. Data from anterior and posterior channels were considered separately to evaluate hemodynamic differences between anatomical regions. All statistical analysis was performed in R (R, The R Foundation for Statistical Computing, Vienna, Austria).

4.4 Results

Twenty participants (i.e., 10 participants in each group) participated in the study. Subject characteristics and results of IPG testing are summarized in Table 4.1 and Table 4.2.

	CG			Anterior				Posterior			
#	Sex	CEAP	Age	OP	EP	VRP	IP	OF	EP	VRP	IP
1	F	N/A	50	15.8	12.35	-0.41	0.48	15.31	16.48	-0.23	0.67
2	F	N/A	27	19.74	12.56	0.17	0.41	17.51	17.24	-0.18	0.33
3	F	N/A	49	51.29	9.2	-0.03	-0.06	37.23	13.29	-0.49	0.31
4	F	N/A	62	17.17	11.06	0.03	0.49	16.93	12.56	0.1	0.54
5	F	N/A	80	15.89	4.2	-0.01	0.19	20.16	5.96	-0.04	0.3
6	M	N/A	71	35.43	3.74	1.51	0.88	36.81	4.94	1.7	0.65
7	M	N/A	64	14.07	9.41	0.38	0.53	17.75	13.23	0.06	0.81
8	F	N/A	43	13.03	14.4	0.42	0.06	16.56	19.26	0.21	0.17
9	F	N/A	63	30.6	7.16	-0.2	0.41	23.66	10.27	-0.36	0.49
10	M	N/A	30	38.49	11.45	-0.6	0.47	40.16	16.32	-1.38	0.57
		Mean	53.9	25.15	9.55	0.13	0.39	24.21	12.95	-0.06	0.48
		SD	17.25	13.05	3.57	0.58	0.27	9.87	4.74	0.76	0.2

Table 4.1: Subject characteristics and computed variables for the control group

In this study, different hemodynamic parameters collected during IPG testing were evaluated to determine which metrics show meaningful differences between persons with and without venous disease.

OP: The ability of OP to measure differences in the venous outflow between groups was poor. Statistical analysis showed no difference between the groups in either the anterior or the posterior channels (Figure 4.5).

VCG				Anterior				Posterior			
#	Sex	CEAP	Age	OP	EP	VRP	IP	OF	EP	VRP	IP
1	F	N/A	50	19.91	9.96	1.19	-0.05	17.8	12.3	2.04	-0.09
2	F	N/A	27	20.56	10.92	-0.26	0.36	21.3	11.64	0.14	0.46
3	F	N/A	49	13.28	8.47	0.15	0.45	15.27	12.09	-1.03	0.73
4	F	N/A	62	31.65	9.83	-0.19	0.1	30.22	13.64	0.25	0.27
5	F	N/A	80	19.92	6.3	0.24	0.21	17.14	8.43	0.37	0.34
6	M	N/A	71	14.03	9.02	-0.29	0.34	15.77	11.48	-0.32	0.47
7	M	N/A	64	22.75	6.31	0.69	0.05	16.22	7.53	1.33	0.07
8	F	N/A	43	5.05	5.09	0.54	0.05	7.72	8.24	1.88	-0.03
9	F	N/A	63	19.51	7.12	0.96	-0.26	18.75	8.47	1.52	-0.15
10	M	N/A	30	8.59	5.71	-0.59	0.11	9.55	5.93	1.31	0.04
		Mean	55.6	17.53	7.87	0.24	* 0.14	16.97	* 9.98	* 0.75	* 0.21
		SD	14.9	7.58	2.03	0.59	0.21	6.18	2.55	1.01	0.29

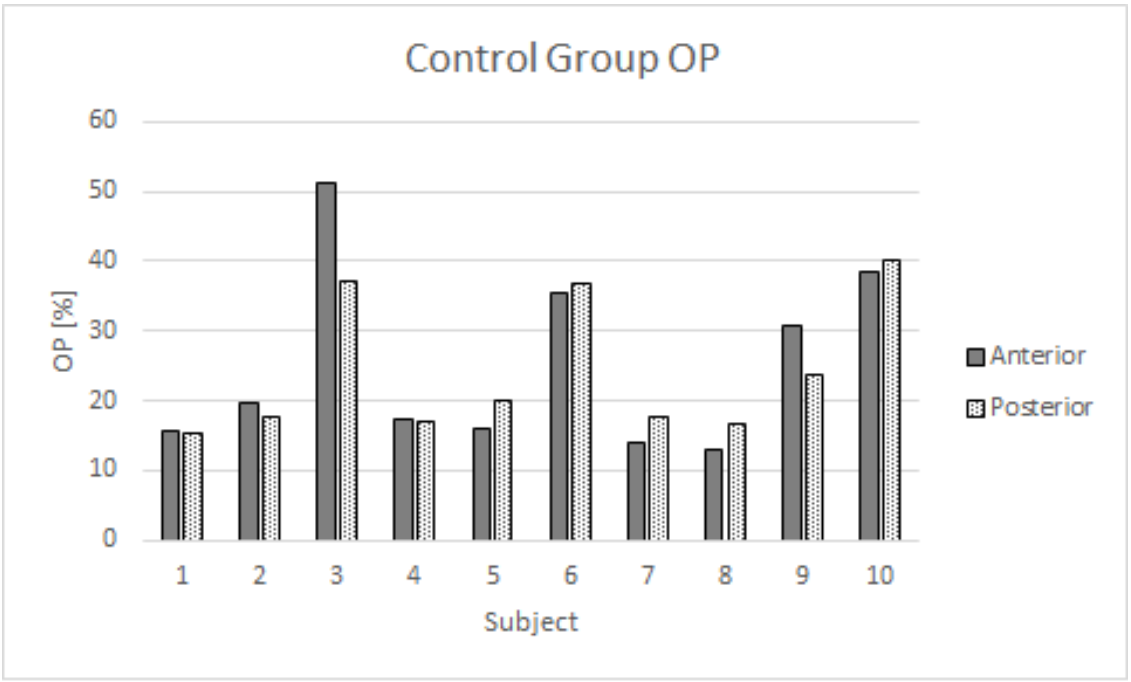
Table 4.2: Subject characteristics and computed variables for the venous compromised group. (* Statistically significant differences measured at the 0.05 level).

EP: The EP was greater for the CG than the VCG group in both the anterior and posterior channels (Figure 4.6). The difference in EP between the two groups in the posterior region was statistically significant ($P<0.05$). In the vascular compromised group, EP decreased with increasing severity of venous disease (Figure 4.7). In the control group, EP decreased with increasing age (Figure 4.8).

VRP: The VRP was lower for the CG than for the VCG in the anterior and posterior channels (Figure 4.9). Most of the participants in the VCG had a positive VRP, while most of the participants in the CG had a negative VRP. The difference between groups in the posterior channel was statistically significant ($P<0.05$).

IP: The IP was the best performing parameter in differentiating between groups, with a greater IP for the CG than for the VCG in both the anterior and posterior channels (Figure 10). The difference between groups was statistically significant for both channels.

Figure 4.5: Outflow Percent for Control Group and Vascular Compromised Group



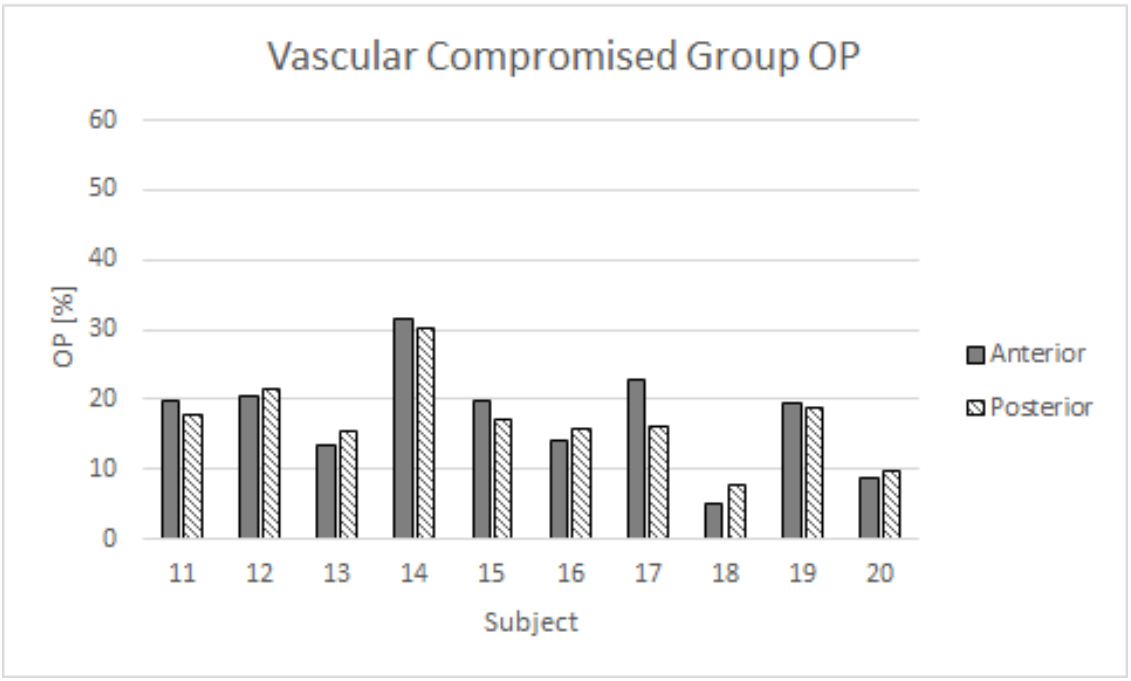
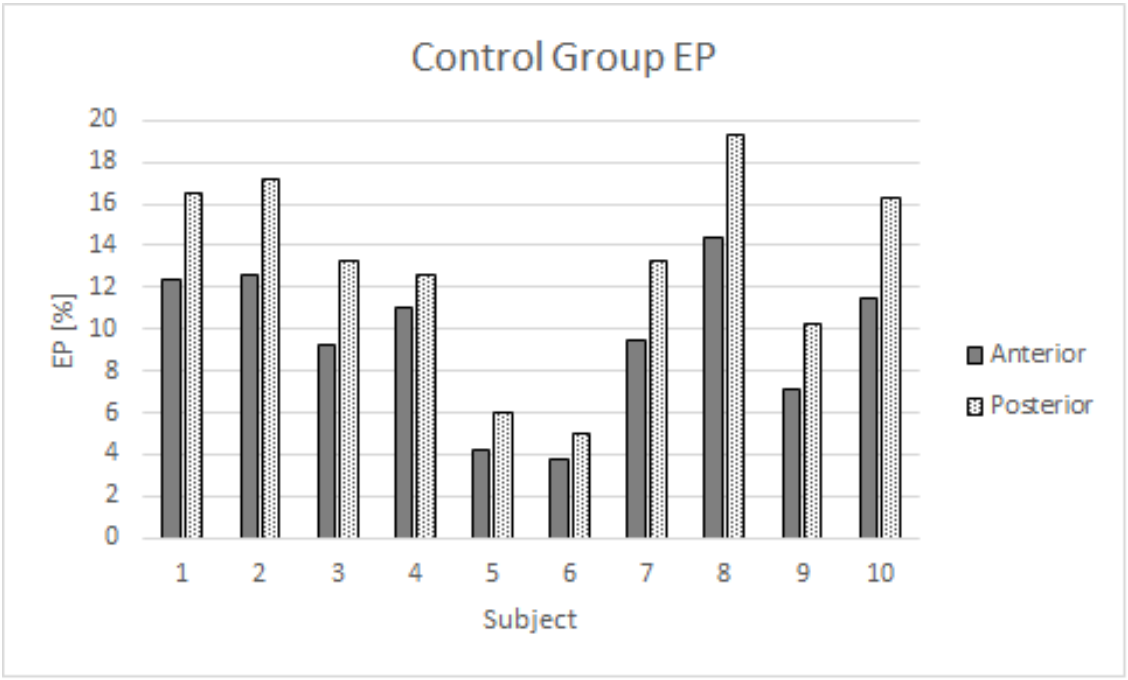


Figure 4.6: Ejection Percent for Control Group and Vascular Compromised Group



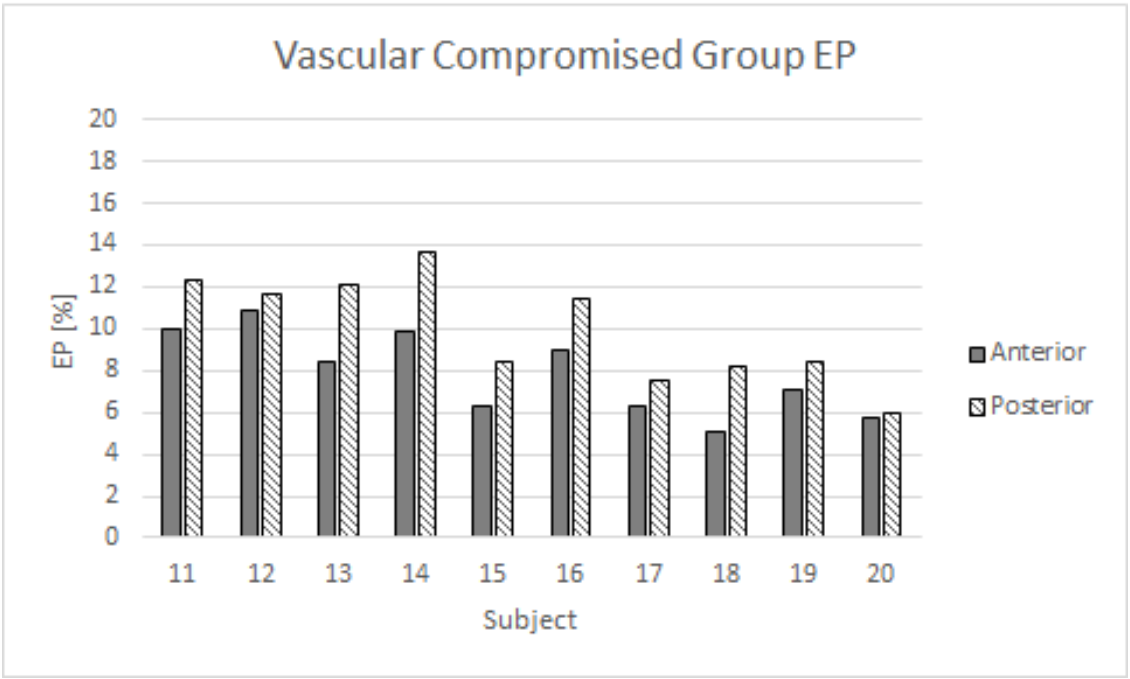


Figure 4.7: Ejection Percent for the Vascular Compromised Group, arranged by increasing severity of venous disease

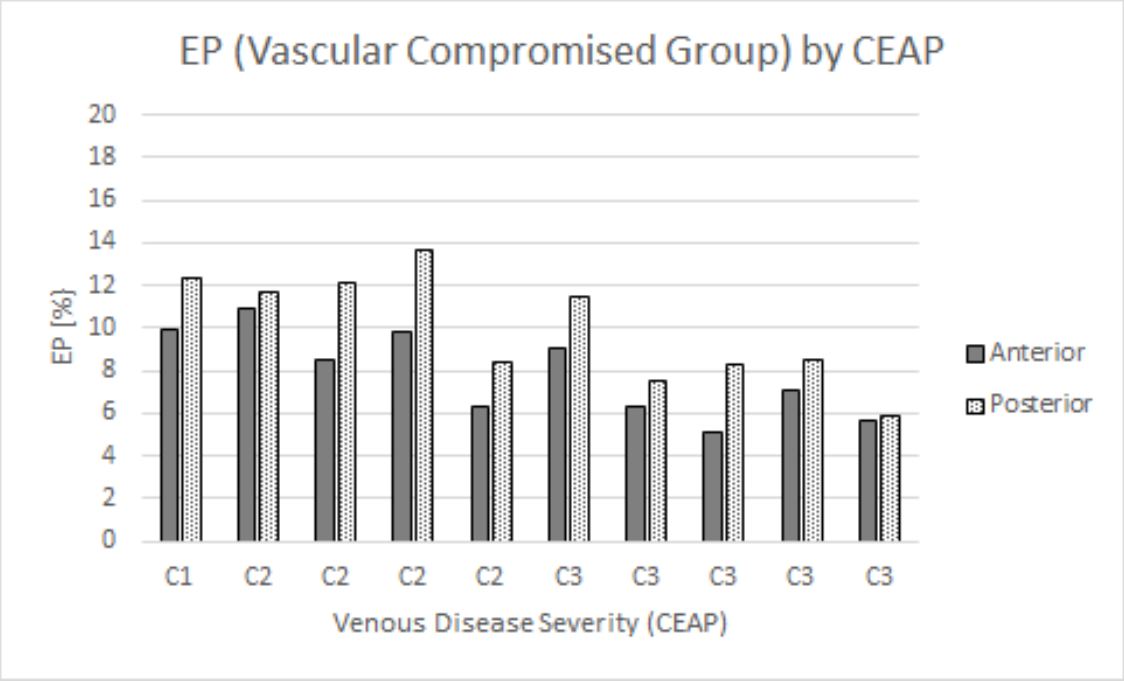


Figure 4.8: Ejection Percent for the Control Group, arranged by increasing age

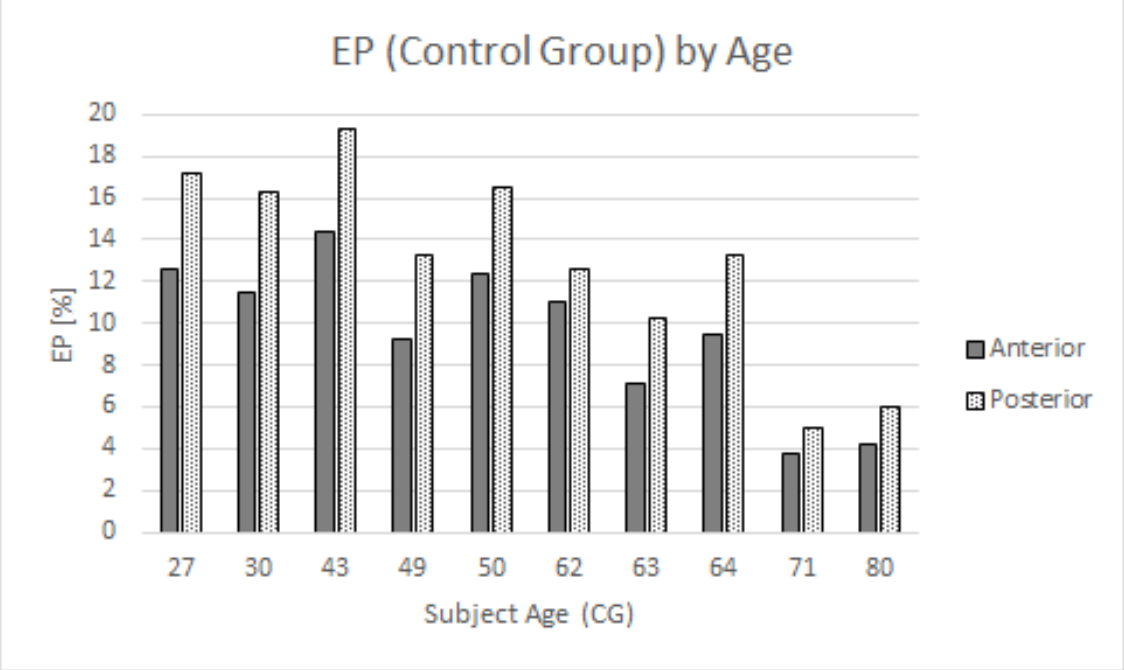
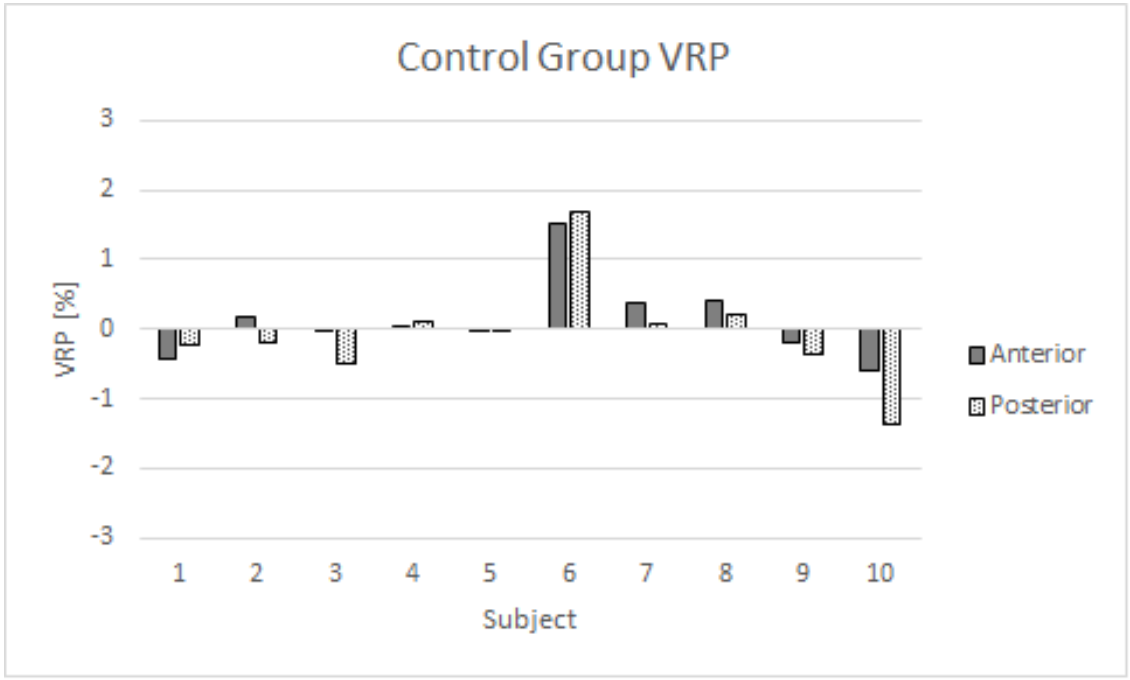


Figure 4.9: Venous Reflux Percent for Control Group and Vascular Compromised Group



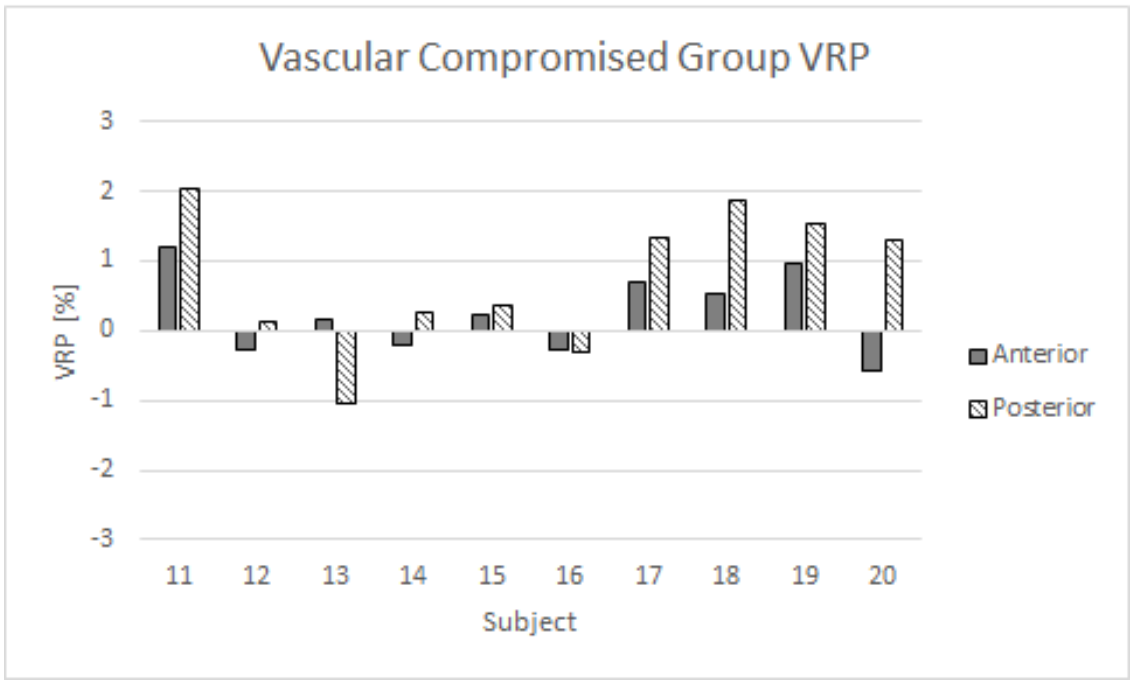
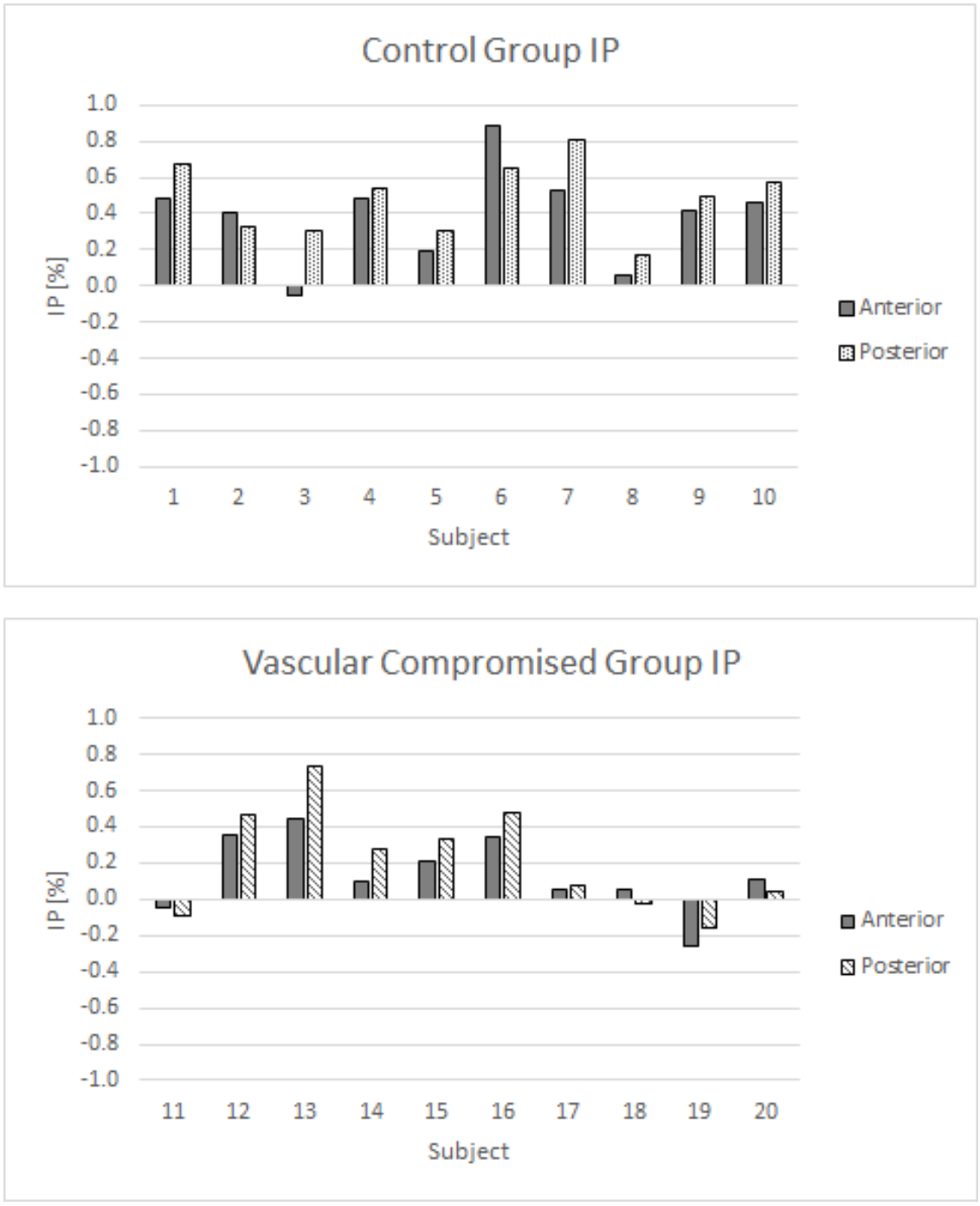


Figure 4.10: Inflow Percent for Control Group and Vascular Compromised Group



4.5 Discussion

This study demonstrated the use of a novel [IPG] device to quantify hemodynamic characteristics in the lower extremity of non-diseased and vascular compromised individuals. Hemodynamic variables reported in this study were derived from physiological parameters of the venous system, and had varying levels of usefulness at differentiating between venous flow characteristics in the two groups.

In this study, OP was similar between the two groups, and between the anterior and posterior regions within each subject. In previous APG studies, reduced venous outflow after pressure cuff release was primarily indicative of venous obstruction [138]. As neither group in our study protocol had previously diagnosed obstruction, the similarity in OP between groups is expected. Previous outflow studies using a proximal pressure cuff have shown mixed results, and its utility as a diagnostic test for obstruction is controversial [140, 138, 141]. While IPG potentially provides a more direct measurement of venous outflow than other methods, the dependence of all plethysmographic measurements on the testing protocol cannot be overlooked. As OP was not useful in differentiating between groups in this study, and its analogous APG measurement has demonstrated mixed utility, future studies should perhaps identify a different test protocol to better characterize venous obstruction.

Our hypothesis that EP would characterize ejection capacity of the calf muscle was supported by the greater values of EP in the CG than in the VCG. EP was greater in the posterior region than the anterior region for all participants in all groups (Figure 4.6). This was likely due to the greater concentration of large deep veins in the posterior muscle compartment and accordant capacity to remove blood from the limb during calf muscle contraction [1]. A decrease in the ratio between anterior and posterior EP may suggest proximal insufficiency or even blockage and simultaneous collateralization in the deep veins of the posterior leg compartment. As the degree of previously diagnosed venous disease increased in the VCG, the EP showed a similar decrease (Figure 4.7). This result suggests EP may provide a measure of the clinical severity of venous disease, a result that should be investigated in future

studies with greater numbers of participants. In the CG, EP decreased with subject age (Figure 4.8), which was likely related to age-linked skeletal muscle loss [142, 143].

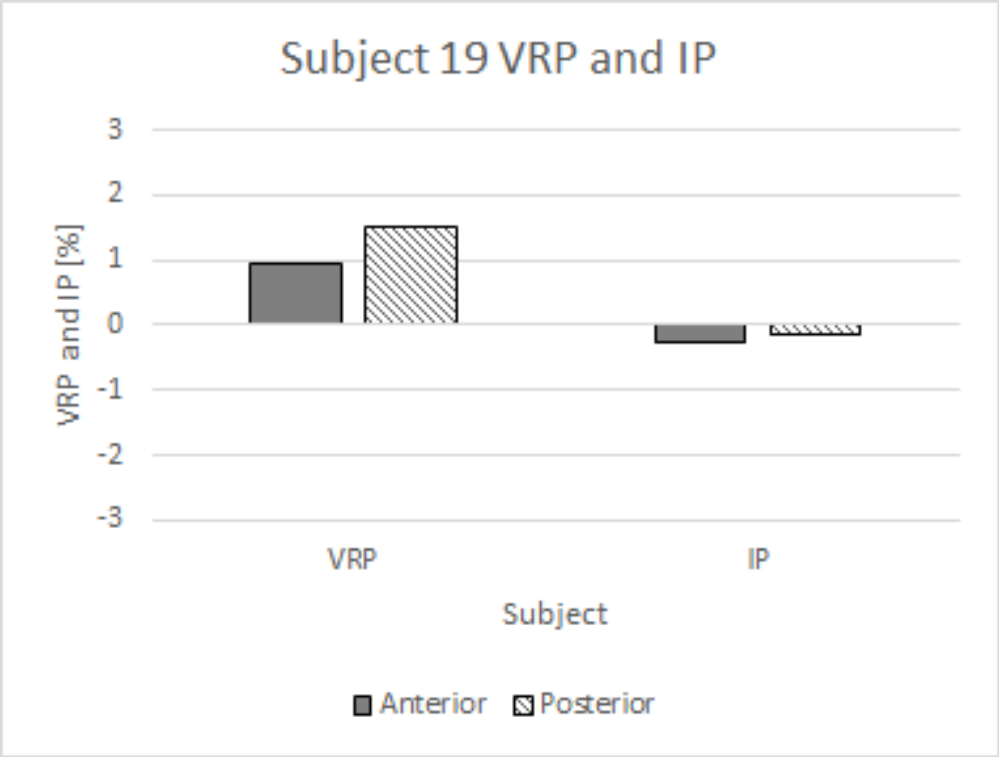
VRP and IP were hypothesized to quantify the reduction in venous volume and pressure during cycles of calf muscle pump activation and indicate whether venous reflux is present. In the VCG, most individuals had a positive VRP, indicating that the blood volume in the lower leg actually increased when the calf muscles relaxed during the ten-tiptoe maneuver. This increase was likely due to retrograde flow caused by incompetent venous valves, indicating that VRP may be useful in identifying the location and extent of reflux. IP was greater in the CG than in the VCG, indicating a greater decrease in venous blood volume over the ten-tiptoe maneuver. In combination, VRP and IP potentially may provide insights into the severity of venous reflux. When deep veins are incompetent, tiptoe maneuvers simply result in an oscillation of blood and no reduction in pressure [71]. This reflux and inability to reduce venous pressure and volume would be indicated by a high value of VRP and low or negative IP value. An examples of this condition in the current study is subject 19 (Figure 4.11).

Clinical Application

The present study illustrates that IPG may be suitable as a diagnostic instrument, as it showed an ability to differentiate between hemodynamic parameters in non-diseased control participants and vascular compromised participants with mild venous disease. This measure is non-invasive, and accurately quantifies the blood volume change in the lower limb in response to patient movement or external perturbations. Further, the use of multiple sensing channels improves upon previous plethysmographic methods and offers the ability to differentiate hemodynamic measurements by anatomical location [60]. In this study, we reported on anterior and posterior compartment hemodynamics, the relationship between which may indicate whether venous insufficiency is occurring in the superficial or deep veins.

A limitation in this study was consideration of all participants with diagnosed venous insufficiency as simply "venous compromised." This analytical step was taken to allow sta-

Figure 4.11: Subject 19, A female with superficial insufficiency in the GSV and incompetent below-knee perforators. High VRP and negative IP may indicate retrograde flow and an incompetent muscle pump



tistical comparison between two relatively small sample sizes. Because participants were divided into two groups, we could not reliably assess whether individual hemodynamic results calculated using IPG correlated against the previously diagnosed severity and location of venous disease in the venous compromised group. Further studies are warranted with a more comprehensive comparison of IPG test results relative to current gold-standard (e.g. duplex ultrasound, Ambulatory Venous Pressure (AVP)) diagnostic tests. A concurrent study using IPG and AVP would validate venous volume measurements calculated from IPG while providing further insight into the relationship between volume and measured venous pressure.

Future Directions

This initial study was designed to assess the utility of different hemodynamic parameters measured using a novel impedance plethysmograph. Evaluation of these parameters focused on their physiological relevance and ability to differentiate between healthy individuals and people with a compromised lower extremity venous system. In this study, EP, VRP, and IP were shown to differentiate between the two groups, and in the case of EP, provide a hemodynamic measure that may be indicative of venous disease severity. Future studies should involve comparison against other global measures of vascular hemodynamics (e.g., APG) or gold-standard measures of the clinical severity of venous insufficiency (e.g., AVP). While recent results suggest that APG is not warranted for use in the diagnosis of chronic venous obstruction in the lower limb [138], IPG may provide insight into hemodynamic changes caused by obstruction. This greater utility is due to its ability to measure direct blood volume changes, and the flexibility of implementation afforded by multiple sensing channels. Simultaneous measurements of different channels arranged serially along the length of the limb could potentially identify regions where venous blood flow stagnates, similar to localization techniques used during nerve conduction studies [144]. To our knowledge, this is the first multichannel plethysmographic instrument with a demonstrated ability to measure lower extremity venous parameters indicative of a disease state. As IPG provides both hemodynamic and anatomic information, it is well suited to use as a clinical diagnostic instrument.

A major drawback of all clinical plethysmographic methods is their sensitivity to indefinable or inconsistent patient movements. Even under ideal test conditions, any hemodynamic measurement is only a brief snapshot of the vascular state of the subject. Transient or mild symptoms may be missed for a multitude of reasons, delaying diagnosis and treatment for the patient. To address these limitations in clinical implementation, hemodynamic studies should extend beyond the lab to long-term monitoring of the venous system under patients normal daily activities.

The IPG instrument used in this study is small, portable, and noninvasive. The electrodes used to measure the blood volume in the leg are low profile, and can be worn under clothing or compression stockings. The instrument can be worn on a waist belt for long periods without patient discomfort. These advances over previous systems allow for the possibility of long-term, extra-clinical hemodynamic monitoring. Such a measurement would be beneficial in that it would provide an in-situ characterization of the hemodynamics and vascular operation of the subject under normal daily conditions. Further, long-term monitoring would mitigate small perturbations to the vascular system, making the measurement more resilient to limb movements than current plethysmographic techniques. Long-term studies that follow a structured set of extra-clinical activities should be considered to better understand how venous hemodynamics are influenced by real-world conditions.

4.6 Conclusion

This study evaluated the ability of a novel impedance plethysmograph to measure global venous parameters during a set of structured, in-clinic procedures. Further, we determined the utility of different hemodynamic parameters by their capacity to differentiate between healthy and unhealthy venous characteristics in a matched samples study. Extending from previous plethysmograph research, this IPG instrument can directly measure blood volume change, and calculate direct hemodynamic characteristics rather than derivative parameters. Simultaneous measurement of venous blood flow in different anatomic locations is possible through multiple-channel operation and may be implemented to increase efficiency of follow-up imaging studies. This could lead to faster and more reliable diagnosis, and ultimately, better health outcomes for people with venous disease.

Chapter 5

AIM 3 - THE VALUE OF AMBULATORY HEMODYNAMIC MEASUREMENTS BY IMPEDANCE PLETHYSMOGRAPHY FOR QUANTIFYING THE SEVERITY OF CHRONIC VENOUS DISEASE. FOUR CASE STUDIES

5.1 Abstract

Background

Impedance Plethysmography (IPG) is a noninvasive method for measuring extremity hemodynamics, previously studied for its diagnostic utility in identifying the incidence and severity of venous disease. As with other vascular imaging methods, its use is limited to the clinical setting. This study assessed the ability of a portable IPG device to measure venous hemodynamic parameters during an extended extra-clinical testing protocol.

Study Design

Case Series

Methods

Participants underwent long-term venous testing using a multi-channel portable impedance plethysmograph. They completed a two-hour controlled activity protocol with period of low activity, high activity, and rest. This protocol was repeated on three different days; during the second day, they wore prescribed compression stockings to measure how a commonly used intervention influenced lower extremity venous hemodynamics.

Results

Four individuals with increasing severity of diagnosed venous disease participated in this study. During the two study sessions when compression stockings were not worn, all participants experienced an increase in lower limb blood volume. This increase was similar for each participant between the two days. When compression was worn, participants experienced a decrease in lower limb blood volume, especially during periods of low activity. The number of steps required to reduce venous volume to a minimum increased, correlating to the severity of previously diagnosed venous disease in the participants. Venous volume increase immediately after cessation of high activity was higher for participants with mild venous disease.

Conclusions

This study demonstrated that a portable IPG device can measure venous hemodynamics during extended out-of-clinic activity. This IPG measurement quantified the differences between use and non-use of compression stockings in all four participants and measured their efficacy during different levels of activity. Extraclinical hemodynamic monitoring has the potential for diagnostic use or outcomes assessment in persons with mild venous disease.

5.2 Introduction

Advances in imaging modalities have greatly expanded the realm of clinical venous evaluation. Duplex ultrasonography (DUS), computed tomography (CT), magnetic resonance venography (MRV) and various plethysmography methods are utilized for the diagnosis of venous obstruction or valvular insufficiency [10]. In particular, plethysmographic methods are capable of indirectly measuring global hemodynamic characteristics in the lower limb. Most commonly used, air plethysmography (APG) is a noninvasive test that measures volumetric changes in the calf using a calibrated air cuff [137, 136]. APG has previously been validated for quantifying valve incompetence [50, 51, 145], and investigated for measuring

the incidence or outcomes of venous obstruction in the lower limb [138, 146]. A limitation of APG, however, is that it measures an indirect volumetric analogue to venous flow, and the results can be susceptible to indefinable patient actions or equipment failures [147]. APG also cannot provide information about the anatomical location of the disease, limiting its use as a standalone diagnostic measure. Despite its limitations, plethysmography is the only existing non-invasive imaging modality that can measure global venous hemodynamics in the extremity [10].

Introduced in the 1940's [139], Impedance Plethysmography (IPG) gained traction through research by Wheeler [148, 149, 150], and others [151, 133] for its potential use in the measurement and diagnosis of venous disease [152]. IPG and other noninvasive techniques have clinical appeal as they present a safer alternative to more invasive methods such as venography [70, 150] or ambulatory venous pressure [20, 153]. Despite significant initial interest and widespread research, inconsistent results and technical limitations with IPG instrumentation has resulted in limited recent investigations into the clinical utility of IPG techniques, especially as a stand-alone diagnostic test [154, 155, 156]. The role of IPG in diagnosis of DVT also decreased when direct duplex imaging was introduced, as that modality provided a more direct (anatomic) assessment of venous obstruction and reflux.

One limitation inherent to all imaging modalities used for vascular diagnosis is that they must be used in a controlled clinical setting by a specifically trained technologist or sonographer. This limits the investigation of hemodynamic characteristics to a short time in the clinic, and can make the results susceptible to day-to-day variability [94, 142]. Plethysmographic tests are also somewhat difficult to perform reliably, as they require good patient compliance to ensure accurate results [94]. Uncontrolled actions (e.g. coughing or postural inconsistency) by the patient can necessitate the retesting or discarding the recorded data [138]. These effects can be somewhat mitigated by limiting patient movement, such as by using a tilt-table for examination [157, 158]. Long-term extraclinical plethysmographic monitoring has the potential to better quantify global hemodynamics in patients by continuously measuring their lower extremity blood-volume over several hours. This novel diagnostic

measurement is made possible by recent instrumentation advances in low-profile, portable, battery-powered impedance plethysmographs (Chapter 4). The purpose of this case study was to assess the utility of hemodynamic parameters extracted from extraclinical IPG measurements on four participants with varying levels of previously diagnosed venous disease. This study also measured the effect of compression stockings on hemodynamic characteristics and overall blood volume in the lower limb.

5.3 Methods

Participants

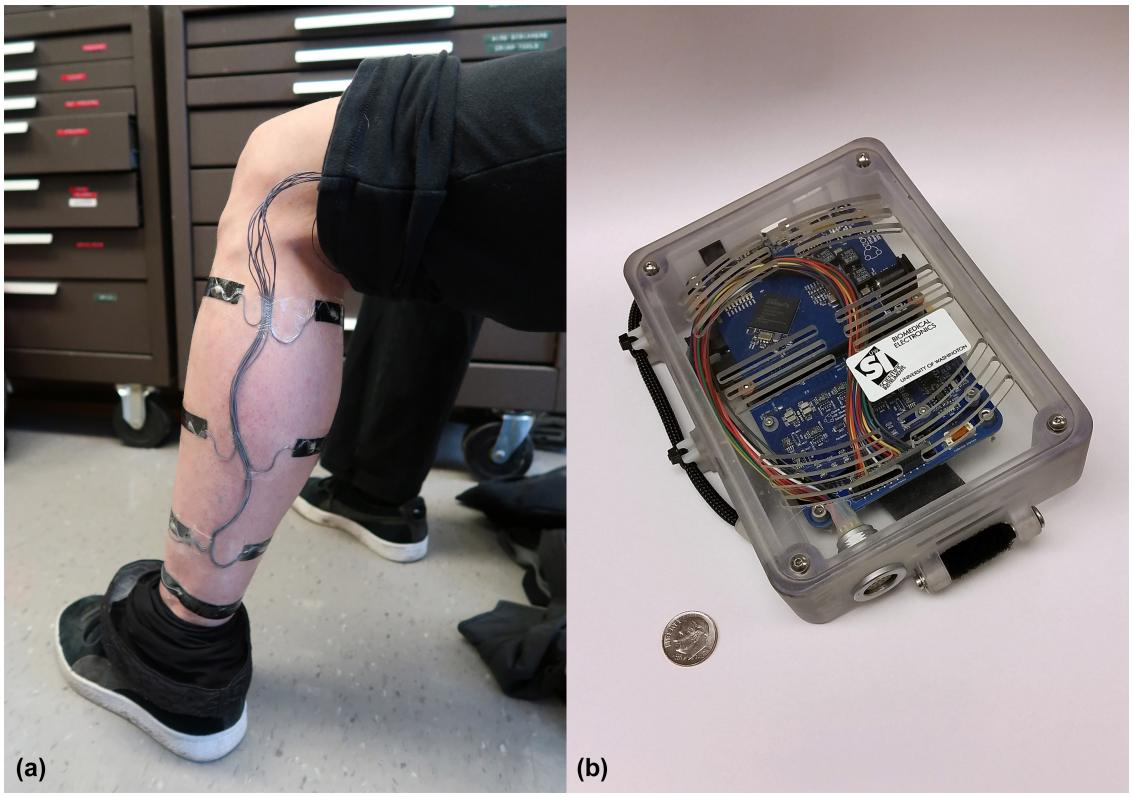
Patients referred to the University of Washington Vascular Surgery Clinic for evaluation of suspected lower extremity venous insufficiency were considered for this study. Inclusion criteria were a prior diagnosis of chronic venous disease that fell within the range of C0-C4 according to the Clinical, Etiology, Anatomy, and Pathophysiology (CEAP) classification [6]. Inclusion criteria also included the prescription and daily use of lower leg compression stockings and the ability to sit, stand, and walk for short durations unassisted over a two-hour period. Exclusion criteria included patients with current venous ulceration, or the presence of metal orthopedic implants within the limb that could distort IPG measurements. Clinic staff contacted potential participants that met inclusion criteria, informed them of the study, and invited them to participate. If an individual expressed interest in the study, informed consent was obtained and their complete vascular history was recorded.

Measurement Methods

IPG measurements in this study were recorded using a low-profile, battery powered four-channel bioimpedance device, This utility of venous hemodynamic measurements using this IPG device were previously evaluated during a short clinical protocol (Chapter 4). IPG measurements were recorded continuously at a rate of 30 samples/second throughout the study protocol. The anterior and posterior surfaces of the calf were instrumented using low

profile measurement electrodes with the wires routed proximally up the limb using Tegaderm. The IPG device was placed on a hip-mounted belt (Figure 5.1 (a)) and connected to the electrodes. The use of low profile measurement electrodes and flat wire routing allows unobtrusive venous measurements underneath normal clothing and during concurrent compression stocking use. (Figure5.1 (b))

Figure 5.1: Instrumented subject. Low profile measurement electrodes and wire routing up leg and under clothing (a) Portable IPG system (b)



Test Procedure

Study subjects completed a standardized controlled-activity test protocol three times on three different days. For two of the days (A1, A2), they were asked to not wear their prescribed compression stockings prior to or during the test. During the other test (B1), they

wore their prescribed compression stockings both prior to and during the study protocol. On each of the three days, the participant arrived at the test center at 11am and sat for 10 minutes to stabilize his or her heart rate. During this time, the participant answered questions about their vascular health history, prescription medications, and diet. The subject's body mass and blood pressure were recorded, and they temporarily removed their compression stockings, if worn (Test B1). The participant's skin was cleaned (Red Dot Trace Prep; 3M) and IPG electrodes were placed on the skin using anatomical markers to ensure consistent placement from test to test. (Figure 5.1(a)). After placing measurement electrodes research staff measured the spacing between the electrodes and circumference at each electrode.

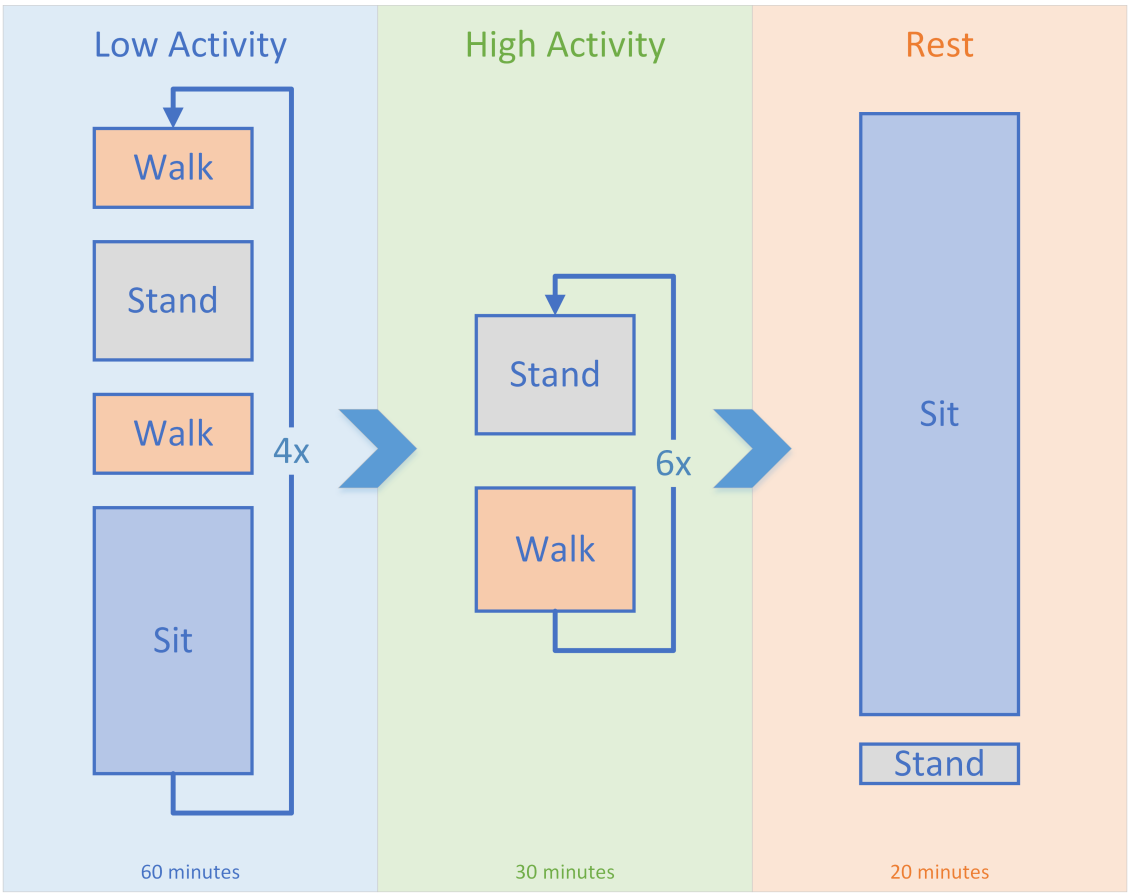
After being instrumented with the IPG system, subjects participated in a scripted 2 hour protocol developed to simulate a portion of a normal day. This included periods of low activity, high activity, and rest (Figure 5.2). Periods of low activity primarily consisted of sitting interspersed with short walking and standing activities. During high activity, the subject alternately walked, stood, and climbed stairs. During rest, subjects sat comfortably with no interruption. At the completion of the test, research staff removed electrodes and instrumentation and downloaded data for analysis.

Variables of Interest

Overall Blood Volume Change is defined as the percent change in blood volume measured at discrete points throughout the test session. Ten different 180 second stand periods throughout the test were identified and absolute blood volume was extracted from the end of each stand (Figure 5.2). Percent volume change was calculated according to Equation 5.1, with the volume at the first stand used as the reference (denominator). Results are presented for comparison as percent changes, as subjects all had different lower leg sizes. Blood volume data from anterior and posterior channels were calculated and evaluated separately to evaluate anatomical variances in hemodynamic trends.

$$BloodVolume\%Change = 100\% * \frac{Stand_X - Stand_1}{Stand_1} \tag{5.1}$$

Figure 5.2: Protocol Flowchart Periods of low activity, high activity, and rest. Overall blood volume trends were calculated by extracting volume points at the end of each stand. Stands 1-4 during low activity, Stands 5-9 during high activity, Stand 10 at the end of the rest section



It was hypothesized that subjects with venous insufficiency would increase in overall blood volume during course of the testing protocol, as their venous valves and muscle pumps are unable to remove blood from the lower extremity against the blood hydrostatic column. This blood volume increase is commonly manifest in mild venous disease as edema, or feelings of aching or heaviness [159]. With the use of compression stockings, it was hypothesized that overall blood volume would not increase as much as without use, or result in a blood volume decrease during the study protocol.

Steps to Steady State is defined as the number of steps required for the lower limb

volume to reach steady state after walking is initiated. The blood volume data for the first walk were extracted, and a Savistky-Golay 2nd order polynomial filter was fit to the data to represent the overall mean volumetric trend. Steady state blood volume was defined to have been reached when the mean blood volume trend became horizontal (analytically calculated when the 2nd discrete derivative of the trend reaches a minimum, Equation 5.2). This steady state condition was considered valid if two successive steps did not result in a further decrease in blood volume.

$$Time_{SS} = Time\Big(minimum\Big(mean\,blood\,volume\frac{d^2vol}{dt^2}\Big)\Big) \tag{5.2}$$

The number of steps taken by the subject to reach steady state was calculated from the ambulatory blood volume signal by counting the number of volume peaks prior to achieving steady state (Equation 5.3), and verified against the walking cadence calculated using a Fast Fourier Transform (Equation 5.4).

$$Steps_{SS} = Number\,of\,Peaks < Time_{SS} \tag{5.3}$$

$$Steps_{SS} = Walking\,Cadence\Big[\frac{steps}{second}\Big] * Time_{SS}[seconds] \tag{5.4}$$

It was hypothesized from previous studies on ambulatory pressure measurements that the number of steps required to reach steady state blood volume would correlate to the efficiency of the lower extremity muscle pumps at reducing the pressure and venous blood volume during walking. In the absence of venous hypertension and valvular insufficiency, the lower limb will reach a steady state venous pressure in 7-12 steps [160], and steady state volume shortly after. Diseased veins with incompetent valves will accordingly require a longer time and more steps to reach steady state

Refill is defined as the percent increase in limb volume after the first 60 seconds and over the entire rest period. Following the 30 minute long high activity portion of the test, the participant was asked to sit calmly with their feet on the floor and knees bent at 90 degrees

for a 15 minute rest period. The absolute blood volume was measured at the beginning of the sitting period and used as a reference to calculate the percent volume change over the whole recovery period. Refill after 60 seconds (Equation 5.5) and over the whole sit period (Equation 5.6) was calculated.

$$Refill_{60s} Volume\, Change = 100\% * \left(\frac{Volume_{60s} - Volume_{begin}}{Volume_{begin}} \right) \tag{5.5}$$

$$Refill\, Volume\, Change = 100\% * \left(\frac{Volume_{end} - Volume_{begin}}{Volume_{begin}} \right) \tag{5.6}$$

These variables quantify the rate of venous refill after activity cessation. With a functioning muscle pump, walking removes blood from the lower extremity veins and decreases the intravenous pressure [161]. After activity cessation, blood volume refill begins quickly, followed more slowly by an increase in venous pressure. In persons with venous disease, walking produces slight to no decrease in pressure and volume, and after activity cessation, volume increases slowly due to already high venous pressures. We hypothesized that these characteristics would be reflected in the 60s and total refill, with increasing severity of venous disease correlating to a slower increase in blood volume during the rest period.

5.4 Results

Four subjects participated in this study, with a diagnosis of C0, C2, C3, and C4. The subject classified as C0 had no history of venous disease, and was included in this study for comparison to the other subjects. Subject characteristics are included in Table 5.1. All subjects with diagnosed venous disease had previously been prescribed compression stockings and reported daily use. All subject test sessions were scheduled to begin at 11am, and took approximately 3 hours to complete, including preparing instrumentation and undergoing the scripted activity protocol. All subjects completed the three test sessions within a two-week period. One subject repeated the protocol a fourth time using a new compression stocking.

Overall Blood Volume Change All subjects increased in overall blood volume over

the course of the test when compression stockings were not worn (Session A1 and A3). This observation was true for both the anterior and posterior regions in the leg. (Tables 5.2, 5.3, 5.4, 5.5, and Figures 5.3, 5.4, 5.5, 5.6) Overall blood volume decreased over the course of the test for three of the four subjects when compression stockings were worn (Figures 5.3, 5.4, 5.6). Subject 3 did not show a decrease in overall blood volume while wearing compression stockings. However, overall blood volume increased, but at a lower rate compared to the two sessions where compression stockings were not worn (Figure 5.5).

Subject #	Subject 1	Subject 2	Subject 3	Subject 4
Age	30	47	43	52
Sex	M	F	F	M
Mass [kg]	74.8	72.5	60.3	116.6
Activity [hrs/day]	17	17	18	18
Resting Pulse	65	85	79	70
Resting BP	115/82	106/78	108/75	127/85
Smoking	No	No	No	No
Compression Style	Knee	Waist	Waist	Knee
Prev. Intervention	No	No	Yes	Yes
Complaint Duration	0	1	4	3
Incompetent Loc.	None	Superficial	Superficial/Deep	Superficial/Deep
Highest CEAP	C0	C2	C3	C4
Comorbidities	N/A	N/A	N/A	Diabetes Type I

Table 5.1: Participants characteristics and vascular history

Figure 5.3: Overall blood volume change trends for Subject 1

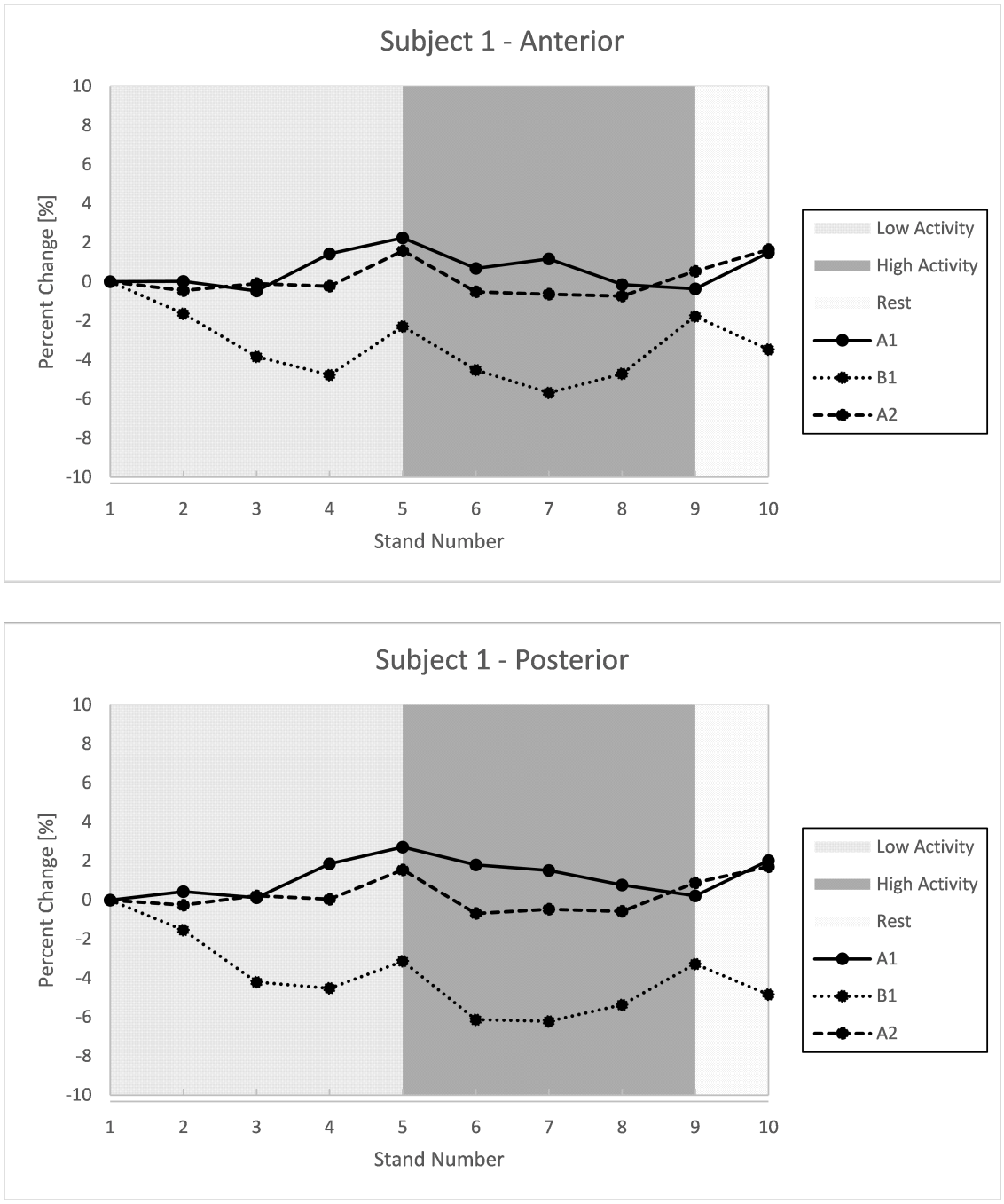
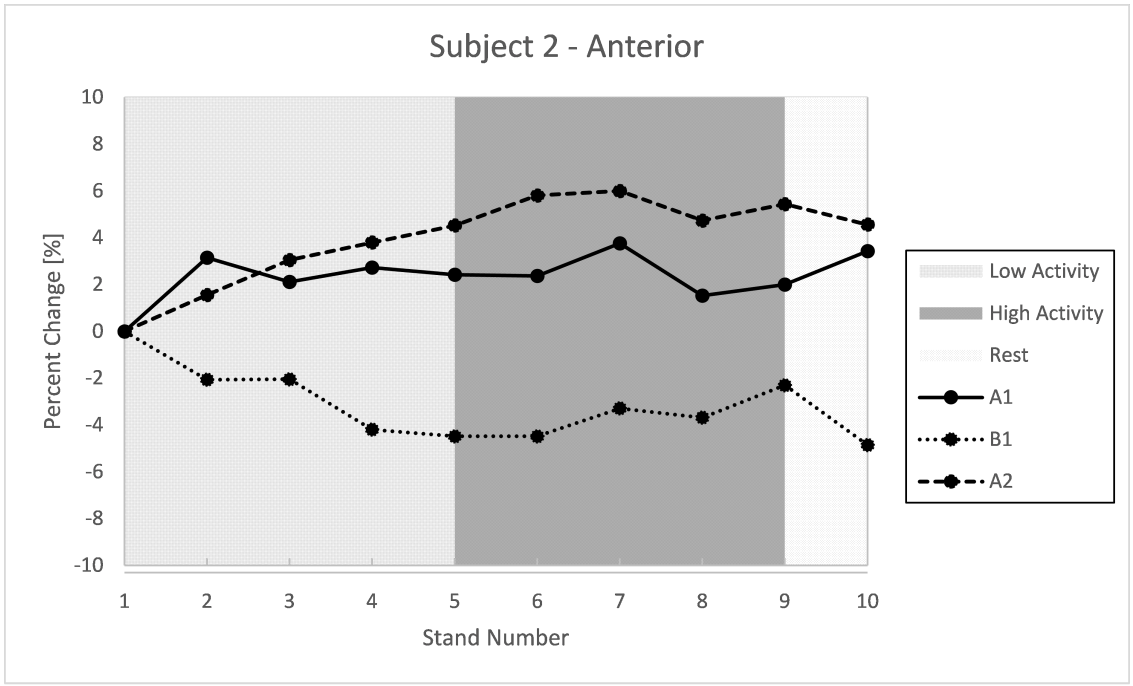


Figure 5.4: Overall blood volume change trends for Subject 2



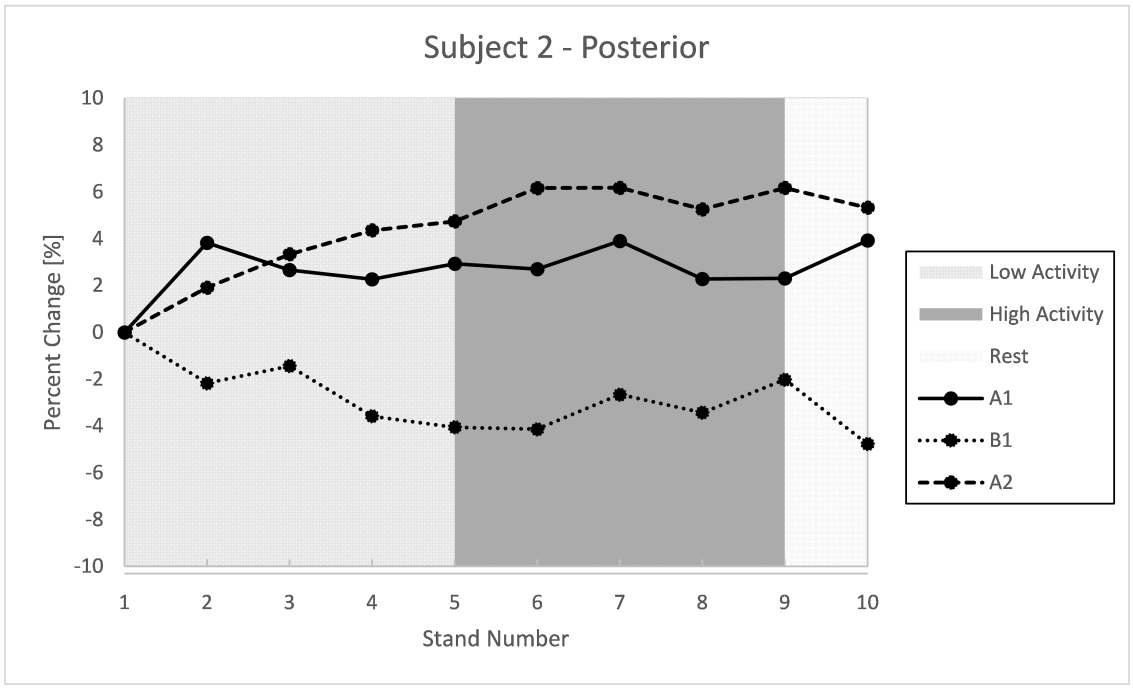


Figure 5.5: Overall blood volume change trends for Subject 3

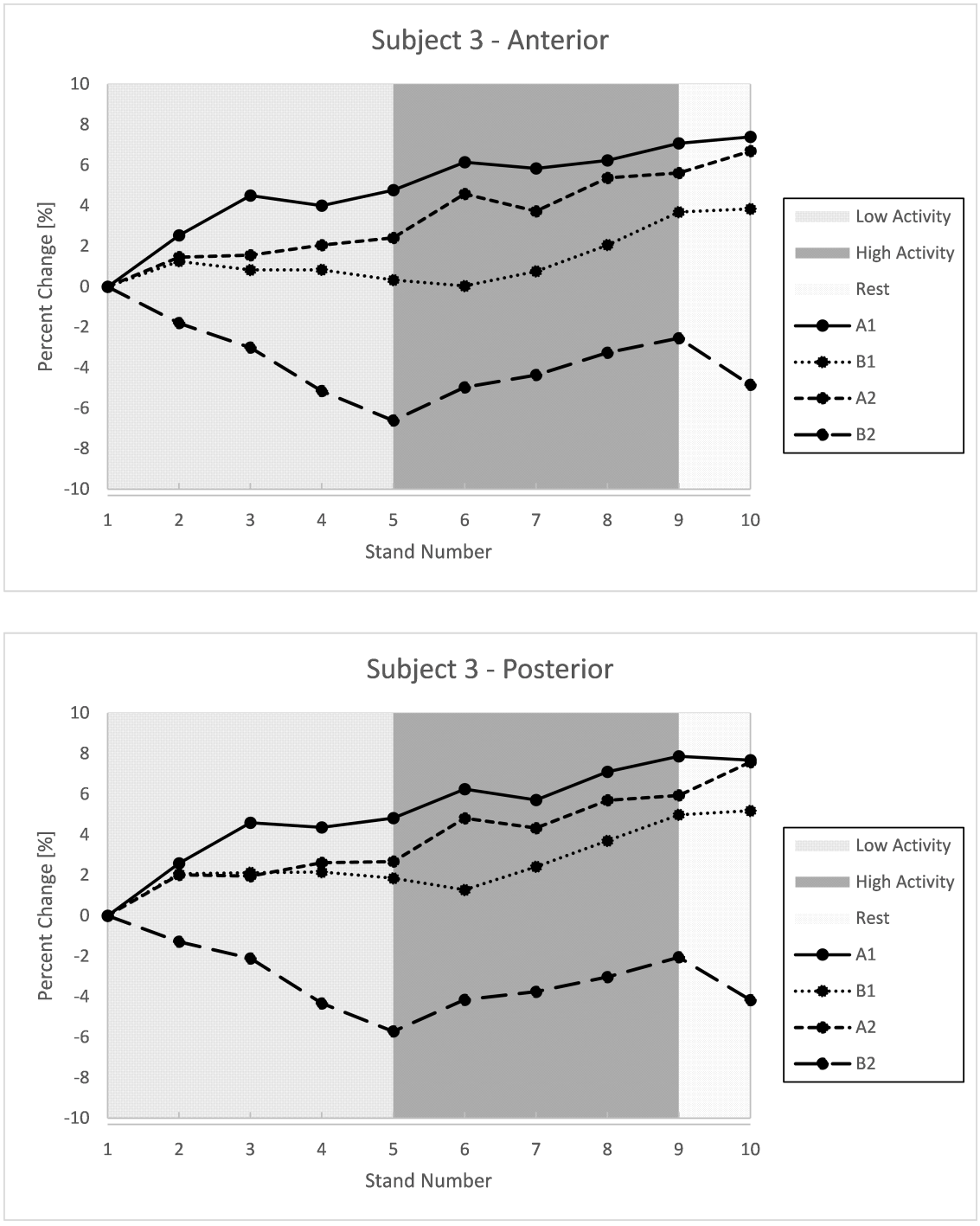
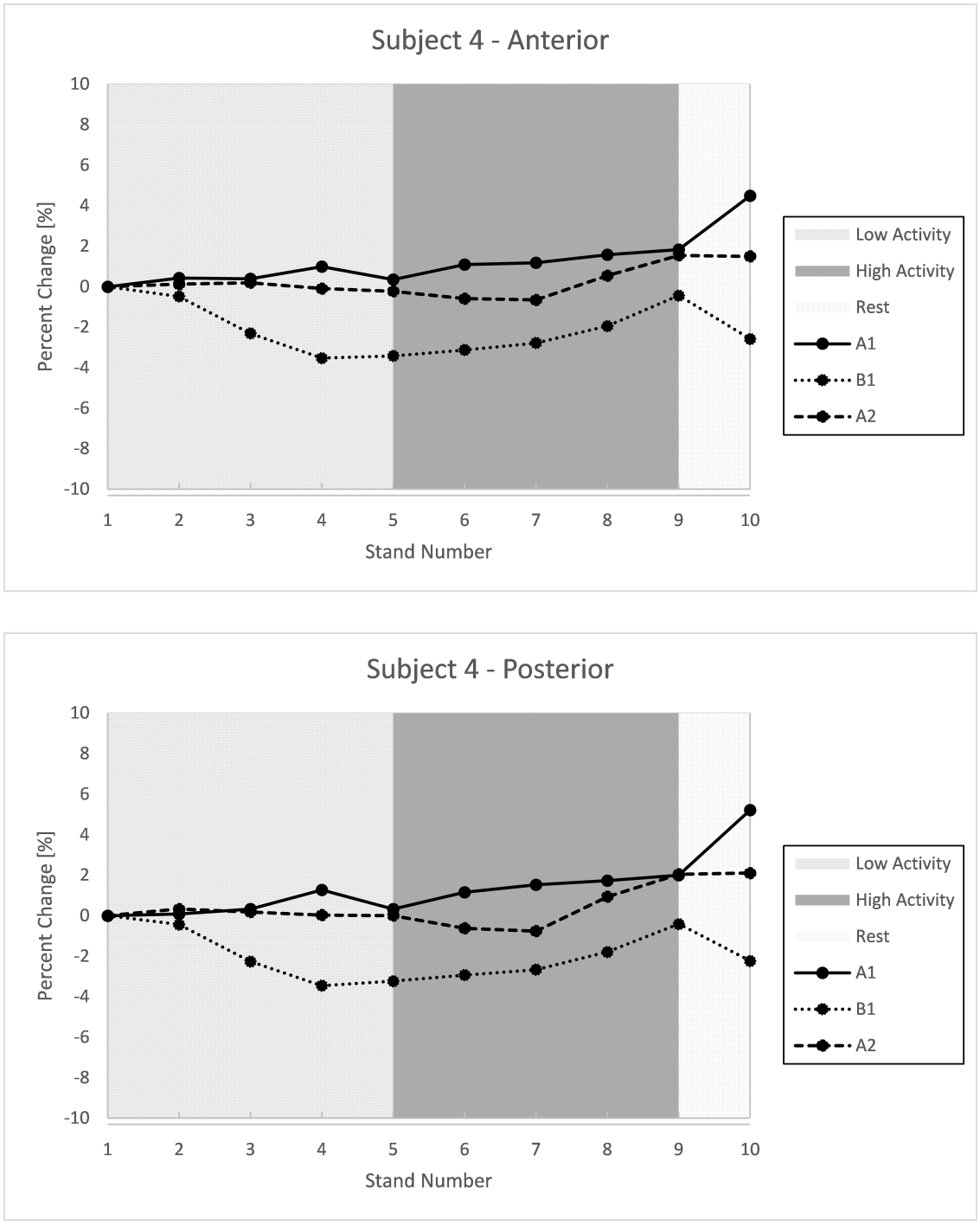


Figure 5.6: Overall blood volume change trends for Subject 4



Participant 1	Session A1		Session B1		Session A2	
Stand	Anterior	Posterior	Anterior	Posterior	Anterior	Posterior
1	0	0	0	0	0	0
2	0.02	0.44	-1.64	-1.53	-0.45	-0.25
3	-0.47	0.13	-3.83	-4.2	-0.09	0.23
4	1.43	1.87	-4.77	-4.53	-0.23	0.05
5	2.25	2.73	-2.29	-3.13	1.59	1.56
6	0.68	1.81	-4.53	-6.13	-0.51	-0.68
7	1.18	1.52	-5.68	-6.21	-0.64	-0.46
8	-0.15	0.78	-4.71	-5.37	-0.73	-0.57
9	-0.35	0.23	-1.77	-3.27	0.54	0.89
10	1.49	2.03	-3.47	-4.83	1.65	1.72

Table 5.2: Total blood volume trend for Subject 1

Participant 2	Session A1		Session B1		Session A2	
Stand	Anterior	Posterior	Anterior	Posterior	Anterior	Posterior
1	0	0	0	0	0	0
2	3.15	3.82	-2.06	-2.17	1.56	1.92
3	2.12	2.66	-2.04	-1.44	3.05	3.34
4	2.72	2.27	-4.2	-3.58	3.8	4.36
5	2.42	2.93	-4.49	-4.05	4.52	4.74
6	2.36	2.7	-4.49	-4.14	5.8	6.16
7	3.76	3.9	-3.29	-2.65	6	6.18
8	1.52	2.29	-3.68	-3.43	4.73	5.26
9	2	2.31	-2.30	-2.01	5.43	6.17
10	3.42	3.93	-4.85	-4.77	4.56	5.33

Table 5.3: Total blood volume trend for Subject 2

Participant 3	Session A1		Session B1		Session A2		Session B2	
Stand number	Anterior	Posterior	Anterior	Posterior	Anterior	Posterior	Anterior	Posterior
1	0	0	0	0	0	0	0	0
2	2.53	2.58	1.25	2.07	1.46	2.02	-1.8	-1.28
3	4.49	4.59	0.82	2.12	1.56	1.95	-3.01	-2.11
4	4	4.36	0.83	2.16	2.05	2.62	-5.15	-4.32
5	4.76	4.82	0.33	1.85	2.41	2.67	-6.61	-5.71
6	6.15	6.25	0.04	1.28	4.58	4.81	-4.97	-4.15
7	5.84	5.72	0.76	2.42	3.73	4.33	-4.36	-3.75
8	6.23	7.11	2.06	3.7	5.37	5.7	-3.26	-3.02
9	7.07	7.88	3.69	4.98	5.62	5.94	-2.54	-2.05
10	7.4	7.68	3.84	5.18	6.7	7.58	-4.85	-4.16

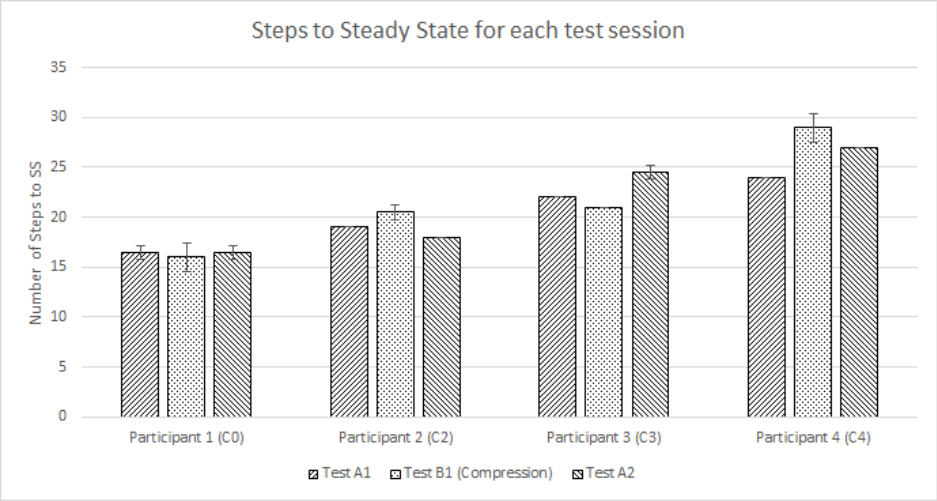
Table 5.4: Total blood volume trend for Subject 3

Participant 4	Session A1		Session B1		Session A2	
Stand	Anterior	Posterior	Anterior	Posterior	Anterior	Posterior
1	0	0	0	0	0	0
2	0.43	0.08	-0.48	-0.44	0.12	0.33
3	0.39	0.33	-2.31	-2.26	0.19	0.18
4	1	1.27	-3.52	-3.45	-0.09	0.03
5	0.36	0.32	-3.42	-3.23	-0.23	0.01
6	1.09	1.15	-3.12	-2.93	-0.59	-0.62
7	1.18	1.53	-2.78	-2.66	-0.65	-0.76
8	1.57	1.73	-1.94	-1.78	0.55	0.94
9	1.83	2	-0.44	-0.41	1.55	2.05
10	4.49	5.21	-2.58	-2.23	1.49	2.11

Table 5.5: Total blood volume trend for Subject 4

Steps to Steady State During the first walk, all subjects reached steady state blood volume within 30 steps (Table 5.6, Figure 5.7). The minimum number of steps to reach steady state blood volume was 16, which corresponds to values reported in the literature for healthy subjects [162]. Across the four subjects, the number of steps required to reach steady state blood volume generally increased with increasing degree of previously diagnosed venous insufficiency. The use of compression stockings did not have a uniform effect on the number of steps required to reach steady state. In two subjects, use of compression stockings increased the number of steps required, and in two subjects, it decreased the number of steps (Figure 5.8).

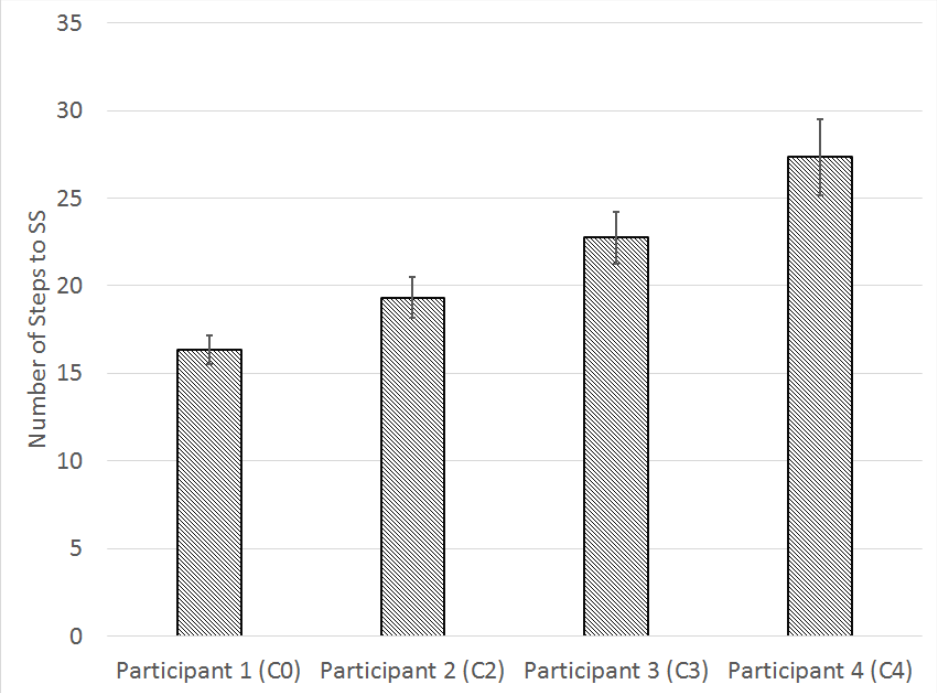
Figure 5.7: The number of steps to steady state for individual sessions



Participant	All Sessions	Test A1	Test B1	Test A2
1	16.33 (0.82)	16.50 (0.71)	16.00 (1.41)	16.50 (0.71)
2	19.33 (1.15)	19.0 (0.00)	20.50 (0.71)	18.00 (0.00)
3	22.75 (1.48)	22.00 (0.00)	21.00 (0.00)	24.50 (0.71)
4	27.33 (2.19)	24.00 (0.00)	29.00 (1.41)	27.00 (0.00)

Table 5.6: Steps to Steady State (means and standard deviation between anterior and posterior)

Figure 5.8: The number of steps to steady state averaged over all three tests

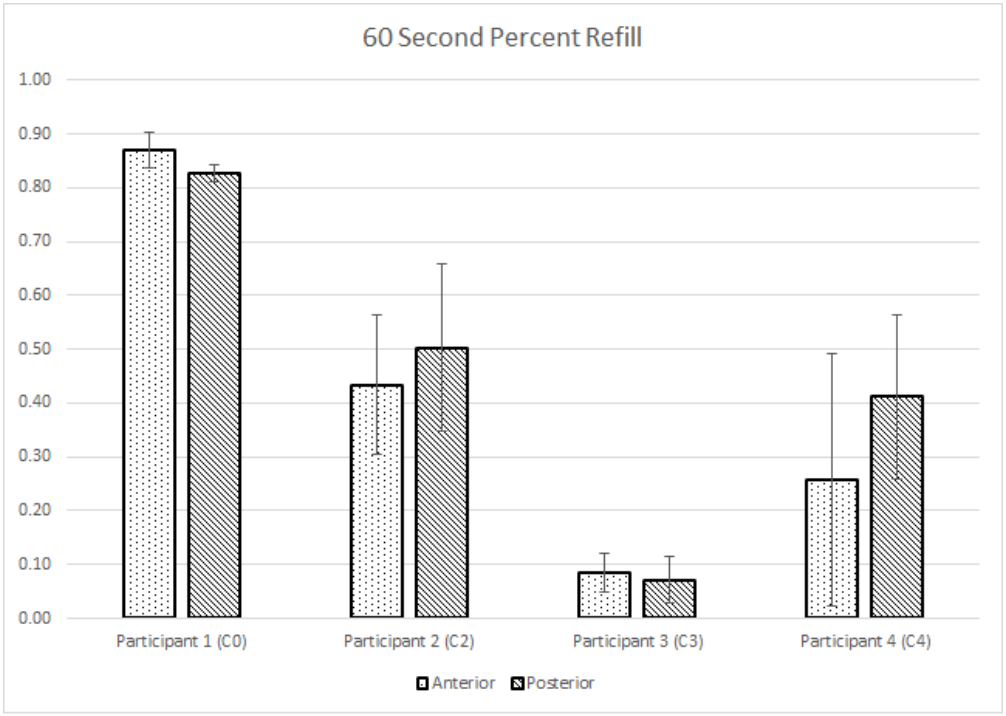


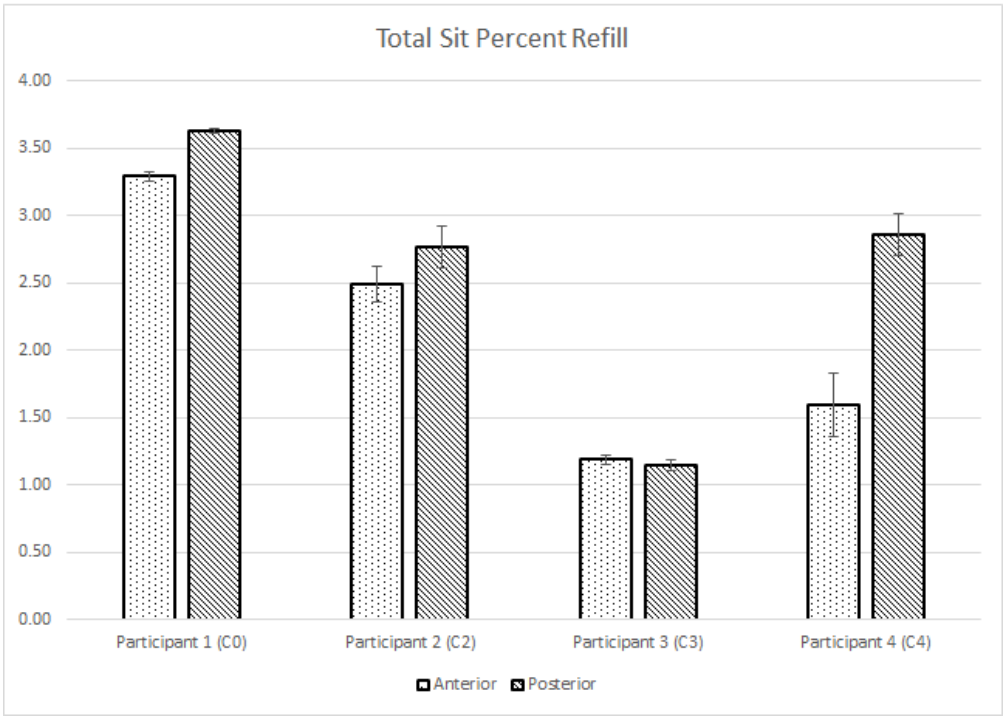
Refill During the final sitting period, refill at 60 seconds was greatest for subject 1 (no history of venous disease) and lower for subjects 2, 3, and 4 (Table 5.7, Figure 5.9). Overall blood volume gain over the 15-minute rest period varied by subject, from 1.2% to 3.6%. Refill trends and percentages were similar between the anterior and posterior regions of the limb, but with greater percent gain measured in the posterior channel.

Participant	Anterior		Posterior	
	60s	Total	60s	Total
1	0.87 (0.03)	3.29 (0.01)	0.83 (0.02)	3.63 (0.26)
2	0.43 (0.13)	2.49 (0.72)	0.50 (0.15)	2.77 (0.77)
3	0.09 (0.07)	1.19 (0.47)	0.07 (0.04)	1.15 (0.46)
4	0.26 (0.23)	1.59 (0.07)	0.41 (0.15)	2.89 (0.07)

Table 5.7: 60-second and 15-minute venous refill percentage

Figure 5.9: Blood volume refill during the rest period. 60s refill (a) and total refill (b)





Individual Results and Discussion

Subject 1 (C0) presented with no prior history of venous disease. During test protocols when compression stockings were not worn, Subject 1 increased in lower limb blood volume over the two test sessions. This increase was the lowest of any of the subjects. During the walk period, Subject 1 reached steady state in the fewest steps, an outcome that was consistent whether compression stockings were worn or not. Subject 1 also had the greatest blood volume refill following cessation of activity at both 60s and overall.

Subject 2 (C2) had previously been diagnosed with mild reflux in the great saphenous vein (GSV) and presented with superficial varicosities above the knee. When compression stockings were not worn, the lower limb blood volume increased by approximately 4 percent in both the anterior and posterior sections of the limb. This blood volume increase primarily occurred during the low activity sections of the test. During the high activity section, lower limb blood volume was maintained or slightly decreased, suggesting a functional calf muscle pump. When compression stockings were worn, the limb decreased in volume during the low activity portion, but followed a slightly increasing trend during high activity similar to when compression stockings were not worn (Figure 3, b). During the steady state analysis, Subject 2 reached steady state in approximately 18 steps when not wearing compression stockings and in 21 steps with compression use.

Subject 3 (C3) was previously diagnosed with both superficial and deep chronic venous insufficiency (CVI) and had their GSV stripped approximately 1 year prior to the study. Subject also had minor pelvic reflux. During the two test sessions when compressions stockings were not worn, the lower limb blood volume increased by nearly 8 percent in both the anterior and posterior portions of the limb. When compression stockings were worn, blood volume increased by 4 percent. When queried on the age of their compression stockings, subject could not remember but reported that they were prescribed at least 12 months prior. As a further exploration, subject repeated the study protocol wearing a set of compression stockings identical to their prescribed pair, but new. When wearing the new

compression stockings, overall blood volume decreased by four percent over the course of the test, in contrast to the trend observed when she wore the (∼12 month old) compression stockings (Figure 5.5). Of note, when the new compression stockings were worn (Test B2), limb volume decreased during low activity, but increased during high activity at a similar rate to when compression stockings were not worn. This result is not unexpected considering the subject's previous history of proximal reflux and suggests that the muscle pump and compression combination were not sufficient to attenuate blood volume rise during high activity.

Subject 4 (C4) was previously diagnosed with a femoral deep venous thrombosis (DVT) and underwent anticoagulation therapy approximately 2 years prior to participating in this study. This subject was diagnosed with secondary venous insufficiency and prescribed compression therapy. During test sessions when compression stockings were not worn, the overall lower limb blood volume increased slightly, with the majority of the increase occurring during the long sit following the high activity portion of the test. When compression stockings were worn, overall blood volume decreased slightly over the test, but not to the same extent as the other subjects. Similar to Subject 3, limb volume decreased during low activity and sitting periods, and increased during high activity similar to when compression was not worn. Subject 4 required the most steps to reach steady state regardless of whether compression stockings were worn, requiring nearly 10 steps more than Subject 1.

5.5 Discussion

Participants selected for this case study series had increasing levels of disease severity from Subject 1 (no previous diagnosis of venous disease) to Subject 4 (DVT and secondary venous insufficiency). This range of disease severity was designed to facilitate preliminary evaluation of how the hemodynamic characteristics measured with IPG change with increasing level of venous disease to better inform future studies with larger participant cohorts.

In the vascular clinic, calf muscle pump efficiency is measured through quantification of the reduction in ambulatory venous pressure (AVP) or limb volume (APG) while the partic-

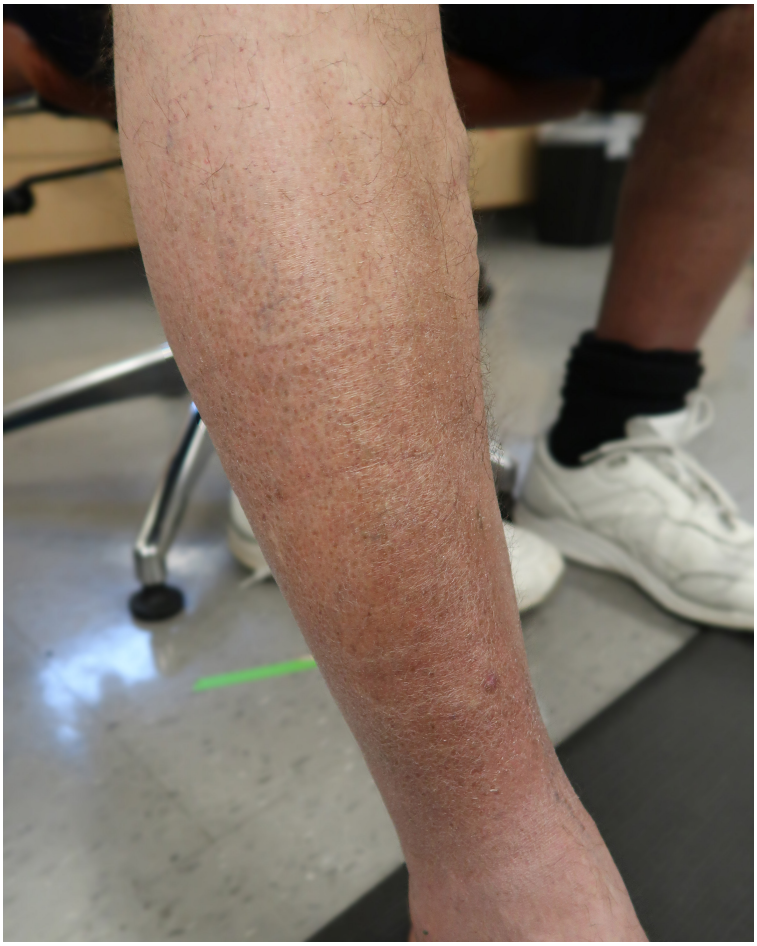
ipant performs tiptoes or walks in place. In this study, we expanded from this measurement by measuring the number of real-world steps required for the subject to reach steady state blood volume. This approach is useful as it provides an approximation of the effectiveness of the primary driver of blood return. The number of steps required to reach steady state was lowest for the subject with no prior history of venous disease, and highest for the patient with the greatest degree of venous insufficiency, following the hypothesized trend.

In this study, the venous refill characteristics were measured during a 15 minute sit following 30 minutes of high activity. The 60 second and total refill percent were highest for the subject with no venous disease and declined with increasing degree of venous disease for both the anterior and posterior regions of the leg, with the exception of Subject 4. Subject 4 had prior venous obstruction, and was classified with C4 level of secondary venous insufficiency. This outlying behavior can be at least partially explained by previous research that showed deterioration in venous hemodynamics parallels clinical severity up to the point of skin changes caused by venous hypertension [125]. In the case of Subject 4, who showed hyperpigmentation and eczema (Figure 5.10), results throughout this study were generally inconsistent between test sessions, and were often outliers from the trends demonstrated in other subjects. This result suggests that ambulatory impedance plethysmography may not be appropriate for subjects with venous disease above a certain threshold, as hemodynamic measurements may not reflect clinical severity.

Clinical Application - External Compression Stockings

Previous studies have evaluated the effect of graduated elastic compression stockings on the hemodynamics of the lower limb using APG and AVP [163, 164]. These studies were limited to tiptoe maneuvers, or treadmill walking within the lab however, and so the global hemodynamics were only quantified during rhythmic exercise in a limited setting. In this study, we expanded the measurement of the lower extremity hemodynamics to an extra-clinical setting. Of note, use of external graduated compression over several hours seems to decrease lower limb blood volume and stasis during periods of low activity (primarily sitting and stand-

Figure 5.10: Subject 4 prior to electrode instrumentation. Skin color changes and eczema are evident in the lower leg



ing) in all subjects. With high activity (30 minutes of standing and walking), compression stockings attenuate the rise in lower extremity blood volume as compared with not using compression, but at different levels for each individual. In this study, we also measured how two different compression stockings can have varying levels of influence on venous hemodynamics. This result validates previous studies into the variability of compression stocking interface pressures and highlights the importance of not using old or ineffective stockings [165, 166].

Future Research

The purpose of this study was to expand the paradigm of venous monitoring beyond postural and rhythmic maneuvers in the vascular clinic to a multi-hour protocol approximating normal daily activities. We suspect that longer monitoring periods during normal activity will result in greater understanding of the overall extent of venous disease in patients. It is unclear at this early stage how long the monitoring period will need to be for accurate measurement of parameters indicative of the presence of disease, but the current study period of 2 hours was sufficient for hemodynamic differentiation between subjects. The IPG device used during this study is capable of up to 12 hours of continuous monitoring, potentially measuring venous function over the course of an entire day. During unscripted activities, relevant hemodynamic parameters may be more difficult to extract, and future studies should involve the use of automatic activity classification using additional sensing modalities to enhance interpretation of the results.

This study was limited in scope and number of participants, and was intended to investigate the utility of hemodynamic parameters extracted from out-of-clinic activities. These parameters were evaluated for their relationship to previous diagnoses, and relationship to plethysmographic standards. In particular, Steps to Steady State correlates well with previously diagnosed disease severity and is similar between test sessions. Future studies may investigate this or a related parameter over multiple walking periods throughout the day to determine if parameters stay constant throughout or if there is a gradual degradation due to fatigue or other factors. This study also reported individual hemodynamic responses to the use of compression stockings. Future investigations should include larger populations to better quantify the efficacy of portable IPG at measuring whether prescribed compression has the expected therapeutic effect. In this study, results suggest that the efficacy of compression stockings as a primary treatment for chronic venous disease vary by subject and by the age/condition of the stocking.

5.6 Conclusion

Measurement of the global severity of venous disease is limited, with current technologies requiring either postural compliance or a rhythmic test protocol during a short session in the laboratory or clinic. In patients with early stage venous disease, symptoms and indicators may be transient, delaying referral to a vascular specialist for diagnosis and intervention. Noninvasive continuous monitoring of vascular flow in the lower limb may improve the diagnostic efficiency of patients with early-stage venous disease. Results from this study suggest that relevant hemodynamic parameters can be extracted from normal daily activity using a portable IPG device. These measures are more robust to small indefinable patient actions that usually cause issues during plethysmographic measurements and may provide increased diagnostic efficiency in persons with early stage venous disease.

Chapter 6

CONCLUSION

6.1 Venous Disease

The burden of lower extremity venous disease is significant, affecting between 10 and 35 percent of the population of the United States. Current methods for the measurement and diagnosis of venous disorders are powerful, but limited to operation by a trained specialist in the clinical setting. Early stage venous disease can present with a variety of symptoms, many of which may be transient or manifest only in response to certain environmental stimuli. This presents a challenge for early diagnosis, as many patients do not receive a referral to a vascular specialist until venous disease has progressed to the point of visible changes in superficial veins. This difficulty is also in part due to a deficit in public knowledge about the prevalence of venous disease, its risk factors, and early indications.

Previous studies have shown that earlier diagnosis and intervention leads directly to improved patient outcomes, better quality of life, and decreased overall health care expenses[14]. The purpose of this dissertation work was to develop and implement new measurement modalities by which researchers and clinicians can better measure the hemodynamics of the lower extremity during normal everyday activity outside the laboratory. This was accomplished by developing new instrumentation for physiological monitoring and then validating them in participant studies on venous-compromised individuals. The development of accurate diagnostic tools for assessing mild venous insufficiency will increase the standard of care of patients by enabling earlier detection and identification of mild venous disease, especially for patients in lower resource settings.

6.2 Research Summary

The first aim in this dissertation focused on the development of portable, unobtrusive bioinstrumentation platforms for use in the study and clinical evaluation of persons who suffer from chronic venous disease. A small modular datalogger was developed to easily implement a variety of different sensors during in lab or out of lab test sessions. This sensing platform, named the ECHO system, can run for up to 96 hours on a single charge, save data to an SD card, and sample onboard or peripheral sensors at variable rates up to 40Hz. The ECHO can measure participant characteristics with a force sensing resistor, infrared rangefinder, inductance proximity sensor, a 6-axis IMU, or temperature sensor. Simultaneous sampling of multiple sensors is possible for rich data collection during controlled protocols in lab or during uncontrolled normal daily activity. A portable bioimpedance platform was adapted from previous amputee studies and adapted for measurement of vascular hemodynamics in able-bodied persons. The OZONE is a battery powered bioimpedance plethysmograph that can directly measure changes in lower extremity blood volume. This system uses low-profile measurement electrodes worn under the participants clothes or external compressions stockings for unobtrusive long-term vascular monitoring. The ECHO and OZONE are powerful and modular research tools created to facilitate in-situ study of behavioral and vascular characteristics.

The second aim of this dissertation assessed the use of a novel multi-channel impedance plethysmograph for the measurement of venous parameters in a controlled laboratory setting. Twenty participants were recruited for participation in the study; ten participants with a previous diagnosis of mild venous disease and ten matched controls. Study participants had a cuff occlude and release venous outflow while lying supine to measure venous obstruction. They then performed a series of tiptoe maneuvers to measure the efficacy of the calf muscle pump and degree of retrograde venous flow. Study results showed statistically significant differences in hemodynamic parameters that quantify venous function between the groups. Those considered vascular compromised had lower levels of blood ejected dur-

ing tiptoe maneuvers, higher amounts of reflux during muscle relaxation, and slower blood volume refill after activity cessation. Blood volume changes were measured in the anterior and posterior region of the limb and differences between the two regions were quantified. The posterior region showed greater differences between healthy and vascular compromised individuals, likely due to the clustering of deep veins in the posterior muscle compartment. This represents an improvement from other plethysmographic techniques that do not have the ability to differentiate between different anatomical locations. This also addresses one of the primary reasons that IPG use was discontinued in favor of more anatomically specific ultrasound diagnostic studies Anatomic localization of hemodynamic parameters may enhance clinical diagnostic efficiency by identifying the region in which venous insufficiency is occurring for follow-up focused imaging studies.

The third aim of this dissertation extended from clinical testing of the impedance plethysmograph to measure lower extremity hemodynamics during a two hour extraclinical activity protocol. Four participants repeated a standardized study protocol on three different days. During the second study session, they wore graduated compression stockings to determine the effect of compression stockings on lower extremity blood volume changes. Subjects with an increasing level of venous disease from C0 to C4 were recruited to study how calculated hemodynamic parameters change as disease severity increases. When compression stockings were not worn, blood volume increased over the test session, especially during periods of low activity or rest. When wearing compression stockings, blood volume did not rise as much in one subject (who was wearing old compression stockings), and decreased in the other three. The subject whose blood volume increased repeated the second session with a new pair of compression stockings, during which blood volume decreased similar to the other subjects. The number of steps needed to reach steady state blood volume was calculated for each subject to quantify calf muscle pump function. The number of steps increased with increasing severity of previously diagnosed venous disease, whether external compression was worn or not. This direct measure of calf muscle pump function is the first reported measure of hemodynamic efficiency during extra-clinical testing conditions.

6.3 Future directions

Venous disease presents a unique difficulty in early diagnosis and timely intervention. There is no pathognomonic symptom unique to venous disease and no standard test to quantify the global extent and severity of disease in the lower extremity. Future efforts should utilize advances in measurement technology to standardize clinical diagnostic procedures in pursuit of a single noninvasive test. Impedance plethysmography has the potential to fill this role, but there are several barriers yet to be overcome.

The research in this dissertation involved validation of IPG measurements in small, carefully-selected populations and individual case studies. Future efforts should expand from these initial studies to larger populations and greater diversity in the clinical manifestations of venous disease. As with all plethysmographic techniques, outcomes reported using IPG are derivative; artificial variables calculated from a blood or leg volume signal and hypothesized to quantify the actual underlying hemodynamic characteristics. The clinical utility of these calculated variables is questionable, and for any given parameter, there are usually multiple studies with contradictory reports on its usefulness. The measures reported in this research effort therefore should be refined and further validated against a gold standard test, such as concurrent AVP. IPG will not achieve any sort of meaningful adoption until it is shown to provide clinicians with simple, accurate, and clinically relevant metrics of venous flow.

Previous plethysmographic studies have shown that hemodynamic deterioration generally parallels venous disease progression up to the point where venous hypertension causes skin changes (CEAP C4). From a clinical perspective, current plethysmographic tests are therefore unsuitable as a standalone diagnostic for all levels of vascular disease. IPG directly measures blood volume changes in the limb and through the selective use of different current injection frequencies, can differentiate between extracellular and intracellular fluid. The underlying pathophysiology of cellular changes due to venous hypertension is not well understood, but is thought to involve damage to capillary basement membranes and subsequent local hypoxia and tissue loss [167]. IPG measurements of intracellular fluid changes over

a period of time or in response to a prescribed action may provide a quantitative measure of the severity of CVI at the cellular level, thereby overcoming the limitations inherent to indirect plethysmographic methods. Further investigations of spectroscopic bioimpedance measurements would add greatly to the clinical impact of this research and further validate the utility of IPG over other noninvasive hemodynamic measurement techniques.

A common complaint with clinical plethysmographic techniques is their susceptibility to indefinable or non-repeatable actions by the patient studied. Deep inspiration, coughing, and muscle twitching can easily alter the hemodynamic parameters of the lower extremity and result in an abnormal or unusable test result. Long-term hemodynamic monitoring has the potential to address these small system perturbations by averaging out their effect over a long test session. In this research effort, venous blood flow was measured during a structured activity protocol over a two-hour period. This is an improvement over clinical tests, but is still reliant on an observant researcher and compliant test subject. Hemodynamic parameters extracted from these structured activity tests were designed to be extended to long-term unstructured activity monitoring, but further study is needed to assess their utility and refine their clinical application.

External graduated compression stockings are widely prescribed for the treatment of chronic venous insufficiency. Despite their widespread prescription as a proven treatment, patient compliance is generally poor [168, 169, 170, 171]. Additionally, compression stockings deteriorate over time and lose their effectiveness. Limited research has been performed in this area, but prior studies show that compression stockings lose their clinical efficacy in as little as four months [172, 166]. In this research effort, we measured the difference in hemodynamic behavior for a new compression stocking and a $\sim$12 month old compression stocking. Portable IPG could be used to evaluate the therapeutic effect of different levels of compression stockings in individual subjects. This evaluation of a common disease intervention could refine clinical recommendations for compression stocking use and provide an objective measure of its individual impact on venous disease.

This research has introduced the novel paradigm of extraclinical vascular monitoring with

a goal of increasing diagnostic ability in early stage or presymptomatic venous disease. In the future, this vascular monitoring could take place over several days or even weeks, to quantify and understand influences and rates in disease progression. Research using current IPG tools will help refine the interpretation and understanding of this long-term vascular data, but advances in instrumentation are also needed before widespread adoption may occur. Miniaturization and package integration will enhance the utility and clinical usefulness of IPG, and decrease the reliance on a trained technician. Advances in the field of wearable electronics will help drive this effort, as will advances in long-term monitoring electrodes [173]. These system improvements will enhance extraclinical vascular monitoring, increase diagnostic efficiency, and enhance outcomes in persons suffering from venous disease.

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Appendix A

AIM 2 PROTOCOL

Vascular Testing – Ejection Fraction

Subject ID:		**Date:**
Consent Form #:	**Signed:** □	**Data File:**
Electrode Scheme:	**Harness ID:**	**Study Staff:**
Note: All timers are initialized and synchronized to AG Monitor Computer in Lab (synchronized to NIST)		
Session Start Time:		**Stopwatch Start Time [Protocol 1]:**
Session End Time:		**Stopwatch Start Time [Protocol 2]:**

Setup – In Lab

- □ Sync AG Monitor Computer in lab to NIST
- □ Instrument subject with MIZ Electrodes on anterior and posterior of limb. Cover with Tegaderm, and record subject limb measurements.
- □ Sync stopwatch with Topaz Surface Tablet computer, record start time above
- □ Begin video capture

Protocol Summary

- The testing protocol involves a short routine that will take place in the Sanders Lab or in the Vascular Laboratory at the University of Washington. The subject will follow a structured set of activities while characteristics of their vascular system are monitored using bioimpedance. These activities include lying down and raising their leg, sitting, standing, and doing tiptoes.

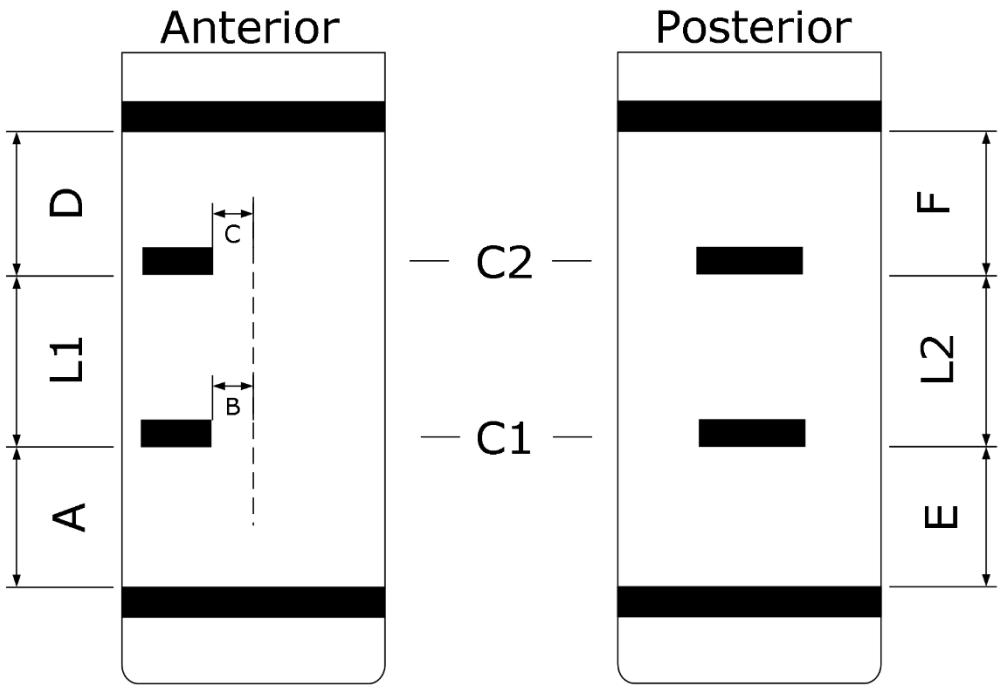
Experimental Protocol – Venous Volume				
	TIME	**ACTIVITY**	**LOCATION**	**NOTES**
1.		Sit	→ Lab – Orange Chair	Place Electrodes
2.		Lie	→ Lab – Hospital Bed	Place Thigh Tourniquet
3.	0:00	Lie	→ Lab – Hospital Bed – Leg Elevated	Begin Test
4.	1:00	Lie	→ Lab – Hospital Bed	Inflate Thigh Tourniquet
5.	3:00	Lie	→ Lab – Hospital Bed	Deflate Thigh Tourniquet
6.	4:00	Lie	→ Lab – Hospital Bed	End Test

Vascular Testing – Ejection Fraction

Experimental Protocol – Venous Filling Index [VFI]				
	TIME	**ACTIVITY**	**LOCATION**	**NOTES**
1.				
2.		Lie	→ Lab – Hospital Bed	
3.	0:00	Lie	→ Lab – Hospital Bed – Leg Elevated	Begin Test
4.	3:00	Stand	→ Lab – Next to Bed	Weight supported on opposite leg
5.	5:00	Heel Raise	→ Lab – Next to Bed	
6.	5:05	Stand	→ Lab – Next to Bed	Weight supported on opposite leg
7.	7:00	10 Heel Raise	→ Lab – Next to Bed	
8.	7:15	Stand	→ Lab – Next to Bed	Weight supported on opposite leg
9.	9:00	Lie	→ Lab – Hospital Bed – Leg Elevated	
10.	12:00	Lie	→ Lab – Hospital Bed	**End Test**

Electrode Placement

Limb	
L □	R □
A=	
B=	
C=	
D=	
E=	
F=	
L1=	
L2=	
C1=	
C2=	



Appendix B

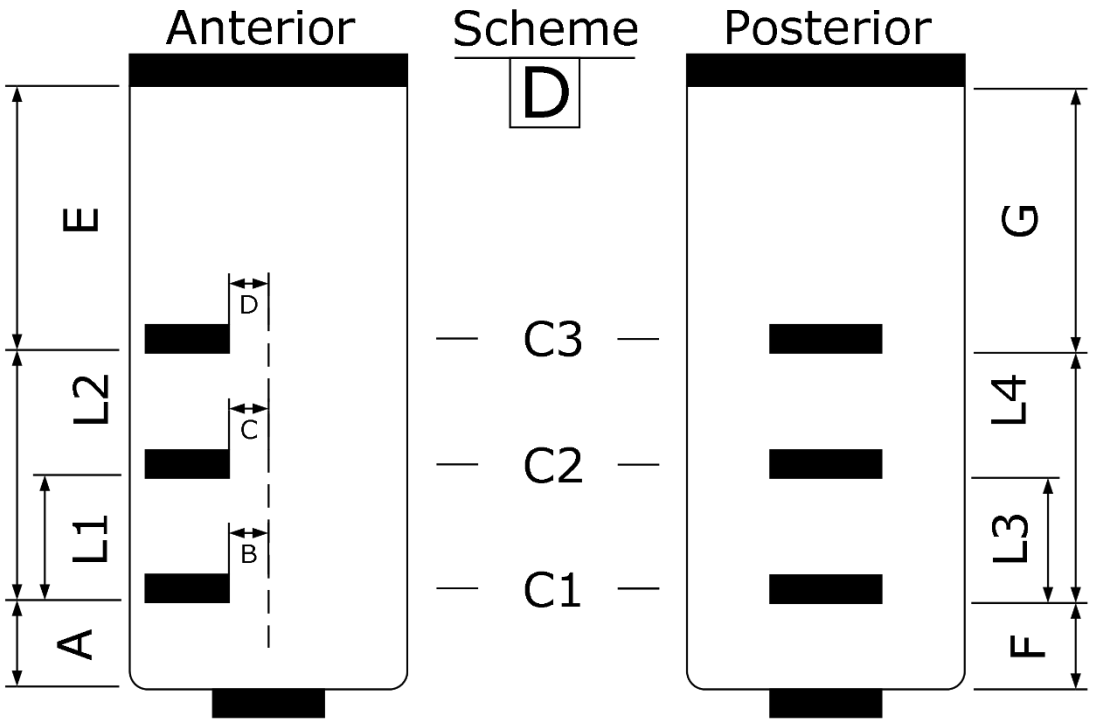
AIM 3 PROTOCOL

2 Hour Venous Study

Subject:	Date:	Harness ID:	Instrument: OZONE
Electrode Scheme: **OZONE 4-Channel**		Personnel:	
Ant. Channels:	Board No.:	Current Injection (circle): PROXIMAL or DISTAL	

Electrode Placement

Limb	
L □	R □
A=	
B=	
C=	
D=	
E=	
F=	
G=	
L1=	
L2=	
L3=	
L4=	
C1=	
C2=	
C3=	



Notes:

2 Hour Venous Study

Protocol Summary

READ TO SUBJECT ON FIRST VISIT

This testing protocol involves a 2 hour routine in the afternoon, following a structured set of activities in and around the University of Washington campus, and will be accompanied by research staff. We will have you do various activities ranging from sitting to standing or walking. There will be periods of long sits, during those sits we need you to remain calm and still. You are allowed to read a book, talk with the research staff, text or use your phone, but no phone calls may be made. We may ask you to stop talking at various times in the day, please understand that this is for the sake of data collection.

Setup – In Lab

- ☐ Sync AG Monitor Computer in lab to NIST
- ☐ Sync stopwatch with computer in lab, record start
- ☐ Place accelerometer at ankle. Initialize data collection.
- ☐ Instrument subject with Electrodes on limb. Cover with Tegaderm, and record subject limb measurements
- ☐ Prepare Android Timer
- ☐ Data connectivity
- ☐ Double check electrode placement

Note: All timers are initialized and synchronized to AG Monitor Computer in Lab (synchronized to NIST)

	2HR Venous (A)				
	Start TIME	DURATION	ACTIVITY	LOCATION	NOTES
SETUP		-	Timer Sync -NIST		
		-	Accel Start	IF Applicable: Don Compression	
		-	Start GoPro		
		-	Timer Start		
		-	OZONE START		
		-	OZONE Plug In		
		5min	Beginning Sit	Lab – Orange Chair	

2 Hour Venous Study

Recorder:		Study Protocol [2HRS Duration]			
	Start TIME	DURATION	ACTIVITY	LOCATION	NOTES
LOW ACTIVITY 1i		30s	Walk | **ASK BR**	Toward N400F Commons	
		3min	Stand	Timer Based	
		30s	Walk	N400F Commons Window	
		11min	Sit	Desk Chair by window	
		30s	Walk	Towards N400A Commons	
		3min	Stand	Timer based	
		30s	Walk	To N400A Commons	
		11min	Sit	In sofa chair	
		30s	Walk	To ↓Elevator	
		3min	Stand	In/Near Elevator to floor 1	
		30s	Walk	To South Foege Lobby	
		11min	Sit	In Sofa Chair	
		30s	Walk	Towards Vista	
		3min	Stand	Time dependent	
		30s	Walk	To Bench near vista elevator	
		11min	Sit	On Vista bench	
HIGH ACTIVITY 1		3min	Stand	Stand near elevator floor 1	
		3min	Walk | **ASK BR**	Down elevator, out south exit, G floor to Agua Verde park	
		3min	Stand	Time Dependent / park	
		3min	Walk	Go down boat street turn back halfway	
		3min	Stand	Time Dependent / park	
		3min	Walk	Up 15th sidewalk to bike racks	
		3min	Stand	Time Dependent	
		3min	Walk	To Foege N. 1st floor. Up Stairs	
		3min	Stand	Time Dependent / stairs	
		3min	Walk	↑Stairs / 4th floor lab / pace hall	
LONG SIT 1		15min	Sit	Brown Chair	
		1min	Stand	Lab	
		3min	Walk	↑Stairs / 5th floor / ↓Stairs	
		1min	Sit	Brown Chair	END PROTOCOL

VITA

CHRISTIAN B. REDD

Department of Bioengineering
Box 355061
3720 15th Ave NE
Seattle, WA 98195

Telephone: (801) 742-1319
Email: credd@u.washington.edu

EDUCATION

University of Washington, Seattle, Washington — March 2017
PhD Bioengineering

University of Utah, Salt Lake City, Utah — May 2011
MS Mechanical Engineering

University of Utah, Salt Lake City, Utah — May 2010
BS Mechanical Engineering

RESEARCH INTERESTS

Bioinstrumentation, medical imaging, wearable sensors, robotics, real-time rehabilitation, bioimpedance spectroscopy, vascular diagnostics

AWARDS AND HONORS

UW Business Plan Competition – Finalist, Best Retail Prize	May 2015
UW Alaska Airlines Environmental Innovation Challenge – Finalist	March 2015
UW Science and Technology Showcase – 2nd Place, Best Communicator	January 2015
University of Utah Outstanding Teaching Assistant	May 2011
University of Utah Mechatronics Competition – Best Design Award	April 2009
University of Utah First Year Engineering Competition – 2nd Place	April 2006

PUBLICATIONS

[1] **Redd CB**, Hafner BJ, Zierler RE, Sanders JS. Using multi-channel impedance plethysmography to measure lower limb venous hemodynamics. *Phlebology*, 2017 in preparation

[2] **Redd CB**, Hafner BJ, Zierler RE, Sanders JS. The value of ambulatory hemodynamic measurements by impedance plethysmography for quantifying the incidence and severity of chronic venous disease. 4 case studies. *Journal of Vascular Surgery*, 2017 in preparation

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[10] Parsons E, **Redd CB**, Gandhi MS, Tuckett R, Bamberg SJM, "Liquid cooling system for the vibrotactile threshold device," *The 33rd Annual Intl. Conf. of the IEEE Engineering in Medicine and Biology Society*. Sept 2011

CONFERENCE PRESENTATIONS

[1] "Monitoring donning and doffing with subject activity in transtibial amputee prosthesis users" *International Society for Prosthetics and Orthotics* Lyon, France, June 23, 2015

[2] "Considerations when using force sensitive resistors to measure socket interface pressure" *The 39th Annual Conference of the American Academy of Orthotists and Prosthetists*, Orlando, Florida, February 21, 2013

[3] "Bioimpedance monitoring for a better prosthetic fit" *Military Health Research Symposium, 2012*, Fort Lauderdale, Florida, August 15, 2012

[4] "A wireless sensory feedback system for real time gait modification" *The 33rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, September 14, 2011