Fluid distribution and tissue resistance: response to intermittent pneumatic compression in people with and without primary lymphoedema

By

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ABSTRACT

In primary lymphoedema, inherent drainage anomalies within the lymphatic system cause fluid accumulation in the dermis and subdermal tissues. Fluid accumulation progresses to fibrotic induration in both the dermis and subdermal tissues, which eventually become resistant to treatment, negatively impacting quality of life. However, no studies of primary lymphoedema have investigated dermal fluid accumulation, resulting in a lack of understanding of quantification and response to standard treatment. To better understand the dermis and subdermal tissues in primary lymphoedema, fluid distribution and tissue resistance in the leg and foot were explored in a cohort of 16 people with primary lymphoedema and compared to an age, gender and ethnicity matched group of 16 people with no lymphoedema. Objective clinical measures of lymphoedema—bioimpedance, percent water content, indurometry—provided subdermal information and circumferences provided a commonly used reference point. High frequency ultrasound (HFU) provided a measure of fluid specific to the dermis. The effect of a standardised dose of intermittent pneumatic compression on all measures was investigated and compared between groups.

The aims of this research were firstly to describe differences in these measures in the foot and leg between people with (PLO) and without (NLO) primary lymphoedema. The second aim was to understand the impact of compression on fluid distribution in primary lymphoedema compared to those with no lymphoedema. The final aim was to explore correlation among measures, to understand the relationships between them and identify possible proxy measures that might replace the need for the inaccessible and expensive HFU and provide simple cost-effective information to clinicians about dermal fluid status. Preparatory work for data collection involved establishing a reliable method of using HFU to measure dermal fluid using echogenicity measures.

In the observational study, significantly higher dermal fluid was observed in the foot in PLO compared to NLO. As well, local percent water content (PWC) and extracellular to intracellular fluid ratio (ECF/ICF) throughout the foot and leg were significantly elevated in PLO compared to NLO. Clinically, high dermal fluid measures in the feet of people with primary lymphoedema indicate the need for treatment to reduce fluid in the dermis of the foot before chronic pathological fibrotic changes occur.

An interventional study with the same group of people then compared the within group and between group changes in these outcome measures before and after one standardised dose of intermittent pneumatic compression (IPC). Dosage for IPC was informed by a systematic review. No significant change in dermal fluid occurred in response to IPC at any site in both groups, despite other fluid measures of ECF/ICF and PWC in PLO significantly decreasing after IPC, indicating that the effect of IPC on fluid distribution occurs deeper than the dermis. Lymphatic vessel anomalies in some forms of primary lymphoedema lead to delayed uptake of fluid from the dermis, which may account for the lack of change in the dermal fluid measures in this study. However, as no significant change was observed in dermal fluid in those with no lymphoedema, it appears that IPC does little to promote fluid uptake in the dermis. In addition, there was no response to IPC in the foot, demonstrated by all measures in both groups. Clinically, it appears that the foot requires an alternative and more effective strategy than IPC as applied, to manage the pathological changes commonly seen in the foot due to fluid accumulation in lymphoedema.

In the third investigation, moderately strong significant correlation was found between bioimpedance and percent water content in the leg. Tissue resistance was measured by the Indurometer, which indicated high tissue resistance by a low reading, and was the only measure to significantly correlate with two other measures. Firstly, there was moderate significant negative correlation between indurometry measures (IU) and PWC at the foot, indicating high tissue resistance (low IU) where percent water content was high. Secondly, a negative correlation was found between IU and the echogenic measures of dermal fluid at the posterior calf. This indicates lower tissue resistance (high IU) where there was low dermal fluid. As the Indurometer appears to be influenced by underlying tissue, and confidence intervals generated by bootstrapping were wide, further investigation is required prior to drawing any conclusions about correlation between the Indurometer and fluid measures.

This study demonstrates three original contributions to knowledge. High distal fluid accumulation was quantified by dermal and subdermal fluid measures in the foot and leg in people with primary lymphoedema compared to those with no lymphoedema, for the first time. This is consistent with lymphoscintigraphic descriptions of the distal fluid distribution in primary lymphoedema and supports treatment to the dermis and subdermal tissues in the foot in primary lymphoedema. The second original contribution was the observed lack of response to a single application of compression in the dermis at any site, based on the dosage applied. This raises important clinical

questions about the effect on the initial lymphatics of less than an hour at 60 mmHg pressure as applied by IPC. Fluid uptake in the dermis is known to be influenced by variable pressure, which may not be optimally applied by IPC when supine. Furthermore, anomalous fluid uptake in the initial lymphatics in primary lymphoedema may have influenced the response to compression in PLO. The third original contribution was the lack of response to a single standardised dose of IPC in all measures in the foot. These findings warrant further investigation due to the high level of impact this may have on clinical practice. If IPC is ineffective in changing fluid accumulation in the foot, alternative evidenced treatment strategies are required and clinical practice adapted accordingly.

A focus for future research is to explore the dermal fluid response to other types of compression and other lymphoedema treatment modalities. In particular, investigation of modalities that move the skin, causing variable pressure in the dermis, may identify a treatment mode to reduce dermal fluid. Mapping the movement of fluid in the foot during IPC by ICG lymphography may assist in the development of optimal IPC dosage parameters to address fluid reduction in the foot, particularly in primary lymphoedema, in whom fluid uptake and pathways vary. Future dermal investigations of primary lymphoedema could benefit by additional baseline stratification by imaging or genetic abnormality. Determination of the baseline vessel anomaly or underlying fluid transport issues in future investigations may progress understanding of the dermal response to treatment in primary lymphoedema.

DECLARATION

I declare that on nearing the completion of this thesis, in February 2022 I took up the role of Clinical Advisor for Haddenham Healthcare who are the Australian suppliers of the LymphScanner. The LymphScanner is the updated version of the MoistureMeterD Compact, which was used in this research and which was provided to me for that research by Haddenham Healthcare. I certify that Haddenham Healthcare had no input to the planning, data collection, analysis or interpretation of this research.

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Signed .....J.J.Phillips.....

Date.....12/8/2022.....

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DISSEMINATION OF RESEARCH

Work for this thesis has led to publications and conference presentations as below:

Publications		
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GLOSSARY

For the purposes of this work, the dermis will be referred to in its entirety, describing its appearance or function in lymph drainage and its properties as illuminated by different measures. The term skin will be used in the context of the organ, which is seen and felt, and upon which the interactions of treatment occur.

Glossary and abbreviations	
Term	**Description**
PLO	Participants with primary lymphoedema.
NLO	Participants with no lymphoedema.
UniPLO	Participants with unilateral primary lymphoedema.
BiPLO	Participants with bilateral primary lymphoedema.
Skin	The organ which covers the body, which is seen and felt and upon which the interactions of treatment occur.
Dermis	Comprises the reticular and the papillary dermis, but will be referred to as a whole for the purposes of this thesis.
SkiPL	Skin in Primary Lymphoedema. Short form name for study project.
HFU	High Frequency Ultrasound
LEP	Low Echogenic Pixels

SFB7	Device for measurement of impedance or resistance to a passage of current in bioimpedance measurements.
R_0, R_i, R_{inf}	Resistance measured by the SFB7, according to the frequency approximating zero (R_0), or infinity (R_{inf}). R_0 is the resistance representative of extracellular fluid. R_{inf} is the resistance representative of total body water. R_i is calculated from R_0 and $R_{inf,}$ and is the resistance representative of intracellular fluid.
ECF	Extracellular Fluid.
ECF/ICF	Ratio of extracellular to intracellular fluid. May also be represented as R_i/R_0
PWC	Percent Water Content
TDC	Tissue Dielectric Constant
MMD and MMDC	MoistureMeterD and MoistureMeterD Compact. Devices for measurement of PWC.
Indurometry	An electronic form of tonometry measured by the Indurometer.
IU	Induration Units. Measured by the Indurometer and equivalent to the distance in increments of 0.01mm travelled into the skin using a force of 200g.
Circumference	Measurement taken at regular intervals around a limb to document change in lymphoedema. May be used to calculate limb volume.
Tonometry	The measurement of tissue resistance to compression.

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CHAPTER 1
INTRODUCTION

Lymphoedema of the extremities is recognisable by enlarged limbs due to inadequate fluid transport which leads to fluid accumulation. It is classified as either primary or secondary lymphoedema (International Society of Lymphology, 2020). Primary lymphoedema arises due to an inherent anomaly in the lymphatic system, which commonly presents as swelling of a body part at birth, in the early years of childhood, or adolescence (International Society of Lymphology, 2020). Prevalence of primary lymphoedema is acknowledged to be low (Maclellan et al., 2015; C. Moffatt et al., 2019), or unknown (Vignes et al., 2021). Secondary lymphoedema is more common, and is provoked by damage to the lymphatic system, which, in first world countries, is most frequently seen following treatment for cancer.

Recently it has been accepted that secondary lymphoedema is an expression of a primary weakness in the lymphatic system (Peters & Mortimer, 2021; Rockson, 2019; Rockson et al., 2019). This blurs the historic distinction between these two groups and raises the importance of understanding primary lymphoedema, as underlying the secondary condition. In primary lymphoedema, known anomalies occur in the lymphatic vessels (Sarica et al., 2019) which arise in the dermis, that part of the skin which houses both blood capillaries and lymph vessels, resulting in fluid accumulation in both the dermis and subdermal tissues. The nature of primary fluid transport insufficiency that underpins secondary lymphoedema is unclear, and has been described as a 'constitutional weakness' predisposing to lymphoedema (Peters & Mortimer, 2021). What is known is that dermal backflow occurs in both (Yamamoto, Narushima, et al., 2011): better understanding of the fluid accumulation in the dermis and its response to compression in people with primary lymphoedema will inform and benefit the management of both primary and secondary lymphoedema.

For the purposes of this work, the term 'dermis' includes the upper papillary and lower reticular layers as a whole, describing the appearance or function of the dermis in lymph drainage and its properties as illuminated by different measures. The term 'skin' includes the dermis and epidermis and is used in the context of the organ as a whole, which is seen and felt, and upon which the interactions of treatment occur.

Pathological changes in the skin and tissue fibrosis (International Society of Lymphology, 2020) as well as inflammation (Ly et al., 2017) accompany fluid accumulation in lymphoedema. Fluid accumulation and fibrosis thicken the dermis (Arrive et al., 2018; Ly et al., 2017) and cause skin pathologies (Fife et al., 2017). Subcutaneous tissue that is overloaded with fluid in lymphoedema also leads to inflammation, fibrosis and adipose deposition (Jiang et al., 2018). Such adipose deposition is seen (Brorson et al., 2009; Dayan et al., 2020; Tassenoy et al., 2016) and may be quantified by MRI (Sen et al., 2018). However, there is no standard objective method to measure the relative fluid or fibrotic nature of the dermis (Coutts et al., 2016; Johnson et al., 2014; Sanderson et al., 2015; Tassenoy et al., 2016).

Staging of the severity of lymphoedema, as determined by the International Society of Lymphology (ISL) relies on the subjective description of swelling—whether or not swelling resolves overnight—and tissue changes—a visual description of skin pathologies (International Lymphoedema Framework, 2006; International Society of Lymphology, 2020). Stages of lymphoedema correlate with dermal backflow (Yamamoto et al., 2013), which, together with delayed fluid uptake in the initial lymphatics, occurs in lower limb primary lymphoedema (Sarica et al., 2019).

The stage of lymphoedema, and the associated state of the skin, informs treatment decisions, as the skin is the interface for treatment of lymphoedema. Over time the skin becomes stiff (Gerber, 1998), hard (Bagheri et al., 2005) or thickened (Goss & Greene, 2019) and resistant to compression (Ramsey & Mortimer, 2015), a key treatment for lymphoedema. The lack of a standard objective method to measure fluid in the dermis limits assessment of change due to treatment such as compression (Rockson, 2020). It is unknown how fluid distribution in the dermis responds to compression. This is the case both in the healthy dermis and in primary lymphoedema.

Existing clinical assessment tools provide information regarding fluid within a limb, or at a single point on a limb. Bioimpedance spectroscopy provides a relative measure of extracellular fluid within a whole limb or limb segment. The tissue dielectric constant provides access to the percent water content to a certain depth at any one point on a limb. However, neither of these measures are specific to the dermis and it is not understood what relationship they have to the composition of the dermis and in particular, fluid distribution in the dermis.

Fluid is overtaken by fibrosis as the more dominant feature in the clinical presentation of

lymphoedema as stiffening and fibrosis increase in the dermis (International Lymphoedema Framework, 2006; Ly et al., 2017). A third clinical tool, the Indurometer, has been used in research to provide a measure of tissue resistance (Pallotta et al., 2011). Again, it is not known what relationship tissue resistance measures have to the composition of the dermis.

High frequency ultrasound, used for over thirty years by dermatologists for dermal assessment (Olsen et al., 1995; Serup, 1992; Tan et al., 1982; Waller & Maibach, 2005), is able to provide a relative measure of fluid in the dermis (Gniadecka, 1996; Suehiro, Morikage, Yamashita, Harada, et al., 2017). The balance between resolution and depth of field in high frequency ultrasound results in a superficial focus (Serup et al., 2006) to depict the dermis clearly (Iker et al., 2019; Nedelec et al., 2016; Schuetzenberger et al., 2019). Fluid in the dermis has low echogenicity, reflecting very little ultrasound, and therefore appears to be black on the ultrasound images. Consequently, fluid is distinct from fibrous or heterogenous tissue content, which reflects sound to differing degrees and thus appears brighter, having higher echogenicity (Serup et al., 2006). Hence measurement of black areas of low echogenicity gives an estimate of fluid within the dermis.

However, due to its price high frequency ultrasound is not readily available to clinicians and is rarely found in small or rural and remote clinics. Understanding if there are other clinical measures of fluid distribution or tissue resistance that are correlated to the dermal fluid measure could provide an affordable and accessible proxy measure of dermal fluid.

This research follows 14 years' clinical practice by the investigator treating all forms of lymphoedema in a public clinic. During this time, the lack of information regarding the physiological basis of primary lymphoedema, the effect of compression on the initial lymphatic drainage in the dermis and the measurement of change in dermal fluid distribution in relation to interventions has restricted the ability to target assessment and intervention. Measurement and understanding of the physiological and physical changes in the dermis will underpin targeted treatment.

In summary, the dermis is the site of the initial lymphatic vessels, which are commonly anomalous in primary lymphoedema and a primary lymphatic 'weakness' also underpins the expression of secondary lymphoedema. Despite change in the dermis, from fluid to fibrosis, being known to impede treatment, measurement specific to the dermis is not clinically available. Stanley Rockson, Founding Chair of the Lymphatic Education and Research Network and Director of the Stanford

Centre for Lymphatic and Venous Disorders at Stanford University, recently summarised the lack of information about the dermis:

> . . .despite the well-recognized cutaneous stigmata of the more advanced stages of lymphedema, the degree of cutaneous change, which reflects the response of the end organ of damage in lymphedema, has not been a consistent target for disease quantification or for assessment with regard to treatment response. This likely reflects the historical absence of investigative tools that are suitable for this purpose. (Rockson, 2020)

The foundational premises on which this thesis stands include: 1) Fluid accumulation (N. Liu et al., 2021; Ricci et al., 2021) and fibrotic changes (Di et al., 2016; Sun et al., 2017) occur in the dermis in lymphoedema, both of which impact treatment outcomes (Bagheri et al., 2005; Ramsey & Mortimer, 2015; Tassenoy et al., 2009). 2) Differences in the dermis specifically in those with primary lymphoedema compared to those without lymphoedema are unknown. 3) High frequency ultrasound provides an objective measure of fluid accumulation specific to the dermis. 4) The response of the dermis to compression, a key treatment, is unknown. Understanding the behaviour of fluid in the dermis in response to compression in people with and without primary lymphoedema will provide important information for targeted and effective treatment.

1.1 Research Aims

1.1.1 Fluid Distribution and Tissue Resistance in Primary Lymphoedema

Aim one: To describe and compare the fluid distribution and tissue resistance in the lower limbs of people with and without primary lymphoedema, addressing the research question:

What are the differences in fluid distribution and tissue resistance between people with primary lymphoedema (PLO) and those without lymphoedema (NLO)?

Observational study. This study involved the following measures, taken in both PLO and NLO: (1) low echogenic pixels (LEP) from high frequency ultrasound for dermal fluid, (2) extracellular fluid (ECF) from bioimpedance for limb and segment fluid, (3) percent water content (PWC) from the MoistureMeterD Compact for point fluid, and (4) tissue resistance (induration units: IU) from the Indurometer. It was hypothesised that PLO would show higher fluid measures and stiffer tissues than NLO.

1.1.2 Response to Compression

Aim two: To measure and compare fluid distribution and tissue resistance before and after the application of a standard dose of intermittent pneumatic compression in people with primary lymphoedema.

Does compression change fluid distribution or tissue resistance in either PLO or NLO and if so, is there a difference in response to compression between PLO and NLO?

Intervention study. Fluid distribution and tissue resistance were measured before and after a standardised application of intermittent pneumatic compression (IPC) in both PLO and NLO. It was hypothesised that there would be a decrease in fluid measures and an increase in tissue resistance following IPC in PLO but no change in NLO.

1.1.3 Convergent Validity

Aim three: To investigate the relationship between different measures of fluid distribution and tissue resistance.

Is there convergent validity between clinical measures of echogenicity from high frequency ultrasound, induration units from indurometry, percent water content from the tissue dielectric constant and segmental measures of extracellular fluid from bioimpedance?

Convergent validity analysis. It was hypothesized that there would be some degree of equivalence between fluid measures, and that where fluid measures were high, there would be inverse correlation with tissue resistance measures.

1.2 Thesis Outline

Chapter 2 contains a review of the literature, including the skin in lymphoedema, the current assessment tools of clinical practice, and the use of high frequency ultrasound in assessing the fluid content of the dermis. The effect of lymphoedema treatment by compression is described and intermittent pneumatic compression explored. A systematic review of intermittent pneumatic compression dosage for children and adults with lymphoedema, relevant to this chapter, was published in *Lymphatic Research and Biology* in 2019 and is found in Appendix B.

Chapter 3 describes a pilot study to investigate and establish a reliable method for using high frequency ultrasound for assessment of the fluid content of the dermis in people with and without primary lymphoedema. The methodology for high frequency ultrasound developed in this pilot study was published in *Skin Research and Technology* in 2020 and is found in Appendix C. As well, the reliability of the investigator in using the Indurometer and in circumferential measurement is investigated.

Chapter 4 describes the methodology for the study, including ethical approval, a description of the participants, recruitment process, equipment used, procedure, and statistical analysis. Study documents including ethics approval and the study protocol are found in Appendices D-K.

Chapter 5 reports the results. Supplementary results tables are found in Appendix L.

Chapter 6 discusses and interprets the differences between people with and without primary lymphoedema. The extent to which clinical tools correlate is discussed with reference to the tissue properties described.

Chapter 7 analyses the response to compression of each group, which underpins discussion of the difference in response between people with and without lymphoedema. The limitations of this research are included here.

Chapter 8 brings together the conclusions of this work with the implications for future research and for clinical practice.

CHAPTER 2
LITERATURE REVIEW: THE LYMPHATIC SYSTEM AND THE SKIN; PRIMARY LYMPHOEDEMA; ASSESSMENT AND TREATMENT IN LYMPHOEDEMA

This chapter firstly describes primary lymphoedema, its prevalence and what is known of primary anomalies in the dermis. Secondly, the lymphatic system and the role of the dermis in lymph transport is outlined, before pathological changes that occur in the dermis due to lymphoedema are described. The third major section describes clinical tools for assessment of fluid content and tissue resistance in lymphoedema highlighting the lack of a measure specific to the dermis. High frequency ultrasound is introduced as an objective means to measure fluid in the dermis. The relationship is explored between current clinical measures and clinical staging of lymphoedema with its reliance on visual assessment and subjective tissue description. Lastly, treatment and compression are discussed; particularly, what is known of the response to compression, and the use of intermittent pneumatic compression.

2.1 Lymphoedema

Lymphoedema is a chronic condition with few effective medical or surgical treatments. It therefore requires lifelong conservative management. It affects quality of life, physical function and psychological health (Moffatt et al., 2017; Okajima et al., 2013), social and emotional well-being (Dunberger et al., 2013), financial security (Boyages et al., 2017; Dean et al., 2019) and results in poor health outcomes (Deng et al., 2015; Moffatt et al., 2017). The high impact of lymphoedema on health-related quality of life (Moffatt et al., 2017), has also been identified specifically in primary lymphoedema (Deng et al., 2015; Doubblestein, 2020; Río-González et al., 2021).

Primary lymphoedema most commonly affects the lower limbs. The low quality of life reported by those with lower limb lymphoedema (Noh et al., 2015; Saito et al., 2015) is further complicated by skin conditions in primary lymphoedema (Okajima et al., 2013). Skin hardening and dryness is common in lower limb lymphoedema, and contributes to poor quality of life to a greater degree in primary than in secondary lymphoedema (Stolldorf et al., 2016). Furthermore, fluid accumulation in lymphoedema of the lower limbs is exacerbated by factors such as unavoidable gravity (Taniguchi et al., 2021), and obesity (Costello et al., 2021; Dean et al., 2020; Warren et al., 2007).

Standard conservative treatment for lower limb lymphoedema produces less improvement in vitality, social function and mental health in primary than secondary lymphoedema (Noh 2015).

2.2 Primary Lymphoedema

2.2.1 Characteristics of Primary Lymphoedema

Early descriptions of primary lymphoedema relied on age of onset alone for classification (Greene, 2015; B. B. Lee et al., 2013; Lee et al., 2010): congenital, within two years of birth; praecox, during adolescence; or tarda, later in life, usually over the age of 35 years (Szuba & Rockson, 1998). In contrast, there is a wide range of phenotypes now recognised to express primary lymphoedema (Gordon et al., 2021) (see **Appendix A**). The term primary lymphoedema covers most forms of lymphatic anomaly: syndromic, systemic, congenital or late onset, all resulting in poor lymph drainage and most commonly involving the extremities (Gordon et al., 2020; Schook, Mulliken, Fishman, Grant, et al., 2011; Watt et al., 2017). Increasing numbers of genetic abnormalities associated with distinct phenotypes are being identified (Gordon et al., 2020); however, although the phenotype may be described, the specific anomalies within the lymphatic system associated with each genetic abnormality have not yet all been identified. Consequently, the underlying cause of poor lymph drainage leading to tissue fluid accumulation is commonly unknown. The end product of the abnormality, swelling, indicates the body part affected by fluid backlog, but this may be some distance from the underlying vessel abnormality. Regardless, even if the vessels within the dermis are not anomalous, fluid pressure backlog caused by poor function downstream (Ramsey & Mortimer, 2015) results in dermal backflow (Yamamoto, Narushima, et al., 2011).

Primary lymphoedema is more common in lower limbs than upper limbs, and bilateral lower limb lymphoedema is marginally more common than unilateral lymphoedema, with roughly equal numbers of left and right lower limbs being unilaterally affected (Bourgeois, 2021; Schook, Mulliken, Fishman, Grant, et al., 2011; Watt et al., 2017). However, where lymphoedema is unilateral, the seemingly unaffected leg may not have normal lymph drainage. This has been demonstrated by lymphoscintigraphy, and reported in those with chronic unilateral lower limb lymphoedema of mixed cause (Burnand et al., 2012; de Almeida et al., 2017), as well as in those diagnosed with primary lymphoedema later in life (over 35 years old) (Bourgeois, 2021). Unilateral lymphoedema is seen in those who first present with primary lymphoedema at adolescence, but, in one cohort of 138 people, 23% of those progressed to bilateral lymphoedema (Schook,

Mulliken, Fishman, Grant, et al., 2011). This indicates abnormal lymphatic drainage may occur without clinical signs yet present, which has recently been dubbed 'latent' lymphoedema (Peters & Mortimer, 2021). Therefore, the 'unaffected' lower limb may not be a useful control or indicator of normality in those with primary lymphoedema.

There are differences in fluid distribution between primary and secondary lower limb lymphoedema at initial presentation and in early stages, even though once established the presentation of chronic lymphoedema is similar, regardless of the underlying cause. The anomalies and area affected in overt primary lymphoedema are distinct from the area and part of the underlying lymphatic system known to be altered in secondary lymphoedema. In primary lower limb lymphoedema, the distal leg and foot are commonly first and most affected (Sarica et al., 2019), whereas in secondary lower limb lymphoedema, proximal thigh presentation is common (Yamamoto, Matsuda, et al., 2011). In Milroy's disease, one form of primary lymphoedema, the area affected is generally below the knee (Sarica et al., 2019) and more likely to be bilateral (Schook, Mulliken, Fishman, Grant, et al., 2011). Common forms of primary lymphoedema such as Milroy's disease present at birth or shortly thereafter, whereas Meige's disease and lymphoedema distichiasis both occur after the age of one year (Gordon et al., 2020), often presenting late in childhood or in adolescence (Mortimer, 2010; Watt et al., 2017). The progression observed in primary lymphoedema (Schook, Mulliken, Fishman, Grant, et al., 2011) indicates the detrimental effect of having lymphoedema over a long period. Despite this, studies investigating change over time in primary lymphoedema are rare. No association was found between duration of lower limb primary lymphoedema and type of abnormality seen on imaging (indocyanine green fluoroscopy), although the type of abnormality was associated with age of onset (Yamamoto et al., 2015), as later confirmed with lymphoscintigraphy (Sarica et al., 2019).

Gravity is a factor, as increased dermal reflux is seen in the foot during dependency in Lymphoedema Distichiasis (Mellor et al., 2011). Gravity has effect on all forms of lower limb primary lymphoedema, as understood by its influence on the movement of interstitial fluid (Baish et al., 2022). Primary lymphatic abnormalities such as incompetent valves lead to increased distal intra-lymphatic pressure under the influence of gravity, impeding drainage into lymph vessels (Mellor et al., 2011; Sarica et al., 2019).

2.2.2 Prevalence of Primary Lymphoedema

The global prevalence of primary lymphoedema is uncertain and remains underestimated (C. Moffatt et al., 2019; Peters & Mortimer, 2021), being unknown in some countries (Vignes et al., 2021). Prevalence is thought to be low (Connell et al., 2009; Todd, 2010), with dated estimates varying from approximately 1.15 in 100,000 of those under the age of twenty in one town in USA (Smeltzer et al., 1985), to one in 6000 at one clinic in London, UK (Dale, 1985). Both these figures were reported nearly forty years ago and relate to the local geographical area of study and from a specialised clinic, limiting its generalisability to the wider population. In Sydney Australia, in a more recent review within two tertiary lymphoedema services over seventeen years, 80 of 86 children (93%) diagnosed with lymphoedema had primary lymphoedema (Watt et al., 2017). These numbers provide little information regarding prevalence, as they were drawn from within specialised lymphoedema services. Furthermore, vessel anomalies causing primary lymphoedema may be more common than was previously evident, as increasing numbers of genetic mutations have been described and investigated in the varied phenotypes presenting with primary lymphoedema (Connell et al., 2013; Gordon et al., 2020). Identification of more affected genes may lead to increased numbers of people being diagnosed with primary lymphoedema. In addition, recent advances in the study of secondary lymphoedema have potential to increase the prevalence figures for primary lymphoedema: the selective determinant for those who develop secondary lymphoedema is now accepted to be an underlying predisposition to primary lymphoedema (Leung et al., 2014; Peters & Mortimer, 2021; Rockson, 2019; Visser et al., 2018). Mild underlying drainage weakness in secondary lymphoedema causes the constitutionally vulnerable lymphatic system to develop fluid accumulation following lymphatic affront, explaining why only a proportion of people undergoing cancer treatment develop secondary lymphoedema (Kilbreath et al., 2016; Rockson et al., 2019). The prevalence figures for primary lymphoedema would be greatly increased if those who develop secondary lymphoedema on the basis of having latent primary lymphoedema were included.

Prevalence of primary lymphoedema is considered to be higher than reported, due to poor recognition of the condition by health professionals (C. J. Moffatt et al., 2019; Todd, 2016). Delays in appropriate diagnosis experienced by people with primary lymphoedema (Doubblestein, 2020) complicate the reporting and hence the known prevalence of primary lymphoedema. Recognition of primary lymphoedema in children remains poor (Todd, 2016; Watt et al., 2017), as conditions

such as vascular anomalies are confused with lymphoedema in children (Schook, Mulliken, Fishman, Alomari, et al., 2011). Such lack of recognition in children contributes to the general under-reporting of primary lymphoedema in adults (Moffatt et al., 2003; C. J. Moffatt et al., 2019).

In Australia, poor understanding of primary lymphoedema has also led to misdiagnosis (Watt et al., 2017) and delayed diagnosis (Boughey et al., 2005; Watt et al., 2017). Historically, primary lymphoedema has taken up to nine years to be diagnosed, based on a review of Victorian services from 2003–2004 (Boughey et al., 2005). A more recent review of 86 children with lymphoedema across two tertiary paediatric hospitals in Sydney NSW found thirteen (15%) had been misdiagnosed and the time to diagnosis averaged nine months, but with a wide range from 0 to 145 months (Watt et al., 2017). Diagnostic delay and misdiagnosis are possibly far greater than seen at these tertiary lymphoedema services, as many Australian cities and rural areas have variable access to lymphoedema services, particularly for children (Newsom et al., 2020), who mostly present with primary lymphoedema (Schook, Mulliken, Fishman, Grant, et al., 2011). An informal review of Australian lymphoedema therapists found only eighteen therapists in services providing care to children with primary lymphoedema across five states (Queensland, New South Wales, South Australia, Western Australia and Victoria) (Newsom et al., 2020).

In summary, little data exists about the prevalence of primary lymphoedema in Australia. In conditions where prevalence is low and recruitment to a research study is expected to produce a small sample, people of the same age and gender from a healthy population may be recruited to 'match' participants with the rare condition. Matching participants with healthy controls on key characteristics, helps to control some of the variation within a small sample (Portney & Watkins, 2015, p. 172).

2.2.3 Tissue Information from Diagnostic Imaging

Imaging methods such as lymphoscintigraphy and ICG fluoroscopy focus on fluid conductivity through the vessel architecture. Lymphoscintigraphy has been the imaging reference standard (Armer et al., 2013; Goss & Greene, 2019; Sarica et al., 2019; Williams et al., 2000) and is useful to confirm delayed fluid transport indicating primary lymphoedema when cause of swelling is uncertain (Goss et al., 2019; International Lymphoedema Framework, 2006; Szuba et al., 2003; Vignes et al., 2021; Watt et al., 2017). Functional lymphatic pathways are outlined and dysfunction within the lymphatic system is indicated by delay or failure of dye transport centrally (Williams et

al., 2000). However, interpretation of lymphoscintigraphy varies (Pappalardo & Cheng, 2020) and radiation exposure is a deterrent for use (Hara & Mihara, 2021; Suami et al., 2019). Although fluid distribution may be inferred from dermal backflow and obstructed pathways that are visualised by lymphoscintigraphy, this imaging provides little information about the condition of the skin or subdermal tissues.

In contrast to lymphoscintigraphy, indocyanine green (ICG) fluoroscopy is a shorter test and has the advantage of being non-radioactive (Suami et al., 2019)and providing images of real-time lymphatic contractility (Rasmussen et al., 2010). ICG fluoroscopy depicts drainage pathways and vessel variation (Burnier et al., 2017; O'Donnell et al., 2017; Suami et al., 2019; Suami & Scaglioni, 2018; Tashiro et al., 2016; Wigg & Cooper, 2017; Yamamoto et al., 2015; Yoshida et al., 2020), and so can indicate the lymphosome (anatomical area drained to specific lymph nodes) (Suami & Scaglioni, 2018) whose drainage may be compromised. The highest stage of lymphoedema as defined by ICG lymphography describes few patent vessels and severe dermal backflow (Garza et al., 2019). ICG lymphography demonstrates greater sensitivity than lymphoscintigraphy (Mihara et al., 2013) and identifies lymphatic vessels with a sensitivity of 90% compared to magnetic resonance lymphography (Yasunaga et al., 2021). Dermal backflow predisposes such areas to fluid accumulation and subsequent fibrotic changes. Identification of the lymphosome affected by dermal backflow focusses attention on the tissues in the affected area and enables planning of alternative drainage pathways for manually assisted drainage techniques (Wigg & Cooper, 2017). However, ICG fluoroscopy does not quantify fluid accumulation.

ICG fluoroscopy is not readily available and lymphoscintigraphy is not always carried out to confirm diagnosis (Sudduth et al., 2020), resulting in reliance on clinical presentation alone (International Lymphoedema Framework, 2006; International Society of Lymphology, 2020; Watt et al., 2017; Wigg & Cooper, 2017). Although dermal backflow may be identified, neither of these imaging methods provide a measure of fluid accumulation in the dermis. Furthermore, staging by ICG lymphography does not correlate well with ISL stages (Garza et al., 2019). This indicates that vessel abnormality identified by imaging does not reflect tissue change due to fluid accumulation and fibrosis which is described in ISL staging, so a different method is required to investigate and measure these aspects of lymphoedema. High frequency ultrasound is one such method, providing a discrete way to visualise and measure fluid specifically in the dermis. High frequency ultrasound is described in **Section 2.7**.

2.3 Lymph Transport in the Lymphatic System

2.3.1 Return of Fluid From Lower Limbs via the Lymphatic System

Understanding normal lymph movement is the basis for understanding the impact of abnormal drainage on the dermis in lymphoedema. The lymphatic system comprises a system for one-way transport of capillary filtrate from the extremities back to the central circulation. Once the capillary filtrate, or interstitial fluid, enters the lymphatic vessel, it becomes known as 'lymph'. The superficial lymphatic system consists of vessels that arise in the dermis, travel deeper to the subcutaneous tissue then drain to lymph nodes in the root of a limb (in the lower limb, at the groin), where filtering of lymph takes place (Suami & Scaglioni, 2018). Efferent vessels leaving the node drain to deep nodes and vessels in the trunk. Deep lymphatic vessels are associated with major arterial and venous circulation in the limbs, but there are few connections of the superficial lymphatics with the deep system in the limbs (Suami & Scaglioni, 2018). It is dysfunction of the superficial lymphatic system that is associated with lymphoedema.

From the lower limbs, lymph predominantly drains anteromedially via numerous vessels and anastomoses to the inguinal nodes, before travelling deep to the pelvic nodes (Foldi et al., 2012, pp. 126-129). Variation may occur: ICG mapping has outlined a lymphatic pathway from the lower limb showing drainage occurs across the lower pelvis to the contralateral inguinal nodes (Yamamoto, Narushima, et al., 2011). It is unknown what percentage of lower limbs with primary lymphoedema may drain in that direction. Efferent vessels from deep pelvic nodes lead to the thoracic duct, which ultimately empties into the subclavian vein (Foldi et al., 2003, p. 23), thus returning the lymph to the central circulation. Variation may also occur in pathways from the feet, with four pathways draining the lower limb having been identified from initiating drainage in the foot: anteromedial, anterolateral, posteromedial, and posterolateral (Shinaoka et al., 2020). The anterior pathways are credited with draining the sole of the foot (Pan et al., 2013; Suami & Scaglioni, 2018): vessels interconnect from the anterior (plantar) to the posterior (dorsal) surface of the foot before travelling proximally (Foldi et al., 2012) (pp126) via one of the two anterior pathways from the leg (Shinaoka et al., 2020). Drainage from the foot varies between these pathways, so imaging depicting the active drainage pathway enables treatment to be targeted to the tissue area affected (Wigg & Cooper, 2017). Return of fluid from the lower limbs can be impeded by gravity, compromised venous return, and infection (Fife et al., 2017; International Society of Lymphology, 2020).

Return of interstitial fluid to central circulation is now understood to occur solely via the lymphatic system, in contrast to earlier models which attributed most interstitial fluid return to the venous system under Starling's Law (International Society of Lymphology, 2020; Keeley, 2018; Levick, 2004). Failure of the venous system increases intravenous capillary pressure, which causes greater filtration of fluid into interstitial spaces, and results in an increased fluid load in the tissues (Mortimer & Rockson, 2014). Accumulation of fluid due to venous disease is distinct from lymphoedema by its location on ultrasound images in the upper papillary dermis in contrast to throughout the dermis as in lymphoedema (Gniadecka, 1996).

2.3.2 Lymph Drainage from the Dermis

The skin consists of the outer epidermal layer, and the dermis, which lies directly beneath it. The dermis is divided into two layers. The thin, upper or papillary dermis, beneath the epidermis, is loosely structured, and interdigitates with the lower reticular dermis, which contains hair follicles, capillaries, and lymph vessels (Ribeiro et al., 2017; Ricci et al., 2021; Yousef et al., 2020). The initial lymphatic capillaries project up into the interdigitations between the upper and lower dermis (Ricci et al., 2021; Suami & Scaglioni, 2018) (see **Figure 2.1**). The dermis is the layer of the skin affected by fluid accumulation in lymphoedema of interest to this research.

Drainage of lymphatic fluid from the extremities begins in the dermis, at the point where the initial lymphatic capillaries arise within the dermis before travelling deeper to empty into the collecting vessels in the subcutaneous layer (Suami & Scaglioni, 2018). The blind-ended initial lymphatic vessels consist of a single-cell layer of epithelium, which have areas of loose connection between cells that create gaps, allowing fluid to enter the lymphatic vessel (Breslin, 2014; Jamalian et al., 2017; Jiang et al., 2018; Martin-Almedina et al., 2021). The forces driving lymph uptake are unclear. Previously it was thought that high interstitial pressure would drive fluid into the initial lymphatics (Guyton et al 1971, as cited in Jamalian et al, 2017); however, there is little evidence to support this (Breslin et al., 2018). Subsequently, fluid has been found to move from interstitial space to vessel with as little as 1mmHg difference between the two (Breslin et al., 2018; Schmid-Schonbein, 1990a, 1990b). Current evidence points to the importance of transient pressure variation in the dermis facilitating the opening of endothelial gaps and uptake of fluid into the initial lymphatic (Michel et al., 2020; Mukherjee et al., 2018).

In the healthy dermis, endothelial cell flaps act as primary valves during the process of fluid uptake

into the initial lymphatic vessel (Mendoza & Schmid-Schonbein, 2003). Anchoring filaments are tethered to a cell on one side of the gap, but not the other. When interstitial pressure rises, fluid may press onto the untethered cell, creating a gap, whilst the tethered one is held steady by the anchoring filament, thereby allowing fluid movement into the initial lymphatic capillary (Ikomi & Schmid-Schönbein, 1995; Suami & Scaglioni, 2018). Fluid is also prevented from reflux out of the capillary by the endothelial cell flaps, such that when the capillary is filled, the gap is held closed (Ikomi & Schmid-Schönbein, 1995; Mendoza & Schmid-Schonbein, 2003; Trzewik et al., 2001). Although fluid uptake at the level of the initial lymphatics is known to be defective in some forms of primary lymphoedema (Sarica et al., 2019), it is not yet clear what part of this process is flawed.

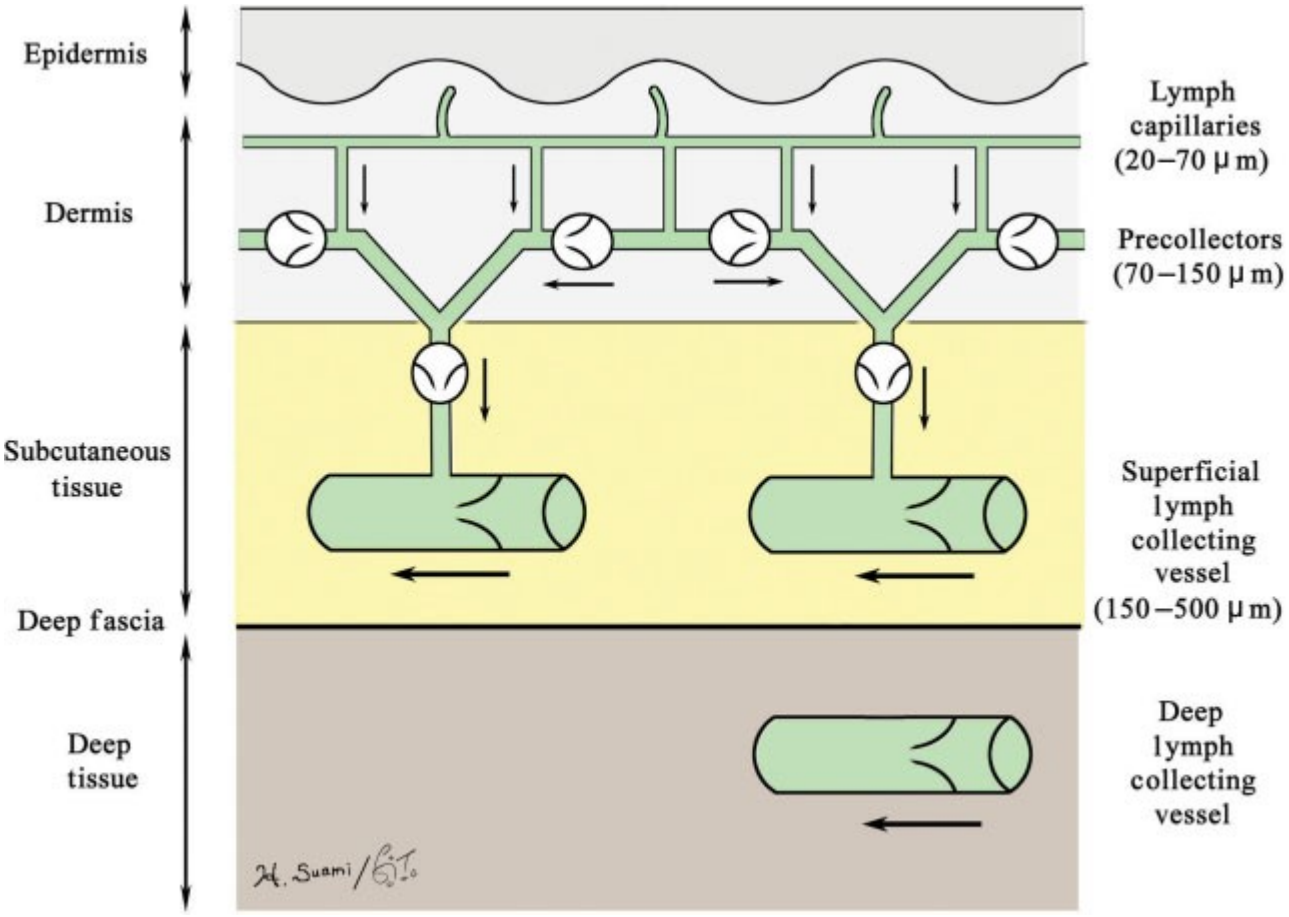


Figure 2.1 Skin and Superficial Lymph Vessels

(From Suami et al (2008), reproduced with permission.)

2.3.3 Factors that Influence Fluid Uptake and Lymph Transport From the Dermis

From the initial lymphatic capillaries, lymph may move in either direction through the network of vessels, until it reaches the deeper collecting vessels (see **Figure 2.1**). In the walls of collecting vessels, smooth muscle contracts regularly, which assists lymph movement and valves at regular

intervals (Suami et al., 2008) ensuring one way flow (Martin-Almedina et al., 2021; Mortimer & Rockson, 2014; Suami & Scaglioni, 2018). These secondary valves divide the collecting vessel into smaller segments known as lymphangions.

Lymph drainage is affected by changes in pressure within the dermis and subdermal tissues. Negative pressure is created in part by the contractions of lymphangions, and plays a role in drawing fluid into and deeper within the lymphatic system (Jamalian et al., 2017; Sloas et al., 2016). The potential for high hydrostatic pressure within the column of lymph in the collecting vessels when standing upright is counteracted by the division of the collecting vessels into the smaller lymphangion segments, and the valves, which prevent back flow (Solari et al., 2020). Fluid movement in deeper vessels is promoted by changes in pressure due to breathing, arterial pulsation, and compression from skeletal muscle contraction (Breslin et al., 2018; Martin-Almedina et al., 2021; Mukherjee et al., 2018; Solari et al., 2020). Gross application of external pressure around the outside of a limb, or compression, assists this fluid movement by working in tandem with muscle contraction and thus plays a key role in fluid management in lymphoedema. Compression provides an outer rigid wall which contains and increases the internal forces created by muscle contraction (European Wound Management Association, 2005; International Lymphoedema Framework, 2012; Mortimer, 2010).

Fluid is subject to both osmotic and hydrostatic pressures at the capillary level. Low capillary filtration pressure in supine (Mortimer & Levick, 2004), decreases the rate at which lymph is formed (Breslin et al., 2018). Gravity is a factor, as increased dermal reflux is seen in the foot during dependency in Lymphoedema Distichiasis (Mellor et al., 2011). Gravity is likely to have effect on all forms of lower limb primary lymphoedema, given the influence of gravity on the movement of fluid through the interstitial space (Baish et al., 2022). Primary lymphatic abnormalities such as incompetent valves lead to increased distal intra-lymphatic pressure under the influence of gravity, impeding drainage into lymph vessels (Mellor et al., 2011; Sarica et al., 2019).

Variation in pressure superficially also assists uptake of fluid into the initial lymphatics. This occurs during intermittent skin stretching that occurs in exercise (Ikomi & Schmid-Schönbein, 1995; Mortimer, 2010), and also during treatment for lymphoedema using manual lymph drainage (Lopera et al., 2017), as seen under ICG fluoroscopy (Suami et al., 2019). Compression applied

externally increases interstitial pressure (Bates et al., 1992; Olszewski et al., 2011), which acts as a counter pressure to capillary filtration (Mortimer, 2010), reducing interstitial fluid accumulation. Therefore, the effect of exercise, gravity and skin movement on fluid in the dermis is eliminated by investigating the effect of compression in supine.

2.3.4 Disruptions to Lymph Drainage in Primary Lymphoedema

The imperfect lymphatic system in primary lymphoedema commonly involves an anomaly in vessels originating in, or impacting, the dermis (Sarica et al., 2019). Vessel abnormalities in primary lymphoedema include smooth muscle disruption in walls of deeper vessels (Mortimer & Rockson, 2014; Petrova et al., 2004) and collecting vessels that are sparse (Arrive et al., 2018; N. Liu et al., 2021), or incompetent (Petrova et al., 2004), (as in Meige's type of late-onset primary lymphoedema) (Mortimer, 2010). Dysfunctional valves, as in lymphoedema distichiasis (Petrova et al., 2004; Sarica et al., 2019) and dilated lymphatic capillaries may be present (Arrive et al., 2018; Pfister et al., 1990). Poor absorption of fluid by initial lymphatics has also been reported in Milroy Disease (Mellor et al., 2010; Sarica et al., 2019). Earlier, these vessels were thought to be absent in Milroy's Disease (Bollinger & Amann-Vesti, 2007), but recent work on the FLT4 genetic mutation has distinguished two sub-types of Milroy's Disease: one with absence of, and the other with poor absorption by, initial lymphatics (Liu & Gao, 2021). The vessel walls in primary lymphoedema become fibrotic, even from early stages, reducing vessel patency as vessels thicken and become sclerosed (as seen in histology of specimens taken during lymphatic surgery) (Barone et al., 2020).

Fluid drainage in primary lymphoedema may therefore be impacted by abnormal vessel numbers (Arrive et al., 2018), vessel function (Sarica et al., 2019) or vessel fibrosis (Barone et al., 2020). Even if the initial lymphatics in the dermis are functional, disruption to drainage proximally (such as valve failure or fibrosis of vessels) can cause dermal backflow (Suami et al., 2019), resulting in fluid accumulation in the dermis. Pathological tissue and vessel conditions in primary lymphoedema, caused by underlying congenital abnormalities (Mortimer, 2010), are distinct from pathologies acquired in response to long term fluid accumulation, such as inflammation (Mikami et al., 2019; Mortimer, 2010).

2.4 Effect of Fluid Accumulation on the Dermis in Lymphoedema

2.4.1 Pathological Changes in the Dermis in Lymphoedema

Inflammation may be present in all types of lymphoedema (Jiang et al., 2018). Interstitial fluid

accumulation in lymphoedema causes progressive fibrosis in the dermis. Fibroblast and inflammatory cell activity are increased with fluid accumulation from early stage lymphoedema (Avraham et al., 2009; Hara et al., 2016; Herrada et al., 2019; Rockson et al., 2019; Yu et al., 2019), altering vascular permeability (Jiang et al., 2018). These changes occur in response to interstitial fluid accumulation, aggravating the fluid accumulation from delayed lymph transport that occurs due to vessel abnormalities in primary lymphoedema.

Fluid accumulation and the early inflammatory changes associated with lymphoedema (Ly et al., 2017) lead to increased collagen deposition (Karayi et al., 2020; Rutkowski & Swartz, 2007). These inflammatory changes cause the dermal stiffening and fibrosis (Daroczy, 1995; Karayi et al., 2020; Ramsey & Mortimer, 2015) which are overt in later stage lymphoedema (International Society of Lymphology, 2020). As progression to later fibrotic stages of lymphoedema occurs, the changes in the dermis become chronic (Carlson, 2014; Daroczy, 1995; Dayan et al., 2018; Grada & Phillips, 2017). Increased collagen disrupts drainage of fluid from the dermis, which continues the cycle by further increasing oedema (Daroczy, 1995; de Cock et al., 2009; Ly et al., 2017; Rutkowski et al., 2010; Szuba & Rockson, 1997; Zaleska & Olszewski, 2017). Accumulated fluid in the dermis predisposes to infection in the dermis, i.e., cellulitis (Fife et al., 2017; International Society of Lymphology, 2020; Mortimer & Rockson, 2014), which also further aggravates both collagen and fluid accumulation (Dai et al., 2016; Jiang et al., 2018; Ly et al., 2017; Rockson et al., 2018).

Pathological skin changes result from both primary and secondary lymphoedema, regardless of the underlying cause of lymphoedema (Brix et al., 2021). In primary lymphoedema, the foot and calf are affected by swelling to a greater degree than the thigh in early stages (Sarica et al., 2019). As primary lymphoedema commonly occurs early in life (Schook, Mulliken, Fishman, Grant, et al., 2011), the foot and calf often exhibit the earliest pathological skin changes, seen clinically in many forms (Fife et al., 2017). Those with lymphoedema of longer duration are inherently at risk of developing cellulitis (Fife et al., 2017; Gordon & Mortimer, 2007; International Society of Lymphology, 2020; Martin-Almedina et al., 2021; Mortimer & Rockson, 2014).

Fluid accumulated in the dermis over the long-term also results in gross pathological skin changes, such as skin fissures, papillomas and fibromas, ‘mossy foot’ (fine papillomatosis), dermal fibrosis, dermal lichenification (hyperkeratosis) and gross papillomatosis (wart-like outgrowths), lymphangiectasia (dilated lymph vessels) and lymphorrhoea (lymph leakage through the skin) (Fife

et al., 2017). Skin thickening and skin folds occur where lymphoedema has progressed to distort the limb shape (Fife et al., 2017; International Lymphoedema Framework, 2006; International Society of Lymphology, 2020), creating lobular outgrowths, which themselves become reservoirs for fluid accumulation (Fife et al., 2017), perpetuating the cycle.

2.4.2 Skin Changes Regardless of Lymphoedema Status

It is important to distinguish lymphoedema-related dermal changes from differences related to age or other conditions, as fluid accumulation is not the only factor to affect the skin over time. The elastic and fibrous components of the dermis change with age over the life span (Smalls et al., 2006). The skin thins with both sun exposure and age, particularly after the fifth decade (Caetano et al., 2015; Gniadecka & Jemec, 1998; Lasagni & Seidenari, 1995; Shuster et al., 1975). Other age-related structural changes include the accumulation of water, seen as a subepidermal low echogenic band on ultrasound (Gniadecka, 2001).

Dermal properties such as dermal thickness and collagen content vary not only with older age but also in the young. Information on dermal properties in children is sparse, as studies investigating dermal properties with age rarely include children (Waller & Maibach, 2005), focussing instead on the effects of aging. Changes in collagen structure are reported in the first years of life (Visscher et al., 2017) and children in those years are also reported to have thinner skin (de Rigal et al., 1989; Seidenari et al., 2000) than adults, as well as differences in the elastic and collagen components of the matrix (Adamsic and Fiser-Herman (1967), as cited in (Seidenari et al., 2000)). Skin thickness can alter the echogenic appearance of the dermis, as thin skin can appear 'denser', or have higher echogenicity, due to the collagenous matrix (Olsen et al., 1995), whereas thick skin can have lower echogenicity on ultrasound due to fluid (Eisenbeiss et al., 2001). There is clearly an interplay between fluid distribution and collagen content in dermal thickness, both of which affect echogenicity and vary with extreme age, whether old (Gniadecka, 2001) or young (Seidenari et al., 2000; Visscher et al., 2017).

Swelling is also associated (Douglass et al., 2018) with venous disease, which is more common over the age of forty (Davies, 2019; Prochaska et al., 2021) and which is also linked with pregnancy (Rasmussen et al., 2020). Venous disease has some distinctions from lymphatic swelling, such as depositing haemosiderin in the skin and causing cutaneous sclerosis (Priollet, 2006) as well as other dermal changes such as ulceration, telangiectasia or atrophie blanche (Davies, 2019; Dean,

2018). Menstrual cycles are also associated with swelling (Douglass et al., 2018).

Alterations occur in the skin due to conditions not related to lymphoedema. Fibrosis may occur in the skin due to scarring from trauma, radiation, or surgery that precedes the development of secondary lymphoedema. Pathological conditions or connective tissue disorders involving fibrosis or collagen, such as Marfan Syndrome (Meester et al., 2017; Neptune et al., 2003; Sano et al., 2019) or Ehlers-Danlos (Bowen et al., 2017), also alter the composition of the tissue matrix. Other conditions such as eczema, psoriasis or dermatitis cause inflammatory tissue changes (International Lymphoedema Framework, 2006; Korman, 2020), as does rheumatoid arthritis (Chua-Aguilera et al., 2017). Differences in skin thickness and collagen content have been reported in the skin of people of different ethnic origin (Langton et al., 2014). The epidermis of African Americans is thicker than that of Caucasians and the low echogenic band associated with aging is less evident in African Americans (Querleux et al., 2009). Although differences in skin thickness between ethnic groups have been found in some body sites (for example, the skin of the thigh was found to be thicker in Caucasians than in Asians (Laurent et al., 2007)), yet others have reported no difference in dermal thickness between ethnic groups (Querleux et al., 2009). However, variation in age-related processes in the skin, as well as underlying structural differences, are evident between ethnic groups (Querleux et al., 2009).

Therefore, to understand changes in the dermis due to primary lymphoedema alone, people with venous disease or other pathological skin conditions should be excluded and participants restricted to young people over five but under forty years from a single ethnic group.

2.5 Lack of Objective Dermal Measures in Assessment of Lymphoedema

Assessment of lymphoedema has historically focussed on the size of a limb using circumferential measurement, which continues to be a useful simple although gross measure of change. However, this form of assessment does not provide any information about tissue and fluid changes that occur in lymphoedema (Johnson et al., 2014; Niwa et al., 2021; Tassenoy et al., 2016) in the dermis.

With such focus on limb size and volume (Belgrado et al., 2010; Vignes et al., 2021), little attention has been given to the condition of the skin (Rockson, 2020). Clinically the change from normal skin to mild stiffening to fibrosis and gross papillomata, may be visually described and photographed,

but its objective measurement remains limited (Hara & Mihara, 2018; Sanderson et al., 2015; Tassenoy et al., 2016; Yu et al., 2019). Two manual clinical tests are used to determine the presence of lymphoedema: the pitting test (International Lymphoedema Framework, 2006), and Stemmer's sign (Goss & Greene, 2019; Vignes et al., 2021). During the pitting test, a finger is pressed into the tissues, causing an indentation when the test is positive, but it varies in its clinical application (Johnson et al., 2014; Sanderson et al., 2015). A positive Stemmer's sign occurs when the skin is too thick to pick up between thumb and finger (Goss & Greene, 2019). Both tests lack standardisation (Sanderson et al., 2015), and are categorical, describing merely the presence of lymphoedema by positive results. They are indicators of physiological change, rather than providing a measure of the physiological change itself. There are no established methods of specifically measuring tissue changes in lymphoedema (Coutts et al., 2016; Johnson et al., 2014; Sanderson et al., 2015; Tassenoy et al., 2016) which indicate the transition from fluid to fibrosis whether in subdermal tissues or the dermis. This study provides the opportunity to explore those physiological differences in people with and without primary lymphoedema.

Staging of lymphoedema rests on these categorical tests and visual assessment. The International Society of Lymphology (ISL) stages essentially describe the severity of lymphoedema based on clinical observations, which chart the transition from fluid accumulation to overt fibrotic changes in the tissues by palpation and visual description. ISL staging includes a pre-clinical stage 0 (International Lymphoedema Framework, 2006; International Society of Lymphology, 2020; O'Donnell et al., 2017), where lymphatic system abnormality is noted, but is not visible clinically. Stage 0 may exist in the 'unaffected' limbs of those with unilateral primary lymphoedema, where abnormality is seen lymphoscintigraphically (Bourgeois, 2021) and lymphoedema may be latent (Peters & Mortimer, 2021).

Fluid accumulation, or swelling, is noted in early stages by the presence of pitting (see **Table 2.1,** p41). By late stage II (known also as IIB), pitting may not be present due to skin and tissue stiffness, and fibrosis is assumed to be the cause. Determining when lymphoedema transitions from early to late stage II, particularly by use of the pitting test, is not consistent between therapists (Sanderson et al., 2015), resulting in variability of stage identification. By stage III, the skin has hardened, gross limb distortion and pathological skin conditions may be present (International Lymphoedema Framework, 2006; International Society of Lymphology, 2020). Hence, the progression from fluid to fibrosis first appears clinically during stage II, but the capacity

to measure this progression is at present limited (Johnson et al., 2014; Sano et al., 2019), except for noting the presence or absence of pitting. The skin changes seen in late stage II are reported to begin at the cellular level early in fluid accumulation (Avraham et al., 2009; Carlson, 2014; Jiang et al., 2018). An objective measure of dermal fluid may contribute to the understanding of this progression and to the reliability of correctly staging lymphoedema, particularly in distinguishing between early and late stage II. Relatively recently, since this study began, skin stiffness measured by the Skin Fibrometer has been found to correlate with ISL stages of lymphoedema, detecting significantly higher stiffness in higher stages of secondary lower limb lymphoedema (Sun et al., 2017). This device potentially provides a means of objectively staging lymphoedema with a quantitative measure of skin resistance.

Studies investigating fluid in lymphoedema do not always distinguish between early and late stage II in study participants, despite the variation in the balance between fluid and fibrosis within stage II lymphoedema. Recently fluid assessed on MRI was reported as variably present in women of the same clinical stage (II) with secondary upper limb lymphoedema (Niwa et al., 2020). A similar finding was earlier described by Suehiro et al (2016), also investigating women with upper limb lymphoedema, who reported the subcutaneous echo-free space (SEFS), indicating fluid accumulation on ultrasound, was found in only half (50%) of the arms examined, despite them all being stage II (Suehiro, Morikage, Yamashita, Harada, Samura, et al., 2016). Although Suehiro et al (2016) deduced that subcutaneous echogenic grading increased in all lymphoedematous limbs, due to fibrosis and chronic inflammation, the detection of fluid in only some of the stage II arms possibly indicated a difference between those with early stage II, with relatively more fluid, while others, with no SEFS and relatively greater echogenicity, may be late stage II, with relatively less fluid. However, such observations were of subcutaneous tissue, which undergoes other complex changes in lymphoedema such as the deposition of adipose tissue (Brorson et al., 2006; International Society of Lymphology, 2020). In contrast, thickening in the dermis occurs due to fluid (Mellor et al., 2004) or collagen deposition and disruption (Dai et al., 2016; Di et al., 2016), but little information is available on dermal fluid, particularly in lower limb primary lymphoedema.

The earlier ISL stages have the least impact, oedema being reversible. Hence, the aim is to identify and begin treatment in these reversible stages in which fluid accumulation is dominant (Stout Gergich et al., 2008), before the longer-term chronic fibrosis becomes pervasive. However, there is no objective measure to detect changes in dermal fluid and on which to base treatment decisions.

Stage of lymphoedema, as indicated by tissue resistance or fibrosis, affects treatment response (Bagheri et al., 2005; Ricci et al., 2021). Treatment is difficult to evaluate when small study sample sizes result in lymphoedematous limbs of different stages being combined in the one study. In a study investigating dermal thickness and limb volume following five days of intensive treatment, participants varied in stage, from stage I (3%), to stage II (56.7%) and stage III (40%) with no stratification for stage (Hacard et al., 2014). Furthermore, staging descriptions vary from study to study. The staging in Hacard et al (2014), appeared to be based on ISL staging (Hacard et al., 2014). In contrast, Zaleska and Olszewski (2018), investigating tissue change after IPC, described staging as 'pitting oedema in the foot and lower half of the calf' in stage II, whereas in stage III, all the calf is involved, as well as the skin being 'hard in the foot and ankle area' (Zaleska & Olszewski, 2018). Use of one universal staging system, to enable comparison of research findings, could facilitate identification of an objective measure of lymphoedema stage. An objective measure of tissues in lymphoedema, such as dermal fluid or tissue resistance, could be added to ISL staging for specific classification of lymphoedema. Such categorisation based on objective measures could underpin treatment targeted to the tissue state.

Table 2.1 ISL Stages of Lymphoedema Described by Swelling and Skin Change.

Based on ISL stages of lymphoedema (International Lymphoedema Framework, 2006; International Society of Lymphology, 2020)

Table 2.1 ISL Stages of Lymphoedema Described by Swelling and Skin Change		
Stage	**Swelling**	**Skin change**
Stage 0	**At risk: no swelling**	**Nil**
Stage I	**Swelling subsides overnight**	**May be pitting**
Stage II **Late stage II**	**Swelling does not subside** **May or may not be pitting**	**Pitting is manifest** **Tissue fibrosis more evident**
Stage III	**Pitting is absent**	**Tissue is hard (fibrotic)**

2.6 Clinical Assessment Tools

Among the many clinical assessment tools, those of specific interest to this study are measures of fluid and tissue resistance as they relate to the aims of this project. Current clinical assessments most commonly include circumferential measures which may also be used for volume calculation

(Australasian Lymphology Association; Neligan, 2016; Wang et al., 2017) and bioimpedance for early detection of lymphoedema and its progression (Koelmeyer et al., 2019; Shah et al., 2016). Circumferences provide a clinical reference point, used in the documentation of change due to treatment or progression. Measures of extracellular water, in three dimensional regions in the limbs or limb segments, are extrapolated from bioimpedance. Bioimpedance was included in this dermal study to provide the backdrop of extracellular fluid distribution in the leg and foot segments of the lower limb, as well as in the lower limb as a whole.

Two tools provide further information regarding the fluid content and tissue resistance in lymphoedema. The MoistureMeter is a localised spot measure of percent water content of the tissues to a fixed depth beneath the probe, and the Indurometer provides a spot measure of tissue resistance. The Indurometer, being a research tool, is not commercially available but provides an objective measure of comparative change over time in tissue resistance (Piller and Birrell (2004) in (Pallotta et al., 2011).

The use of ultrasonography is increasing in clinical studies of lymphoedema (Bok et al., 2016; Dylke et al., 2018; Hacard et al., 2014; Iker et al., 2019; Kim et al., 2021; Lee et al., 2020; Niimi et al., 2014; Niwa et al., 2021; Tassenoy et al., 2011), having been used in dermatology for over thirty years (Agner & Serup, 1990; de Rigal et al., 1989; Doldi et al., 1992; Schmid-Wendtner & Burgdorf, 2005; Seidenari et al., 1991; Serup et al., 2006). The following sections describe what is measured by each of these tools.

2.6.1 Circumference Measures

Circumference measures are a gross measure of limb size. They allow comparison of affected and unaffected limb size (Australasian Lymphology Association; Kojima et al., 2019), and comparison of the size of an affected limb over time in those with lymphoedema (Australasian Lymphology Association; Fukushima et al., 2017; Karafa et al., 2020; Wu et al., 2021). As well, they are used in many settings to assess change in limb swelling in acute injuries and in healthy populations. When used in long-term tracking, variation in body weight over time, and maturity from a child to an adult must be taken into consideration.

When considering lymphoedema, a limitation of circumference measures is that they include all tissues within a limb: bone, muscle, skin and subcutaneous tissues. Circumferences taken at small set intervals such as five centimetres can indicate the distribution of a size difference compared to

an unaffected limb. However, inter-limb differences are not specific to fluid as muscle and adipose tissue may contribute to that size difference. Circumferences therefore provide little information about the tissue size or location of fluid accumulation within specific tissues.

Measures of circumference by tape measure may show high inter-operator reliability (Taylor et al., 2006), but reliability must be established for each operator. An alternative measure, bioimpedance spectroscopy, is able to detect fluid accumulation with more sensitivity than volume calculated using circumferences (Ridner et al., 2022).

2.6.2 Bioimpedance Spectroscopy: A Measure of Fluid Distribution

Bioimpedance spectroscopy involves passing a small current with a multi-frequency range of 4 kHz to 1 MHz through the tissues and measuring resistance to the passage of that current. Differences in impedance between bone, fat and muscle tissue enables bioimpedance to be used in the assessment of body composition, in the critically ill (Khalil et al., 2014; Price & Earthman, 2018), healthy populations (Mattila et al., 2007; National Institutes of Health, 1996) and those with lymphoedema (Sierla et al., 2018).

Measures of resistance are inversely proportional to fluid in body compartments (Ward, 2019; Ward, Winall, et al., 2011): the more fluid present, the lower the resistance (or impedance). Currents at low frequencies do not pass through the cell membrane; therefore, measures of resistance to low frequency currents are an inverse representation of extracellular fluid (ECF) (Thomas et al., 1998; Ward, Winall, et al., 2011). As the fluid in lymphoedema accumulates in the extracellular space, bioimpedance therefore provides a method for quantifying that fluid accumulation (Cornish et al., 2002; Ward et al., 2009).

Bioimpedance is a widely used clinical tool for lymphoedema measurement that has claims of being worthy of the gold standard measure (Ward, 2009). It provides a surrogate measure of fluid attributed to lymphoedema, not just within the dermis but in subcutaneous and other tissues within a whole limb (National Lymphedema Network, 2011b; Sierla et al., 2018), or a limb segment (Svensson et al., 2017). Bioimpedance has been particularly useful as a baseline measure to detect gross extracellular fluid changes in a limb or limb segment over time (Cornish et al., 2001; Koelmeyer et al., 2019; Suehiro, Morikage, et al., 2018a; Suehiro, Yamamoto, et al., 2019), or in response to external factors, such as therapy (Brix, Apich, Roessler, et al., 2020; Cho et al., 2020; Do et al., 2017; Donahue et al., 2017). In particular, bioimpedance has been important in early

detection of lymphoedema following surgery for cancer (Cornish et al., 2001; Koelmeyer et al., 2019).

Bioimpedance measurements are contraindicated in pregnancy or in the presence of a pacemaker. The presence of metal in the body, such as a joint replacement, is merely a precaution as, once introduced, it may affect the comparison of measures over time (ImpediMed Limited, 2016). Bioimpedance measures ideally require more than 30 minutes for stabilisation of extracellular fluid (Gibson et al., 2015), to allow for equilibrium to occur following the orthostatic response to the change in posture from standing to supine (Barantke et al., 2008).

In lymphoedema, excess fluid indicated by bioimpedance has correlated significantly with circumferences (Cho et al., 2020), limb volume calculated from circumferences (Cornish et al., 2002; Suehiro, Morikage, et al., 2018a) and volume measured either by perometry (Ward et al., 2009) or other three-dimensional body scanning (Taniguchi et al., 2021). Compared to perometry, bioimpedance demonstrates a sensitivity of 73% and specificity of 84% for detection of lymphoedema in the upper limbs (Bundred et al., 2015). More recently, specificity of 97.4% was reported in detecting unilateral leg lymphoedema (Yasunaga et al., 2020). In the lower limbs, decrease in limb volume over time (Suehiro, Morikage, et al., 2018a) and in response to treatment is detected by bioimpedance with sensitivity (Coroneos et al., 2019; Do et al., 2017). Bioimpedance has been used to detect reduction in extracellular fluid following decongestive therapy for secondary lymphoedema in both upper and lower limbs. A far greater response was seen using bioimpedance (ECF) measures (48.3% reduction) than in limb volume calculated from circumferences (13.8% reduction) (Coroneos et al., 2019). It is this sensitivity to change that has led to the use of bioimpedance in early detection of fluid accumulation (Koelmeyer et al., 2019) and to assess limb change in response to therapy (Coroneos et al., 2019).

High intra-rater reliability (ICC >0.999) was demonstrated using bioimpedance in healthy lower limbs of young people (8–21 years old) when tested three times on the same day (Douglass, Graves, & Gordon, 2017). Reliability was lower but acceptable in those with lymphoedema (ICC 0.69) also when tested three times consecutively, with sensitivity of 66% and specificity of 99% compared to bioimpedance taken in healthy women (Fu et al., 2013). Bioimpedance is known to be less stable in certain situations: measures taken two weeks apart showed concordance of 0.517 following exercise (Lin's coefficient), and 0.628 following a drink of coffee (Timmer et al., 2019).

The highest reliability in the latter study was found between measures taken 10 minutes apart on the same day (Lin's concordance coefficient 0.935) (Timmer et al., 2019). Hence studies taken on the same day have high reliability but must control for caffeine use prior to taking bioimpedance measures.

2.6.2.1 Bioimpedance Outcome Measures

Measures of resistance across the range of frequencies are charted in a Cole plot, and the model extrapolates the resistivity to zero at the low end (R_0), and to infinity at the upper end (Ri_{inf}) (Ward, 2015) (see **Figure 2.2**). Representation of ECF is from low frequencies, ideally from a frequency of zero, which is instead inferred from modelling (Ward, 2015). Resistance measured at high frequencies (extrapolated to infinity on the Cole plot, R_{inf}) is representative of total body fluid (Ward, 2006, 2019). The resistance that represents intracellular fluid (known as R_i) may be calculated once R_0 and R_{inf} are known (Steele et al., 2018).

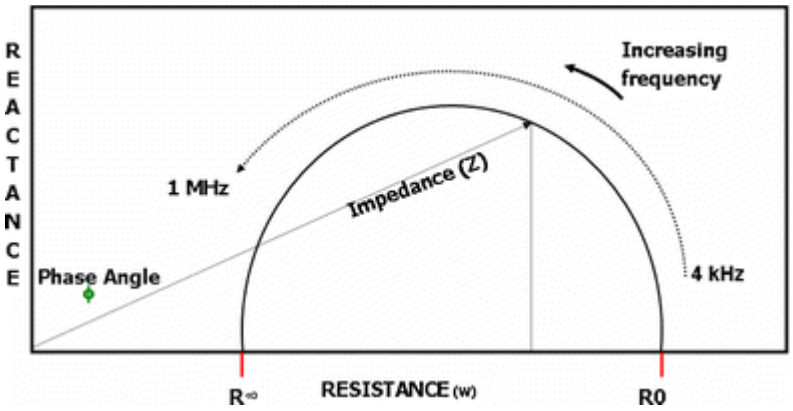


Figure 2.2 A Cole Plot Shows the Relationship between Impedance (Z), its Components Resistance (R) and Reactance, and Frequency.

(Reprinted by permission from Springer Nature Customer Service Centre GmbH : Springer Nature, Breast Cancer Research and Treatment, S. L. York et al (2008). Single frequency versus bioimpedance spectroscopy for the assessment of lymphedema. *Breast Cancer Res Treat, 117*(1), 177-182. https://doi.org/10.1007/s10549-008-0090-6)

Values of R_i and R_0 may then be used in the ratio R_i/R_0, or ECF relative to ICF, which represents the fluid accumulation in lymphoedema (Thomas et al., 1998; Ward, 2006; Ward, Winall, et al., 2011). Measures of ECF (R_0) alone vary according to other factors such as limb volume, muscle size and hydration (Brantlov et al., 2017b; National Institutes of Health, 1996), although inter-limb ratios of R_0 alone, investigated ten minutes apart, showed high reliability (Timmer et al., 2019).

The ratio of ECF to ICF (R_i/R_0) can be used for comparisons of differently sized limbs, such as affected to unaffected or upper compared to lower limb (Cornish et al., 2002; Dylke & Ward,

2020). As well, the R_i/R_0 ratio allows for comparisons between limb segments (Cornish et al., 2002; Ward, 2006) and accounts for variations in individual fluid status, such as size and hydration, which can affect measures of resistance (Khalil et al., 2014). Greater variation over time occurs in R_i/R_0 than in R_0, but this was found over eighteen months of measures (Steele et al., 2018). R_i/R_0 ratios (ECF/ICF) in lymphoedematous lower limbs were significantly different from those in healthy lower limbs of similarly aged controls, regardless of limb dominance (Ward, Winall, et al., 2011). Limb dominance was also found to have little effect on bioimpedance in unilateral leg lymphoedema (Cornish, Eles, et al., 2000; Ward, Dylke, et al., 2011b). Therefore, comparative measures taken in the same body posture over a one-hour period reduce variability due to hydrostatic or electrolytic changes in body fluid.

Bioimpedance ratios in normative data have been used to set thresholds for detecting lymphoedema against which those at risk for lymphoedema are compared (Steele et al., 2018, 2019; Ward, Dylke, et al., 2011a). Bioimpedance measures for such thresholds commonly involve ratios between an affected limb and unaffected limb. Correspondingly, to detect unilateral lower limb lymphoedema in one lower limb, the mean inter-leg R_0 ratio within a healthy sample is used as the comparator to that ratio in people at risk of lymphoedema, with minimal effect from gender (Ward, Winall, et al., 2011). However, reference to a 'normal' side is not possible where bilateral lower limb lymphoedema is present (Cornish et al., 2002; Steele et al., 2019; Ward, Winall, et al., 2011).

Bilateral presentation is common in primary lymphoedema (Watt et al., 2017). Even in unilateral lymphoedema, a seemingly unaffected limb may have abnormal drainage and consequently higher ECF than limbs in healthy controls, rendering it inappropriate as a comparator for establishing the presence of lymphoedema. Such limbs are described as having latent, or stage 0 lymphoedema (Yasunaga et al., 2020). For measurements of bilateral lymphoedema, ratios comparing a leg to the arm on the ipsilateral side (rather than to the other leg which is also affected) have been suggested for detecting fluid accumulation (Ward, Winall, et al., 2011). Confirmation of such a method has only recently been published (Steele et al., 2018, 2019).

2.6.2.2 Segmental and Whole Limb Bioimpedance Measurement

Normative bioimpedance data utilise full limb measures, which are tested against secondary lymphoedema populations, to establish thresholds for lymphoedema identification (Steele et al.,

2019; Ward, Dylke, et al., 2011b). Full limb bioimpedance measurement protocols incorporate the thigh and the leg, which is appropriate in secondary lower limb lymphoedema, given that the thigh is earliest affected (Sarica et al., 2019; Vidal et al., 2016; Yamamoto, Matsuda, et al., 2011). The distal leg is known to be affected in early primary lymphoedema (Sarica et al., 2019), but the foot is not included in full limb bioimpedance measurement protocols. Consequently, the measurement of the distal limb segments, the foot and the leg, are appropriate for comparisons involving early stage primary lymphoedema.

Little has been published about bioimpedance in segments of the lower limb. In the upper limb, lymphoedema has been detected with more sensitivity in 10cm segments than in whole limbs (Svensson et al., 2017). High concordance (Lin's correlation) was found between bioimpedance and volume, measured with perometry, in distal segments (r_c=0.78) (Czerniec et al., 2011). Upper limb segmental studies have included the hand (Svensson et al., 2015), but lower limb segmental studies have reported only the calf and thigh (Suehiro, Morikage, Yamashita, Harada, Ueda, et al., 2016; Taniguchi et al., 2021), not the foot. Established methods use anatomical landmarks for accurate placement of electrodes for whole limb bioimpedance. Using anatomical segments, electrode placement is standardised, and intra-operator variability minimised (Cornish, 2006). Anatomical landmarks used as boundaries of limb segments enable proportionate comparison (Ward et al., 2009). Both of the above-mentioned segmental studies of leg and thigh used anatomical landmarks for boundaries of these segments (anterior ankle, upper edge of patella or lateral condyle at the knee and groin or anterior superior iliac spine) (Suehiro, Morikage, Yamashita, Harada, Ueda, et al., 2016; Taniguchi et al., 2021). Although bioimpedance measures in the foot have not been reported, the hand was measured in its entirety, and, despite its irregular shape, had excellent test-retest reliability (ICC 0.967) (Svensson et al., 2015).

2.6.3 Tissue Dielectric Constant: A Measure of Fluid

The tissue dielectric constant (TDC) provides a measure of skin and tissue water at a specific site and is more sensitive in detecting localised fluid changes in lymphoedema than limb volume assessment by water displacement (Karlsson et al., 2019) and bioimpedance (Lahtinen et al., 2015). TDC is measured using the MoistureMeterD or its Compact version, in which an electromagnetic wave of 265 MHz is emitted from a co-axial probe of 20mm diameter which rests on the skin. Most energy is absorbed by tissue water, so the TDC depends on the water volume in the tissues beneath the probe (Mayrovitz et al., 2013). A measure of TDC is produced at the depth

at which much of the energy has dissipated, dependent on the size of the probe (Mayrovitz, 2015). The penetration depth of the MoistureMeterD Compact is between 1.5 and 2.5mm (Mayrovitz, 2015), or approximately 2mm (Mayrovitz et al., 2019), although it is reported as 2.5mm depth in the manufacturer's manual (Delfin Technologies, 2016). TDC measured by the MoistureMeterD Compact (MMDC) will therefore incorporate both the dermis and some of the subcutaneous tissue in the lower limbs, where total skin thickness (epidermis and dermis combined) in healthy adults measures less than two millimetres (Nedelec et al., 2016).

TDC is a dimensionless measure dependent on both the permittivity and conductance of the tissue. However, as the conductance contributes little to this value, the dielectric constant is influenced mainly by the permittivity, which is dependent on the water molecules in the tissues, relative to that of a vacuum (Mayrovitz, 2015). Where no water is present (as in a vacuum), TDC would be one, whereas the TDC of pure water would be 78.5 (Nuutinen et al., 2004). Significant difference in TDC has been reported between healthy and early stage 0 lymphoedema at depths up to 2.5mm, indicating sensitivity to fluid in the dermis and upper subcutaneous tissue (Mayrovitz et al., 2008).

Much information regarding the variability of this TDC measurement technology in lower limbs has arisen from investigations using the MoistureMeterD (MMD) (using a 23mm diameter probe to 2.5mm depth). The MMD produces TDC values of approximately 5.6% less than those produced by the MoistureMeterD Compact (MMDC, 20mm diameter probe, to 2mm depth) (Mayrovitz, Weingrad, Brlit, et al., 2015). Information from the MMD which is pertinent to the MMDC includes the behaviour of TDC technology in the lower limbs, particularly around superficial tendons and bones in the foot.

Investigations in the healthy lower limb have shown low standard error of measurement (SEM) at the posterior calf (SEM 1.7) of TDC using the MMD (Jönsson et al., 2019). Test-retest reliability of the MMD is high (Hidding et al., 2016), demonstrated by intra-class correlation coefficient (ICC, single measure) of 0.945 (95% CI: 0.792-0.991) at the lateral leg, taken on five participants a week apart by two raters (Mayrovitz, 2015). Reliability was slightly lower being 0.887 (95% CI: 0.615-0.982) and 0.923 (0.720-0.988), at 1.5 and 2.5mm depths respectively, on the dorsum of the foot, for measures taken one week apart (Mayrovitz, 2015). Intra-rater reliability of repeated measures taken on the same day approximately an hour apart was higher ($ICC_{(2,1)}$ of 0.996 (95%CI: 0.960-

1.000) (Mayrovitz, 2015). TDC measures close to bones and tendons were found to have lower but acceptable reliability in women ($ICC_{(2,1)}$: 0.63-0.93), whereas results were more variable in men ($ICC_{(2,1)}$: 0.21-0.89 (Jönsson et al., 2019). In the latter study, although measures were taken at 14 points across the lower limb, no measures were taken on the dorsum of the foot (Jönsson et al., 2019).

On the dorsum of the foot, there is a relative paucity of subcutaneous tissue and high preponderance of blood vessels, tendons and bone. The latter structures, which have variably higher permittivity than skin (Gabriel et al., 1996), have been suggested as a cause for lower reliability in the foot. A slight change in the angle of the probe on the antero-dorsal surface of the foot would result in different structures being measured (Jensen et al., 2012). A pressure sensor in the compact version contributes to the consistency of the MMDC (Mayrovitz et al., 2019), by showing when pressure is correct before the measurement is taken. Few studies report percent water content (PWC) measures in the foot, instead combining measures from several lower limb sites to produce a mean lower limb value for comparisons (Sun et al., 2017; Yu et al., 2019).

TDC values have been found to vary based on race, with significantly lower values found in the lower limb of Asian compared with Caucasian females (Mayrovitz, Mahtani, et al., 2017). Variation is also found according to anatomical site around the body (Mayrovitz & Luis, 2010) including with the MMDC (Mayrovitz, 2019a). Significantly higher measures were found in a healthy cohort in the antero-dorsal foot (mean TDC 37.8, SD 5.5), than in the medial ankle (TDC 29.0, SD 3.1), or lateral lower leg (TDC 30.5, SD 3.9, $p<0.001$) (Jensen et al., 2012). Where lymphoedema is present, TDC or PWC values are higher than in healthy lower limbs (Mayrovitz, 2019a). However, care must be taken when comparing different body sites, as the absolute TDC value may be higher in normal limbs at some anatomical sites, than in lymphoedematous limbs at other sites. This was demonstrated by the TDC on the dorsum of the foot in healthy limbs (mean 37.8, SD 5.5) (Jensen et al., 2012) being similar to that found in lymphoedematous upper limbs (mean 41.1, SD 8.8) (Mayrovitz et al., 2009).

To account for variation between individuals and body sites within individuals, an inter-limb TDC ratio was suggested to be more practical than absolute values for comparisons between individuals (Yu et al., 2019) and for detection of change against a threshold (Mayrovitz, Weingrad, & Lopez, 2015). However, although this applies in healthy people or unilateral lymphoedema,

differences in lymphatic function between the two lower limbs in primary lymphoedema limit the usefulness of inter-limb ratios for comparison against a lymphoedema threshold reference range (Mayrovitz, 2019a). Furthermore, absolute values were found to have higher reliability than interlimb ratios (Mayrovitz et al., 2019).

2.6.3.1 The MoistureMeterD Compact (MMDC)

The MoistureMeterD Compact (MMDC) converts the TDC to a percentage of water content (PWC) in the calculation:

PWC (%) = 100 x (measured dielectric constant − 1)/ 77.5 (Delfin Technologies, 2016; Nuutinen et al., 2004).

The accuracy of the MMDC is reported to be approximately 3% with a coefficient of variation of less than 5% (Delfin Technologies, 2016). On testing the device against the known standards for water and ethanol, variation has been reported to be closer to 2.5% (Mayrovitz, Corbitt, et al., 2017). The manufacturer recommends calibration tests each two years (Delfin Technologies, 2016), in which the device is tested against the established values for ethanol and water. Any percentage adjustment made to the device may be applied to previous measures and recalculated for consistency across the calibration period (Juha Pärnänen, Delfin Technologies, personal communication, October 9, 2020).

Triplicate measures are advised when using the MMDC for lower limbs (Mayrovitz, 2019b), as the MMDC shows greater variability in PWC in the lower than the upper limb (mean coefficients of variation: 3.22% (foot) and 4.59% (lateral leg), compared with 2.64% (palm of the hand) (Mayrovitz et al., 2019). A standardised position is advised for measures in the foot; the widest area of muscle tissue between bones is between the metatarsals, particularly the first inter-metatarsal space, which avoids bones and major vessels (Mayrovitz, 2019b).

Considering both the depth of the dermis and the depth of penetration of the MMDC is important in understanding what tissue is being measured when using the MMDC. Structures influencing TDC measures vary according to the depth measured, and whether or not lymphoedema is present. Skin thickness varies dependent on body site (Nedelec et al., 2016). The posterior calf was 1.3mm and dorsum of the foot 1.49mm in healthy females (aged 24-41 years, determined using high frequency ultrasound) (Olsen et al., 1995). The skin in lymphoedema is thicker than unaffected

skin (Mellor et al., 2004). Lymphedematous lower limbs show greater average total skin thickness (2.17mm) than in contralateral limbs (1.14mm, using magnetic resonance imaging) and no difference between primary and secondary lymphoedema (Idy-Peretti et al., 1998). Two millimetres has been deemed a threshold for lymphoedema (Arrive et al., 2018), but the threshold varies with site (Dylke et al., 2018). Hacard et al (2014), having examined both upper and lower limbs, reported a range of dermal thickness of 1.1 to 1.3mm in healthy limbs and 2.6 to 2.9mm in the foot and leg below the patella in lymphoedematous limbs.

Given that the MMDC measures to approximately 2mm depth (Mayrovitz et al., 2019) or 2.5mm depth , the tissues being measured will differ between people with and without lymphoedema due to differences in dermal thickness. Therefore, the MMDC is likely to penetrate beyond the dermis to subcutaneous tissue in healthy people, just as the MoistureMeterD (2.5mm depth) is known to do (Nuutinen et al., 2004). In those with lymphoedema in whom the dermal thickness is unknown except that it is greater than in the healthy dermis (Mellor et al., 2004), and the penetration depth of the MMDC is uncertain, the tissues measured are assumed to include relatively less subcutaneous and more dermal tissue than in healthy people.

The relative contribution of dermal fluid to TDC is unclear, but may be inferred from studies measuring TDC at different depths and the relative influence of the adipose content of subcutaneous tissue. Adipose tissue has low water content (Mayrovitz, 2019c), so subcutaneous tissue shows relatively low TDC values (Nuutinen et al., 2004). The relative contribution of the subcutaneous tissue to TDC is greater at depth (Jensen et al., 2012), as indicated in the decreasing TDC values seen at greater depth (Mayrovitz et al., 2008). However, in the lower limb (foot, ankle and lateral calf), BMI has not affected TDC values in healthy people, perhaps due to the low proportion of subcutaneous tissue measured relative to the dermis at these sites (Jensen et al., 2012). In people of high total body fat, TDC measured to a depth of 2.5mm was lower than the median value by 3.3% to 5% in the upper limb (Mayrovitz, 2019c). From this it appears that although BMI did not affect TDC measures, subcutaneous tissue may affect TDC when measured to a depth over 2mm. Given the lack of clarity around the measurement depth of the MMDC (Mayrovitz, 2015) and the variation in dermal thickness dependent on anatomical site and presence of lymphoedema, the exact tissues measured by the MMDC is uncertain.

Age has been found to have little impact on TDC at depths greater than 2mm (Jensen et al., 2012;

Mayrovitz, Grammenos, et al., 2017), whereas at depths up to 1.5mm, TDC increased significantly with age (Mayrovitz, 2010). Depths to 1.5mm may include the dermis alone dependent on site (Nedelec et al., 2016) and increased TDC at such depths is consistent with increased fluid in the dermis seen by ultrasound in those of greater age (Gniadecka, 2001). Therefore, measures taken in a young population and using the MMDC to a depth of 2-2.5mm avoids the effect on TDC of increased dermal fluid with age.

2.6.4 The Indurometer: A Measure of Tissue Resistance

The Indurometer is an electronic hand-held instrument which produces a measure of 'Induration Units' (IU) (Flinders University Biomedical Engineering, 2013; Pallotta et al., 2011). A six-centimetre disc sits on the tissues; on downward pressure by the operator, a small plunger protrudes through the disc into the tissues, until the standardised force of 200g is reached. The device requires daily calibration to a 200g mass (Flinders University Biomedical Engineering, 2013). The lower the IU, the less distance into the tissue the plunger has travelled, equating to stiffer tissue, whereas a high IU reading indicates softer tissues. The pressure of the Indurometer is not held for long enough to cause a pitting-like indentation, but may have effect on subsequent measures (Douglass, Graves, & Gordon, 2017).

The mean coefficient of variation of the Indurometer investigated in lower limb primary lymphoedema was 7.5% and 6.7% in the normal population (Phillips & Gordon, 2016). Further, the intraclass correlation coefficients (ICC) of repeated measures taken on the same day by the same operator were generally good to excellent in both primary lymphoedema (range 0.893 to 0.971) and healthy lower limbs (0.844 to 0.976). Although there were only three people under 45 years of age in the latter study cohort, Douglass, Graves and Gordon (2017) reported excellent reliability in the posterior calves of healthy adolescents in Australia and Myanmar also for measures taken on the same day. (Comparative results for the posterior calves of both studies are in **Table 2.2**).

Table 2.2 Indurometer Reliability Studies

Table 2.2 Indurometer Reliability Studies			
Study / population	**Douglass et al (2017)** **Healthy adolescents n=34** **Mean age 15y**	**Phillips & Gordon (2016)** **LL Primary n=16** **Mean age 47y**	**Phillips & Gordon (2016)** **Healthy population** **n=14** **Mean age 38y**
CoV **Mean (range)**	**30.1%**	**7.5% (Range: 5.4 – 9.9) dependent on site**	**6.7% (Range: 5.3 – 9.7) dependent on site**
ICC (95%CI)	**0.937 (0.901, 0.972) Dominant calf** **0.921 (0.877,0.965) Non-dominant calf**	**0.893 (0.746, 0.961) Posterior calf (R)** **0.925 (0.826, 0.972) Posterior calf (L)**	**0.877 (0.697, 0.957) Posterior calf (R)** **0.844 (0.618, 0.946) Posterior calf (L)**

CoV, coefficient of variation; ICC, Intraclass correlation coefficient.

As lymphoedema progresses, fluid accumulation is associated with fibrosis and disrupted collagen matrix (Rockson, 2001, 2010), which causes concomitant tissue stiffness (Sano et al., 2019). Lack of indentation in tissues may result from the unimpressionable 'toughness' of the skin due to fibrosis, but fluid filled structures can also be a cause of stiff tissues (Belgrado et al., 2010). This suggests that an Indurometer would indent less into lymphoedematous tissues, whether fluid-filled or fibrotic, but previous studies show conflicting results. Sano et al (2019) hypothesised that as the stage of lymphoedema progressed (and tissue induration or fibrosis progressed) so an 'Indentometer' would indent less into the tissue. However, the reverse was seen in their study cohort of mainly women (84%) mostly with secondary lymphoedema (72%), where there was a tendency for an Indentometer to indent further into the tissue of the anterior thigh in more advanced stages of lymphoedema. Furthermore, there was no association between stage and indent distance, despite the decreased number of elastic fibres identified histologically in lymphoedematous skin (Sano et al., 2019). However, Sano et al (2019) used an index of thigh to forearm to indicate induration, in contrast to an earlier study which reported a positive association between skin stiffness and stage of lymphoedema (Sun et al., 2017). Sun et al (2017) used the mean of 20 sites across the lower limb as the indicator of skin stiffness using a Skin Fibrometer, an alternative form of indentation, in a cohort of ISL stages I, II and III secondary lymphoedema. The inclusion of so many sites across the limb may have contributed to this significant finding,

particularly as 12 of 20 sites were below the knee, where tissue resistance is greater than in the thigh (Douglass et al., 2018). Tissue resistance measured by indentation, or indurometry, remains unclear in primary lymphoedema, particularly the response of indurometry to compression.

2.7 High Frequency Ultrasound

High frequency ultrasound (HFU) has been used extensively by dermatologists (Olsen et al., 1995; Serup, 1992; Tan et al., 1982; Waller & Maibach, 2005) to provide a quick and non-invasive assessment of skin condition and its pathologies specific to dermis or epidermis. Although HFU is not common in lymphoedema therapy practices (Mander et al., 2019), ultrasound has been useful to identify differential patterns of fluid distribution within the dermis in different conditions (Iker et al., 2019; Liu et al., 2017; Naouri et al., 2010). Ultrasound is increasingly being advocated for investigation of the tissues in lymphoedema (Cavezzi, 2018; Johnson et al., 2015; Ricci et al., 2021), to assist diagnosis (Erdinc Gunduz et al., 2021), measure the dermis and subcutaneous tissues (Mander et al., 2019; Mellor et al., 2004; Suehiro et al., 2013) and assess response to treatment (Hacard et al., 2014). HFU at 20MHz focuses superficially, producing images that are particularly useful for measuring the dermis (Schuetzenberger et al., 2019; Serup et al., 2006), in contrast to those using lower frequencies (e.g., 7.5MHz) which examine the subcutaneous tissue (Kim et al., 2021). A water-based gel is used as an interface between the skin and the ultrasound probe, and may be applied in a spacer, standardising the distance from the skin and therefore the depth of focus (Serup et al., 2006).

Where tissue changes in density, sound is reflected to different degrees. In HFU images, this is displayed as areas differing in brightness or echogenicity (Serup et al., 2006). This results in the dermis being distinct from the epidermis above and the subcutaneous tissues below (see **Figure 2.3**). Fluid has little variation in density thus its low echogenicity may easily be detected on HFU images (Gniadecka, 2006; Gniadecka & Quistorff, 1996), showing as black. In contrast, tissue which changes in density, for example where collagen is present, echogenicity becomes variable or speckled (Kleinerman et al., 2012). The relative uniformity of the echogenicity of fluid, appearing black, facilitates its measurement. In **Figure 2.3**, it may be seen that the uniformity of the subcutaneous tissue results in a consistent black appearance, whereas in contrast, the variable density of collagen in the dermis gives it a speckled appearance.

The dermis of people with lymphoedema show greater fluid representation on ultrasound images

compared with dermal images of people without lymphoedema (Gniadecka, 1996). Measures of echogenicity have been used to identify lymphoedema (Iker et al., 2019) and the distribution and relatively greater fluid content in the dermis in lymphoedema enabled lymphoedema to be distinguished from lipoedema in a blinded ultrasound image assessment (Naouri et al., 2010).

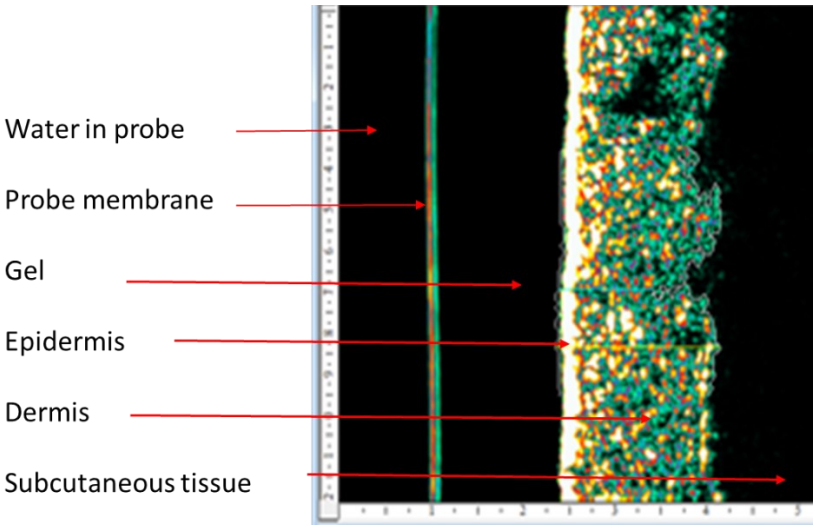


Figure 2.3 Image of Dermis in Healthy Skin Using the DermaScan C High Frequency Ultrasound

The DermaScan C is a 20MHz ultrasound used in many dermatological studies (Caetano et al., 2015; Gniadecka, 2006; Seidenari, 2006; Serup et al., 2006), in which echogenicity is allocated a measure of brightness on a spectrum from 0-255. Consequently, the area (in pixels) of a chosen echogenicity or brightness may be measured, such as the echogenicity representative of fluid. However, echogenicity varies with many factors other than the presence of fluid: mechanical factors (such as ultrasound gain setting, see **Figure 2.4**) (Seidenari et al., 1994; Serup et al., 2006), age (Gniadecka, 2001; Lasagni & Seidenari, 1995; Lee et al., 2016; Seidenari et al., 1994) and differences in body tissue, dermal thickness and site (Nedelec et al., 2016; Olsen et al., 1995).

Age-related changes in echogenicity may be variably present, dependent on whether the site is sun exposed or not (Gniadecka & Jemec, 1998). Proteoglycans, glycosaminoglycans and collagen fibres vary in sun-exposed dermis (Crisan, Lupsor, et al., 2012; Lee et al., 2016), which all affect echogenicity (Crisan, Crisan, et al., 2012). Both inflammatory changes and increased dermal collagen fibres have been seen in the dermis in lymphoedema (Di et al., 2016; Domaszewska-Szostek et al., 2016; García Nores, Ly, Cuzzone, et al., 2018; Hara et al., 2016; Ly et al., 2017) and episodes of cellulitis can change the structural nature of the dermis and hence ultrasonic properties (Dai et al., 2016). Variations in echogenicity between the upper and lower dermis

become more pronounced after the fourth decade (Gniadecka, 2001). As well, variations occur according to body site (Nedelec et al., 2016; Olsen et al., 1995). For example, dermal echogenicity is lower on the dorsal foot compared with posterior leg in young healthy people (24-41 years) (Olsen et al., 1995).

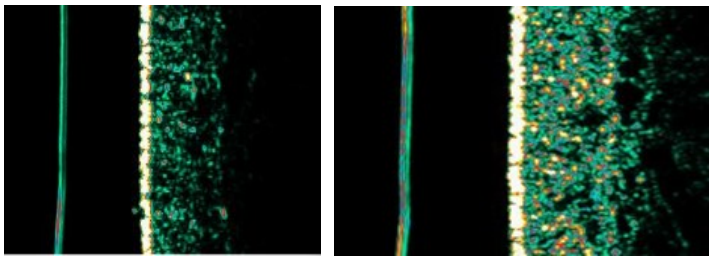


Figure 2.4 Variation in Echogenicity at the Same Site Using Different Gain Settings.

Left: low gain. Right: high gain.

Echogenicity has also been found to vary over the course of a day, although this appears to be associated with the effect of gravity. The dermis was found to decrease in echogenicity over a day in the leg and thigh of young people (aged 17-27yrs) (Tsukahara et al., 2001). However, no change in echogenicity was found in a control group lying supine for 12 hours (Gniadecka, Serup, et al., 1994). Therefore, echogenicity is expected to remain stable with respect to gravity during measurements taken in supine.

2.7.1 Echogenicity Outcome Measures

Studies investigating dermal fluid have used low echogenic pixels (LEP) alone (Gniadecka & Quistorff, 1996; Gniadecka, Serup, et al., 1994) or as part of a ratio of LEP to total pixels in the dermis (giving the area representative of fluid out of the total area of the dermis) (Gniadecka & Jemec, 1998; Gniadecka et al., 1998; Schou et al., 2004). The first method, the LEP count from the whole dermis imaged has been commonly used in studies using the DermaScan C (Crisan, Crisan, et al., 2012; Crisan, Lupsor, et al., 2012; Gniadecka, 2001; Gniadecka, Serup, et al., 1994; Seidenari et al., 1994). However, dermal thickness varies from person to person which affects the area-based comparative measures of LEP from the whole dermis (Gniadecka et al., 1998; Schou et al., 2004). To account for differences in area, Gniadecka and colleagues used two other methods, 1) a ratio of LEP against the total pixel count (Gniadecka & Jemec, 1998; Gniadecka et al., 1998) and 2) divided the dermis into equal halves longitudinally and used a ratio of LEP in the upper to LEP in the lower dermis (Gniadecka, 2001). Both methods involve outlining the dermis, a process that was found to have variable reliability when using HFU, due to the uneven nature of the subdermal

boundary (Naouri et al., 2010; Seidenari et al., 1994).

A fourth method involves setting a standardised area, or region of interest (ROI), and extracting the low echogenic pixels (LEP) in that ROI. The average intensity can also be extracted from the total area of the ROI, which represents the echogenicity in the rest of the dermis. These two measures have been used as a ratio of the segmented LEP to the total ROI intensity (Tsukahara et al., 2001; Veen et al., 2001). However, the echogenicity in the total area of the ROI would vary between individuals due to variation in collagen and such factors as aging (Crisan, Lupsor, et al., 2012; Gniadecka, 2001), photo-damage from sun exposure (Gniadecka & Jemec, 1998) and anatomical site (Ploin et al., 2011; Seidenari et al., 1994), as previously discussed. This would affect the denominator of such a ratio to a greater extent than LEP, which is set to a known range that is attributed to fluid. Therefore, even when controlling for HFU settings and personal factors such as age, both of which may affect echogenicity (Crisan, Lupsor, et al., 2012; Gniadecka, 2001; Seidenari, 2006; Seidenari et al., 1994), a range of echogenicity is seen in different people. This would affect the total intensity of the dermis, but not the segmented area. Hence, a measure of LEP from a segmented ROI provides opportunity to compare echogenicity related to dermal fluid between groups, with the least potential variation due to unrelated factors.

2.7.2 Utilising High Frequency Ultrasound in Lymphoedema

Previous ultrasound investigations of lymphoedema in the leg have examined the subcutaneous tissues (Lee et al., 2020; Niimi et al., 2014; Suehiro, Morikage, Ueda, Samura, Takeuchi, Nagase, Mizoguchi, & Hamano, 2018; Suehiro, Morikage, et al., 2018a, 2018b; Suehiro, Morikage, Yamashita, Samura, et al., 2017) and mixed primary and secondary lymphoedema, in which primary lymphoedema is in the minority (Hacard et al., 2014; Lee et al., 2020; Suehiro et al., 2013; Suehiro, Morikage, Yamashita, Harada, et al., 2017; Suehiro, Morikage, Yamashita, Samura, et al., 2017). Other studies do not report the cause of lymphoedema (Iker et al., 2019; Naouri et al., 2010).

No HFU studies have previously reported echogenicity in the dermis specific to primary lymphoedema. The dermis is known to show low echogenicity in the distal lower limb, when comparing the ankle, calf and thigh to corresponding sites in the healthy dermis (Iker et al., 2019; Naouri et al., 2010). However, neither of these studies specify what type of lymphoedema was investigated and the foot has not been included (Iker et al., 2019; Naouri et al., 2010). In an earlier

study of dermal echogenicity, neither the site nor the type of lymphoedema were described (Gniadecka, 1996). Yet fluid is known to accumulate in the dermis of the foot in primary lymphoedema specifically (Sarica et al., 2019). As well, the presence of fluid in the dermis of the foot relates clinically to the presence of skin changes in the foot, such as the Stemmer sign (Goss & Greene, 2019) and the disruption caused by fluid to the extracellular matrix, collagen and elastic fibres (Carlson, 2014; Daroczy, 1995; Eisenbeiss et al., 2001; Rockson, 2001, 2010; Szuba & Rockson, 1997).

There is a similar lack of data on primary lymphoedema in studies using ultrasound to investigate the response to treatment. Studies utilising ultrasound to investigate the outcome of complex decongestive treatment (CDT) report differences in echogenic response between the upper and lower limbs. Suehiro et al (2019) found that a subcutaneous echo-free space (SEFS), indicative of fluid accumulation, also indicated responsiveness to therapy which included compression, in the upper limb, compared to those with no SEFS (Suehiro, Morikage, et al., 2019). Responsiveness to therapy was measured by volume change after treatment with CDT for over a year. However, in the lower limb, lymphoedema reduction was not limited to those with SEFS, where volume reduction was found even in those without an SEFS (Suehiro, Morikage, Ueda, Samura, Takeuchi, Nagase, Mizoguchi, & Hamano, 2018). Consequently, only studies investigating response to treatment in the lower limb are described here.

Changes in in response to treatment of the lower limb have been investigated using HFU following various aspects of complex decongestive therapy (CDT) in either secondary lymphoedema populations (Niimi et al., 2014) or a mix of primary and secondary (Hacard et al., 2014; Suehiro, Morikage, Ueda, Samura, Takeuchi, Nagase, Mizoguchi, & Hamano, 2018). Multiple treatment strategies of CDT have been applied to a limb over periods varying from days (Hacard et al., 2014) to a month (Niimi et al., 2014). The subcutaneous tissues are commonly the focus for outcome measures (Niimi et al., 2014; Suehiro, Morikage, Ueda, Samura, Takeuchi, Nagase, Mizoguchi, & Hamano, 2018). When the skin is the focus, outcome measures commonly include thickness (Hacard et al., 2014).

Dermal thickness is associated with fluid accumulation in lymphoedema (Mellor et al., 2004; Rockson et al., 2018), so a reduction in dermal thickness may indicate reduction in the fluid accumulation in the dermis. Hacard et al (2014) report a mean reduction in dermal thickness of

15.1% (SD 12.3) following five days of intensive treatment (manual lymphatic drainage, pneumatic compression and bandaging). However, this was a mean value across sites and included both upper and lower limbs with primary or secondary lymphoedema (Hacard et al., 2014).

Therefore, little is known about fluid in the dermis in lower limb primary lymphoedema and there has been no measure of the actual physiological impact of treatment on the fluid content of the dermis.

2.8 Correlation Among Measures of Lymphoedema

No studies have investigated primary lymphoedema exclusively for correlation among measures. Significant associations have been found between fluid measures in mixed groups of primary and secondary, where primary is very much in the minority, or secondary lymphoedema alone. Correlation between bioimpedance (ECF/ICF) and grade of echogenicity (subcutaneous echo-free space, SEFS) grade, ($r=0.67$, $p<.05$) were reported in the thigh and leg in lymphoedema cohort of which only 4 of 68 were primary (Suehiro, Morikage, Yamashita, Harada, et al., 2017). However, grading of echogenicity in subcutaneous tissue appears to be reliant on subjective assessment of ultrasound images (Suehiro et al., 2013), and no blinding of assessors is described in the early establishment of this method (Suehiro et al., 2014).

Although no conclusions can be drawn about correlations with SEFS until reliability of this method is established, further observations of SEFS are relevant. Fluid is associated with both SEFS (which is not seen in healthy limbs) and a raised ECF/ICF ratio, yet Suehiro and colleagues (2017) noticed an association of raised ECF/ICF with SEFS grade 0 (which should indicate healthy limbs, or normal subcutaneous tissue fluid), in the thigh of those with lymphoedema. Suehiro and colleagues surmise that this rise in interstitial fluid may result from an inflammatory component, as yet untested. If so, fibrotic changes from that inflammatory component may affect tissue resistance from early in lymphoedema development, as noted in a cohort infected with lymphatic filariasis but showing no other signs of lymphoedema (Douglass, Graves, Lindsay, et al., 2017). Further investigation by the same group showed significant associations between lower limb volume and bioimpedance in predominantly secondary (44 of 49; five were primary) lower limb lymphoedema ($r=0.67$) (Suehiro, Morikage, et al., 2018a).

A strong correlation between volume change and ECF (R_0) change in the leg of healthy participants

was found over a six-hour period of normal activity (sitting, reading) ($r = -0.79$, $p<0.001$) (Taniguchi et al., 2021). Interestingly, no changes in the volume of the thigh were observed over the same period, suggesting a gravitational effect. The distribution of extracellular fluid in the leg compared to the thigh has been attributed to gravity by bioimpedance in both lymphoedema and healthy limbs (Suehiro, Morikage, Yamashita, Harada, Ueda, et al., 2016).

Similarly, moderate to good correlations have been identified between local tissue water measured by TDC and dimensional measures: inter-arm TDC ratio with inter-limb volume difference ($r = 0.644$, $p=0$) (Y. Liu et al., 2021). Reductions in PWC (TDC) and circumference measures at the calf following complex decongestive therapy (CDT) were also correlated ($r=0.71$, $p=0.002$) (Tugral et al., 2018). However, the above dimensional associations contribute little to understanding dermal fluid distribution in primary lymphoedema.

2.8.1 Correlation of Lymphoedema Measures with Stage of Lymphoedema

Objective measures of tissue alterations in lymphoedema that relate to the stage of lymphoedema are missing (Johnson et al., 2014; Tassenoy et al., 2016). This lack of a measure of the relative contribution of fluid or fibrosis means there is no method to track the gradual change in severity of lymphoedema, as fluid accumulation increasingly converts to fibrosis. The stage of lymphoedema which classifies the fibrotic change in lymphoedematous tissues and is accessible and applicable clinically (International Lymphoedema Framework, 2006), lacks quantification by a physiological measure, instead relying on visual assessment and palpation. Various measures of lymphoedema provide information of quantified physiological difference between limbs with and without lymphoedema. Correlation between such assessment measures and the stage of lymphoedema elucidates the relative contribution of fluid or fibrosis in the tissues which relates clinically to the severity of lymphoedema.

Fluid accumulation has long been identified in the early stages of lymphoedema (ISL stages 0, I and early II) by bioimpedance measures of extracellular fluid (ECF) (Cornish, Chapman, et al., 2000; Koelmeyer et al., 2019), but objectively measuring tissue fibrosis has been challenging. The beginning of fibrotic tissue change is described in ISL Stage II, dividing this category into two: early and late Stage II (International Lymphoedema Framework, 2006). To identify this change with an objective measure of tissue resistance or fluid distribution in the dermis would be useful clinically. Once identified, treatment methods may be adapted to address tissue resistance or fluid

accumulation.

Clinical staging of lymphoedema (ISL) has been compared with inter-limb volume difference, showing a moderate correlation of r=0.579 (p<0.001) (Garza et al., 2019). Interestingly, ISL staging had weak correlation with ICG lymphography staging (based on vessel patency and dermal backflow) r=0.254 (p<0.001) (Garza et al., 2019), suggesting that vessel abnormality is not an indicator of tissue change. Consistent with the latter findings, no correlation was found between ICG lymphography staging and inter-limb volume difference (Coroneos et al., 2019).

Localised tissue water (TDC) showed moderate significant correlation with ISL stage (r=0.571, p=0.000) in a cohort of early stage upper limb lymphoedema (Y. Liu et al., 2021). In contrast, weak correlation of localised tissue water (percent water content, PWC) with ISL stage (rho=0.25) was found in a mixed cohort of upper and lower limb secondary lymphoedema, despite finding moderately strong correlations of the clinical stage of lymphoedema with the excess ECF (rho=0.60) as well as with skin stiffness (rho = 0.63, using a Skin Fibrometer) (Yu et al., 2019). However, differences in the distribution of intracellular and extracellular fluid have been described between the upper and the lower limb (Suehiro, Morikage, et al., 2018a), which suggests that a limb-specific study is appropriate when investigating extracellular fluid using bioimpedance. Nevertheless, the abovementioned study demonstrated the association of PWC with stage of lymphoedema weakened in later stages of secondary lymphoedema, suggested to be due to PWC measuring relatively less as fluid accumulation progressively affects deeper tissue (Yu et al., 2019). In contrast, the association of whole limb measure of bioimpedance with stage remained strong in later stage secondary lymphoedema (Yu et al., 2019). This appears to indicate that the measure of fluid accumulation by bioimpedance throughout the segment remains relative to stage, whereas the point measure of fluid accumulation by PWC may be limited by penetration depth. Similar descriptions of segmental fluid measures by bioimpedance and point measures of percent water content in primary lymphoedema are not available.

An index of induration against age was devised between healthy forearm skin and lymphoedema-affected skin in the lower limb, but no correlation was found with ISL stage (Sano et al., 2019). In the latter study, elasticity was also tested, using a suction device (lifting the skin), whereas induration was tested by a pressure device (indenting the skin). The elasticity to age index tended to increase with stage in lymphoedema-affected skin, but there was no change in the induration to

age index. The change in elasticity over time indicates a change in stiffness, which was not detected by indenting the skin. However, elasticity is rarely used, and is a measure for which currently there are no assessment tools used by lymphoedema therapists. The findings of the latter study may also be explained by the application of pressure to lymphoedematous tissues being affected by other factors than dermal properties alone, such as the resistance of underlying tissue (Douglass, Graves, & Gordon, 2017).

Recently, a strong correlation was found between skin stiffness and stage in lower limb lymphoedema ($r=0.9$, $p<0.01$) (Sun et al., 2017), but combined, averaged values across five sites on the lower limb were assessed, masking the relative distribution of tissue resistance. As well, more than half of the cohort had more advanced (late stage II or stage III) lymphoedema, in which tissue changes are more marked. Staging was supported in the latter study by histologically prepared skin sections: greater collagen content was seen in those with lymphoedema than in the healthy dermis, and collagen content was also seen to increase with ISL stage (Sun et al., 2017).

To detect local tissue changes in relative fluid versus fibrosis in the dermis, Liu et al (2021) suggested that a location-specific tool, that can detect fluid changes over small zones within a limb, may be needed. Tissue outcome measures from the DermaScan C HFU (echogenicity), the MoistureMeterD Compact (PWC) and the Indurometer (IU) provide such location-specific information to different depths. (In contrast, bioimpedance (ECF) provides extracellular fluid information throughout all tissues in a limb or segment through which the current travels.) Association between such location-specific measures provide information about the degree to which they represent similar constructs. An association of a commonly used lymphoedema measure with a dermal fluid measure from high frequency ultrasound could provide information to clinicians about the relative fluid or fibrotic state of the dermis, with clinical implications for treatment of lymphoedema.

2.9 Treatment

Management of lymphoedema is aimed at promoting lymph movement and reducing lymph accumulation (International Lymphoedema Framework, 2006; Mortimer & Levick, 2004; Mosti & Cavezzi, 2019; Vignes et al., 2021). Reduced volume or circumference in the affected limb is the aim of treatment (Mosti & Cavezzi, 2019; National Lymphedema Network, 2011a).

Four main strategies are used for lymphoedema management: compression, manual lymph drainage, skin care and exercise (International Lymphoedema Framework, 2006; International Society of Lymphology, 2020). The first three of these strategies are applied directly to or via the skin. Skin care is aimed at reducing the risk of cellulitis due to breaches in skin integrity, such as in wounds or fungal infections that result in increased swelling (Armer et al., 2013; National Lymphedema Network, 2011a). Tough thickened and inelastic skin, as found in later stage lymphoedema, resists both compression and manual lymph drainage (Bagheri et al., 2005; Didem et al., 2005; Ezzo et al., 2015; McNeely et al., 2004; Ramos et al., 1999; Wozniewski et al., 2001).

Limb elevation is advised (International Lymphoedema Framework, 2006), and even supine lying effectively 'elevates' both lower limbs by counteracting gravity, lowering capillary filtration pressure (Mortimer & Levick, 2004). Lymphangion pumping in supine continues the necessary propulsion of lymph centrally, even though the pumping rate of lymphangions is lower in supine lying than in the upright posture (Olszewski & Engeset, 1980). The effect of elevation in decreasing dermal oedema has been demonstrated in venous oedema: a 15.6% decrease in dermal oedema in the leg was found following three to four hours of leg elevation (Xia et al., 2004). In healthy aged adults, there is an increase in dermal fluid after being upright for two hours, and no change in dermal fluid over 12 hours of lying supine in a mixed age group (age 17-83; median age 18) (Gniadecka, Serup, et al., 1994). The dermal response in primary lymphoedema to either compression or elevation is unknown.

Little data are available describing the effect of elevation on dermal fluid in any type of lymphoedema. Generally, elevation is described as reducing fluid accumulation in the limbs in stage I lymphoedema, has some effect in early stage II, but limited reduction is seen with elevation in late stage II and stage III (Gordon & Mortimer, 2018; International Society of Lymphology, 2020). The lower capillary filtration pressure during elevation (Mortimer & Levick, 2004) influences osmotic pressure and lymph drainage (Solari et al., 2020), thereby reducing oedema formation (Gordon & Mortimer, 2018). When changing position from supine to upright, no change in intra-lymphatic vessel pressure is seen in the healthy foot but the rate of lymphangion contraction increases (Olszewski & Engeset, 1980). The effect of this orthostatic change in primary lymphoedema is unknown, but may possibly cause increased dermal backflow due to impaired inter-lymphangion valves in some forms of primary lymphoedema. During investigation of dermal fluid in supine lying, the possible effects of orthostatic change on echogenicity are avoided.

A decrease in dermal backflow has been observed following decongestive therapy, along with a reduction in intra-lymphatic vessel pressure, but this was in in a mixed group of primary and secondary lymphoedema, after months of treatment (Franzeck et al., 1997). Pressure within the 'micro-lymphatic' vessels is higher in primary lymphoedema (15.0+5.1mmHg) compared to that in healthy vessels (7.9+3.4mmHg, $p<0.001$) in supine (Zaugg-Vesti et al., 1993), but it is unknown what impact high intra-vessel pressure may have on response of dermal fluid uptake to compression.

Change in the contralateral limb must be considered when assessing the effect of treatment, which may instead be at least partly due to elevation. A decrease in volume observed post treatment in a contralateral untreated limb could indicate that significantly less volume change in the treated limb should be attributed to treatment (Mayrovitz et al., 2007).

2.9.1 Compression

Compression, which may be applied via bandages, adhesive wraps, garments, or pneumatic compression devices is applied in both the intensive reduction phase of treatment for lymphoedema, as well as for long-term maintenance of limb size (Armer et al., 2013; International Lymphoedema Framework, 2006; International Society of Lymphology, 2020; Vignes, 2015; Vignes et al., 2021). The modality chosen depends on the aims and phase of treatment, along with client characteristics (Bjork & Ehmann, 2019; International Lymphoedema Framework, 2006), such as the inability to tolerate bandaging resulting in use of the adjustable adhesive wraps, or the inability to don compression garments resulting in regular use of an intermittent pneumatic compression device.

Compression takes effect by increasing interstitial pressure, thereby reducing the rate of capillary filtration from the circulatory system and promoting uptake of fluid into the initial lymphatic capillaries (Mortimer & Levick, 2004; Mosti & Cavezzi, 2019). Externally applied compression, enclosing the limb, combines with skeletal muscle contraction to further increase interstitial pressure (Mortimer, 2010).

There is little previous work investigating the effect of compression on the dermis, particularly echogenicity in the dermis post-compression in lymphoedema. Change in echogenicity following treatment has been assessed mainly in the subcutaneous tissues (Niimi et al., 2014; Suehiro, Morikage, Ueda, Samura, Takeuchi, Nagase, Mizoguchi, & Hamano, 2018; Ueda-Iuchi et al., 2015)

whereas studies of the dermis have focussed on reduction in dermal thickness, as seen following five days' CDT (Hacard et al., 2014). Hacard et al (2014) reported the mean dermal thickness decreased by 15.1% (mean percentage change across three sites: above and below the patella, and the dorsum of the foot). The corresponding mean 4% leg volume decrease indicated that a greater percentage change occurred in the dermis than in the overall leg volume. Although ultrasonic dermal thickness measures correlate with circumferential measures, which may assist in diagnosis (Erdinc Gunduz et al., 2021), some caution may be required in the use of ultrasound measures to assess post-treatment change, based on results from other types of oedemas. For example, in venous oedema, dermal thickness has been reported to increase significantly post-elevation, in conjunction with decreased circumferences, with relatively increased echo density in the dermis, seen as increased echogenicity (Xia et al., 2004). This suggests a change similar to that which happens for instance to a rubber band, which, no longer being stretched around so great a circumference, resumes a shorter, but thicker appearance. Feasibly, if dermal thickening and corresponding increased echogenicity occurred in tandem with decreased circumferences post-compression in lymphoedema, any decrease in fluid that may occur as a result of compression may be offset by the relative increase in echogenicity. However, the effect on echogenicity may not apply to lymphoedema, as venous oedema has different patterns of dermal echogenicity (Gniadecka, 1996) which may respond differently to compression. Similar skin thickening with compression has been observed under compression garments in upper limb lymphoedema (Karakashian et al., 2019). In contrast to the latter study, dermal thickness reduction was reported following treatment in upper limb lymphoedema; however, compression was combined with manual lymph drainage (Uzkeser et al., 2015), with its variable pressure and skin stretch, which possibly added extra effect on the initial lymphatics in the dermis.

Decongestive treatment has been shown to reduce both intra-luminal pressure and the diameter of lymph vessels in a mixed group of primary and secondary lymphoedema (Franzeck et al., 1997). However, changes in vessel pressure and diameter were found after two weeks of intensive combined physical therapy which consisted not only of compression and exercise, but also manual lymph drainage which induces pressure variation in the dermis and increased lymph movement in collecting vessels (Lopera et al., 2017; Olszewski & Engeset, 1980). Tugral et al (2018) reported that thigh, leg, and ankle TDC measures decreased ($p<0.01$) post-treatment in lymphoedema of mixed cause (11 primary and six secondary). Treatment comprised skin care, MLD and exercise as

well as compression, and response was assessed after four weeks (Tugral et al., 2018) providing no information regarding the specific response to compression of fluid measures in primary lymphoedema.

Studying the effect of compression is complicated by variability in application and type of compression. Pressure applied via bandaging or adhesive wraps is inconsistent. During bandaging, the amount of pressure applied varies between therapists (Hara et al., 2020; International Lymphoedema Framework, 2012), and few apply the pressure they are aiming for (Hara et al., 2020). In addition, the shape of the limb affects the amount of pressure applied, depending on the curve of the anatomical site. A limb section with a small radius experiences greater inter-face pressure (applied by the bandage to the surface) than that experienced on a surface with a large radius (Chassagne et al., 2017). This is in accordance with Laplace's Law which states that pressure exerted on a curved surface is inversely proportional in part to the radius of the curved surface to which it is applied (International Lymphoedema Framework, 2012; Troynikov et al., 2010). Further variability in bandaging is dependent on the elasticity of the bandaging material and the friction between its layers (Chassagne et al., 2020).

Compression applied via garments also varies in the amount of pressure applied (Lurie & Kistner, 2014; Ma et al., 2015). Compression garments are classed by the pressure they apply to a limb (graded in mmHg) across a range of pressures. The class of compression applied depends on the presentation and needs of the individual (International Lymphoedema Framework, 2006) and therefore will vary across a group with primary lymphoedema. As well, the stiffness of the material affects the garment's ability to maintain its shape and pressure over time (Chassagne et al., 2020; Partsch, 2012). As is the case in bandaging, the shape to which a garment is applied also affects the pressure transmitted to the body (Karakashian et al., 2019; Troynikov et al., 2010), resulting in variable pressure depending on body shape. Even methods of care, such as how garments are laundered, can affect the compression applied by a garment over time (Australasian Lymphology Association, 2021).

In contrast, pneumatic compression devices can be set to apply a consistent dosage of pressure for a set period of time. Pneumatic compression has been shown to increase fluid movement through superficial vessels, seen using ICG fluoroscopy and a transparent pneumatic sleeve (Adams et al., 2010; Kitayama et al., 2017; Zaleska & Olszewski, 2017). Interestingly, Adams et al (2010) found

lymphatic activity increased on the untreated side as well as that under pneumatic compression, confirming the importance of attention to the untreated side (Mayrovitz et al., 2007).

Drainage of fluid away from a congested limb is facilitated by a gradient of pressure over a limb, with the greatest pressure applied distally, reducing towards the root of the limb (European Wound Management Association, 2005; Flour et al., 2013; Xiong & Tao, 2018). Although not all compression garments deliver this gradient (Reich-Schupke et al., 2009), a graduated pressure is not as important when wearing compression garments during active movement—when muscular contraction generates high pressures (Partsch & Mani, 2019)—as it is in supine lying (Partsch, 2012). Consequently, compression using IPC, which is commonly applied in supine, utilises a distal to proximal pressure pattern (International Lymphoedema Framework, 2006).

Transmission of pressure during IPC has been measured in the subcutaneous tissues and associated lymph movement demonstrated under ICG lymphography (Zaleska & Olszewski, 2018). Variation in pressure within the subcutaneous tissues promotes lymph movement through collecting vessels, and allows for refilling of lymphangions during periods of less pressure (Belgrado et al., 2016; Ikomi & Schmid-Schönbein, 1995; Solari et al., 2020). Such variation in pressure is applied by the inflation and deflation cycle of an intermittent pneumatic compression (IPC) device (Zaleska et al., 2013). Although less pressure was transmitted within the tissues than was applied externally, intermittent pneumatic compression was effective in reducing circumferences in lower limb lymphoedema (Zaleska & Olszewski, 2017). However, the effect of pressure transmitted by IPC on the dermis, and the impact on dermal fluid in particular, is not clear.

Little is documented about the response of the dermis and subcutaneous tissues to compression in primary lymphoedema, therefore the impact of the vessel and fluid drainage abnormalities of primary lymphoedema on treatment by compression is not understood. Early investigations of the impact of compression on dermal echogenicity were reported in lipodermatosclerosis, where a decrease was found in dermal oedema following compression garment use over five days, measured using a 20MHz HFU (Gniadecka et al., 1998). Lipodermatosclerosis differs from primary lymphoedema in that there is venous stasis, extravasation of erythrocytes and haemosiderin-laden cells in the dermis in lipodermatosclerosis, as well as necrotic adipocytes in the subcutaneous tissues (Choonhakarn et al., 2016). Although fibrosis is present in both conditions, in

lipodermatosclerosis the pathological fibrotic changes result in the hardening of the dermis and subcutaneous tissues around the leg, narrowing this area in a characteristic 'inverted champagne bottle' appearance (Choonhakarn et al., 2016). In contrast, the leg and ankle are enlarged in primary lymphoedema (Sarica et al., 2019). Despite this chronic hardening of the dermis in lipodermatosclerosis, Gniadecka and colleagues (1998) demonstrated a decrease in dermal echogenicity, indicating decreased dermal fluid, following compression. This may indicate that compression has effect on the dermal uptake of fluid, but nothing has previously been reported about the effect of compression on dermal echogenicity in primary lymphoedema.

It is widely accepted that lymphoedema responds more successfully to treatment early in its development when fluid accumulation is relatively greater than the fibrosis (International Society of Lymphology, 2020; Shah et al., 2016; Stout Gergich et al., 2008). Stiffness renders tissues resistant to conservative treatment (Bagheri et al., 2005; Brorson, 2012; Brorson, 2015; Warren et al., 2007; Yu et al., 2019). Although little is known about the mechanism (European Wound Management Association, 2005), compression is acknowledged to assist in softening tissues in lymphoedema (European Wound Management Association, 2005; International Lymphoedema Framework, 2012; Mosti & Cavezzi, 2019). As limb volume reduces, fluid in the subcutaneous tissues and dermis reduces (Grada & Phillips, 2017; Johansson et al., 2019; Mortimer & Levick, 2004; Tugral et al., 2018; Zaleska & Olszewski, 2018; Zasadzka et al., 2018), as well as pain and discomfort (Desai et al., 2020), which is associated with primary lymphoedema (Okajima et al., 2013). However, although a change in tissue stiffness was found in deep subcutaneous tissues following compression, along with volume reduction, there was little change in the dermal stiffness, except at the ankle (Zaleska & Olszewski, 2018). All participants had mid to high stage lymphoedema which was secondary to infection, suggesting a high degree of fibrosis or tissue stiffness at baseline (Zaleska & Olszewski, 2018). The contralateral limb was used as the 'healthy' comparator, and baseline measures showed little difference between limbs except at the ankle. Tissue fluid pressures appear to be relatively low (21-28mmHg) in the presence of such fibrosis, despite application of high pressure (120mmHg), as the force applied by IPC was dispersed within the tissues (Zaleska et al., 2017). High IPC pressures (> 80mmHg) were recommended to overcome such tissue resistance (Zaleska & Olszewski, 2017). The effect of lymphoedema stage and fibrosis within the study cohort was acknowledged (Zaleska & Olszewski, 2017; Zaleska et al., 2017).

2.9.2 Intermittent Pneumatic Compression (IPC) Dosage

To determine an optimal standardised dose of IPC for this project, a systematic review was completed. The systematic review has been published and is provided in **Appendix B.**

> Phillips JJ, Gordon SJ. Intermittent pneumatic compression dosage for adults and children with lymphedema: A systematic review. *Lymphatic Research and Biology.* 2019;17(1):2-18 10.1089/lrb.2018.0034

Intermittent pneumatic compression (IPC) is an acknowledged component of the multi-modal treatment for lymphoedema that comprises complex decongestive therapy (CDT) (Armer et al., 2013; International Lymphoedema Framework, 2006; International Society of Lymphology, 2020; Queensland Health, 2014; Shao et al., 2014). IPC comprises an air-filled sleeve applied around a limb attached to a small compressor device. The sleeve is made up of several chambers, which are sequentially inflated to a pre-determined pressure from distal to proximal, then deflated. The pressure and time IPC is applied for, the regularity of IPC use (International Lymphoedema Framework, 2006), as well as recipient factors such as the resistance of the tissues (Guan et al., 2020; Zaleska & Olszewski, 2018; Zhao et al., 2020) all affect treatment outcomes. IPC used in home-based lymphoedema management has been associated with reducing episodes of cellulitis and reduced use of outpatient lymphoedema physiotherapy (Karaca-Mandic et al., 2015, 2017), emergency visits and hospital admissions (Maldonado et al., 2020).

Few studies have investigated IPC alone. Instead, studies have used IPC with other treatment modalities of CDT compared with CDT alone, which limits the evidence for IPC (Franks & Moffatt, 2015). Reduced limb volume and improved function has been found post-treatment, in studies where IPC has been used in conjunction with other modalities such as compression garments (Muluk et al., 2013) in the maintenance phase of lymphoedema (Desai et al., 2020).

Adjustable features of an IPC device, which provide the ability to vary dosage, include the length of treatment time, the pressure applied and the type of compression cycle. The compression cycle can vary in the time for which each chamber is inflated and deflated, as well as the order and direction of inflation of each chamber. For example, inflation of the sleeve may begin by inflating the chamber at the foot, holding the pressure and successively filling all chambers until all chambers up the limb are inflated, then all the chambers are deflated at once. Or, in contrast, once the chamber proximal to the foot (chamber 2) is inflated, then the foot (chamber 1) may

deflate, and the next most proximal chamber (chamber 3) is inflated, and so on up the limb in peristaltic fashion. In addition, a preclearance cycle may be added, which aims to 'decongest' proximal areas of the limb before moving more distally, based on the clearance principles of MLD (Queensland Health, 2014). Not all features are adjustable in every IPC device. Optimal dosage parameters for IPC have not been agreed (Feldman et al., 2012; Maclellan, 2015; Tran & Argáez, 2017; Zaleska et al., 2013), resulting in a lack of guidelines (Feldman et al., 2012).

A systematic review was undertaken to ascertain optimal dosage, searching for studies assessing IPC independent from other treatments. Nineteen hundred and fifty-five records were identified from five data bases (Medline, Embase, CINAHL, Scopus and PubMed), and were screened, with 122 full text studies assessed for eligibility, resulting in 16 that were qualitatively analysed. Studies investigating IPC alone (n=12) or IPC with 'maintenance use' of compression garments between IPC treatments (n=4), were included. Most studies were single case design (before/after) studies or comparative studies without concurrent controls, with only one being higher than NHMRC level III-3. In this controlled study from 1998, participants were not randomised to group, so it was rated Level II. The population characteristics of those studied included more upper limb (n= 338) than lower limb lymphoedema (151), with a mean age ranging from 37.8 (14-80) years (Modaghegh & Soltani, 2010) to 71 (54-83) years (Theys et al., 2015). Most were secondary breast cancer related lymphoedema and some included mixed populations of primary and secondary lymphoedema in upper and lower limbs (Bergan et al., 1998; Pohjola et al., 1995; Raines et al., 1977). Three investigated lower limbs alone (McLeod et al., 1991; Modaghegh & Soltani, 2010; Zaleska et al., 2014). Only one study investigated the use of IPC in children, in a sample of nine, whose mean age was 13 years (range 5.5 to 17 years) (McLeod et al., 1991). Evidence for specific dosage was limited and dated, often based on retrospective clinical reviews of samples with a wide age range. Studies investigated devices that varied in the cycle and amount of pressure applied, as well as the number and style of chambers in the sleeve through which pressure is applied.

No optimal pressure was identified, and in some studies the pressure was adjusted per participant, according to tolerance or comfort (Johansson et al., 1998; Pilch et al., 2009). High IPC pressures of up to 120mmHg have been advocated for lymph movement in lower limbs (Taradaj et al., 2015; Zaleska et al., 2013). Evidence for an optimal pneumatic compression pressure specific to the upper or lower limb is limited. Hydrostatic pressure, contributing to capillary filtration rate, is high

in the lower limb (International Lymphoedema Framework, 2012). Consequently, higher garment compression pressure is applied in the lower than the upper limb (Mosti & Cavezzi, 2019; Partsch & Rockson, 2018); as well, there is greater distance to travel from the lower limb to return to central circulation than from the upper limb. The range of tolerated compression pressure, applied via bandages or garments, differs between upper and lower limbs (Damstra & Partsch, 2009; Partsch et al., 2011). Similarly, studies utilising IPC have differed in pressure for upper and lower limbs. Higher pressures of 40–120mmHg have been applied for lower limb lymphoedema (Feldman et al., 2012) to generate tissue fluid flow (Zaleska et al., 2013). A recent lower limb study demonstrated increased lymph flow with higher IPC pressures (gradient of 120 mmHg at the foot to 96 mmHg at the groin) (Zaleska & Olszewski, 2018). However, those investigated had lymphoedema secondary to infection, who were late stage II or III (Zaleska & Olszewski, 2018). Possibly the more advanced staging of these participants, with stiffened skin and soft tissue due to fibrosis, may have influenced the pressure needed for fluid movement, as stiffness of tissues is known to affect the pressure transmitted to the tissues (Guan et al., 2020).

High pressure applied by IPC is not always tolerated by all participants, resulting in the need to reduce pressure mid-treatment (Johansson et al., 1998; Pilch et al., 2009), which is not ideal for standardising dosage. Zaleska and Olszewski (2018) reported that the tissue pressure required for fluid flow was 25-30mmHg. The intra-tissue pressure has been found to be lower (20-40mmHg) than that applied within pneumatic sleeves (50mmHg), which prompted the recommendation that higher initial pressures are needed for IPC dosage (Zaleska et al., 2013). A recent review of the clinical effectiveness and guidelines for use of IPC concluded that the safe use and efficacy of high pressures in IPC had yet to be ascertained (Tran & Argáez, 2017). Moderate 40-60mmHg compression pressure is recommended for management of lower limb lymphoedema using bandaging or garments (Mosti & Cavezzi, 2019; Partsch et al., 2011), and low to moderate pressure, described as 30-60mmHg (Feldman et al., 2012; International Lymphoedema Framework, 2006), is clinically acceptable for adults. A recent review of guidelines for IPC reported pressures of 40-60mmHg were applied over a treatment time that varied from 30 minutes to two hours (Tran & Argáez, 2017).

Clinical treatment programs for children and adolescents have shorter treatment time and lower pressure, consistent with their shorter limb length and smaller limb circumference. Treatment guidelines for children rely on the practitioner for appropriate adaptation of adult treatment

guidelines (Damstra & Mortimer, 2008). Lower pressure is used in compression garments for children and adolescents compared with adults (personal observation; J Newsom, Deputy Head Physiotherapist Children's Hospital at Westmead, personal communication 2016). Care must be taken when applying pressure to limbs with small circumferences, due to the greater pressure transmitted over a curved surface of smaller than larger diameter (Chassagne et al., 2017; International Lymphoedema Framework, 2012). Limb circumference in children and adolescents will vary according to age and growth. Paediatric studies using IPC have had small sample sizes with a wide range of ages included (Hassall et al., 2001; McLeod et al., 1991) and optimal pressure and cycle time have not been definitive.

Despite some caution about side effects in applying IPC (Boris et al., 1998), no adverse events have been reported in reviews of IPC use in lymphoedema (Feldman et al., 2012; Shao et al., 2014; Tran & Argáez, 2017). Contraindications to IPC use include uncontrolled cardiac, embolic, or thrombotic conditions and metastatic disease , although use is permitted with caution where conditions are controlled (International Lymphoedema Framework, 2006; Queensland Health, 2014). Compression may be used with care in the presence of arterial disease (Hedayati et al., 2015; International Lymphoedema Framework, 2006), which is more prevalent over the age of forty (Bąk et al., 2016).

2.10 Investigation of Dermal Fluid in Primary Lymphoedema

There is agreement that the dermal lymphatics are important in the initial uptake of fluid into the lymphatic system, and that this initial dermal fluid uptake is delayed or abnormal in some forms of primary lymphoedema. As the dermis is where lymphatic drainage begins, the measurement of dermal fluid load is essential to understanding changes in the composition of the dermis related to condition status and interventions. Treatment of lymphoedema necessarily involves strategies to address adipose and fluid accumulation in the subcutaneous tissue as well as reduction of overall volume of the body part affected. However, understanding changes in dermal fluid with treatment such as compression is essential to tailor treatment strategies to address the condition of the dermis. With no objective measure of the dermis, assessment and treatment of lymphoedema focus on the size of the limb. Common clinical assessment tools provide various measures of fluid within the limb, but none are specific to the dermis. The identification of a measure of dermal fluid could enable recognition of early changes indicating progression in stage of lymphoedema,

planning for targeted treatment and assessment of treatment response.

Investigations have described some of the changes in the dermis and tissues as lymphoedema progresses, including the impact of tissue inflammation (Carlson, 2014; Di et al., 2016; García Nores, Ly, Savetsky, et al., 2018), biomechanical changes (Bustos et al., 2020; Polat et al., 2020), and change in lymphatic vessel densities, dimensions and functionality (Barone et al., 2020). Information in primary lymphoedema regarding the state of the dermis, methods to assess it and what changes in the dermis with conventional treatment strategies such as compression, remain limited (Johnson et al., 2014; Niwa et al., 2021; Tassenoy et al., 2016).

This study provides the opportunity to explore physiological differences between people with and without primary lymphoedema. High frequency ultrasound is useful in dermal assessments utilising echogenic properties, although there is no established method for fluid assessment in the dermis using high frequency ultrasound images. Based on this literature review, the following decisions were made about the methodology of this study:

1. A study was first undertaken to establish a method for assessing dermal fluid using HFU in participants with no lymphoedema and the intra-rater reliability of the investigator for that method. This reliability of this method was confirmed in the first ten participants with primary lymphoedema in the main study.
2. Age, ethnicity and gender matching was utilised to minimise potential confounding factors of dermal variations associated with these parameters, due to the expectation of a small sample size.
3. The age of participants was limited to between three and 40 years, due to the effect of age on the skin, and to avoid the possible impact of arterial or venous complications associated with older age.
4. Recruitment was not restricted to adults or to a particular limb, as prevalence of primary lymphoedema is known to be low.
5. Inclusion criteria for those with primary lymphoedema included a diagnosis from a lymphatic or tertiary assessment clinic.
6. Exclusion criteria applied to both those with and without primary lymphoedema and included diseases that affected the skin or connective tissues, renal or cardiovascular system, infective or inflammatory diseases, malignancy and pregnancy.

7. Intermittent pneumatic compression was chosen as the mode of compression, with dosage of 60mmHg for 50 minutes, based on the evidence from a literature review of IPC.
8. Anatomical landmarks were used as boundaries for segmental lower limb bioimpedance measures.

The above review has highlighted the challenge to clinicians and researchers for a practical objective measure of dermal fluid change in primary lymphoedema. The following chapter describes the establishment of a method for use of high frequency ultrasound in assessment of dermal fluid in lymphoedema and the reliability of the investigator for that method.

CHAPTER 3
RELIABILITY OF ASSESSMENT METHODS

High frequency ultrasound (HFU) is used extensively in dermatology to assess the skin (Agner & Serup, 1990; Bagatin et al., 2013; Iyengar et al., 2018; Jemec et al., 2000; Kleinerman et al., 2012; Seidenari & Di Nardo, 1992; Serup et al., 1984; Wortsman, 2012) but rarely has it been used to assess the skin, or more specifically the dermis, in people with lymphoedema assessment. There is no established method for its use (Serup et al., 2006) and investigations of fluid in the dermis have used several different methods (Crisan, Lupsor, et al., 2012; Gniadecka, 2001; Gniadecka & Jemec, 1998; Naouri et al., 2010; Schou et al., 2004; Tsukahara et al., 2001). Consequently, a study was undertaken to establish a reliable method for HFU image capture and measurement of the dermis, based on the manufacturer's standard protocol (Cortex Technology, 2014). Intra-rater reliability of the investigator was then determined using these methods. The process of establishing this method and intra-rater reliability is described below (**Section 3.1**).

A study was also undertaken to determine the intra-rater reliability of the investigator when using the Indurometer and undertaking circumferential measures, which is described in **Section 3.2**.

3.1 Methodology and Reliability of High Frequency Ultrasound

The HFU reliability study presented in this section has been peer reviewed and published:

> Phillips J, Reynolds KJ, Gordon SJ. Dermal thickness and echogenicity using DermaScan C high frequency ultrasound: Methodology and reliability testing in people with and without primary lymphoedema. *Skin Research and Technology.* 2020;26(6):813-823 (full paper in **Appendix C**).

This study consisted of two parts; the first with people who did not have primary lymphoedema (NLO) and then with people who did have primary lymphoedema (PLO). The equipment described initially was used in all studies.

3.1.1 Equipment

The DermaScan C (Cortex Technology, Hadsund, Denmark) HFU was used to assess dermal thickness and water content by echogenicity. It has a frequency of 20 MHz, penetrates to a depth of 13mm with 60 x 150-micron resolution (Cortex Technology, 2014) and has been validated

against MRI (Gniadecka & Quistorff, 1996) to determine both dermal thickness and echogenicity (Bagatin et al., 2013; Gniadecka, 2006; Gniadecka & Jemec, 1998; Laurent et al., 2007). The head of the probe is water-filled and covered by a fine plastic membrane held down by a plastic cap with a slot in it, through which the ultrasound beam is transmitted (see **Figure 3.1a and b**). The slot is filled with water-based gel providing contact between the probe and the skin with a standardised gel thickness for each image.

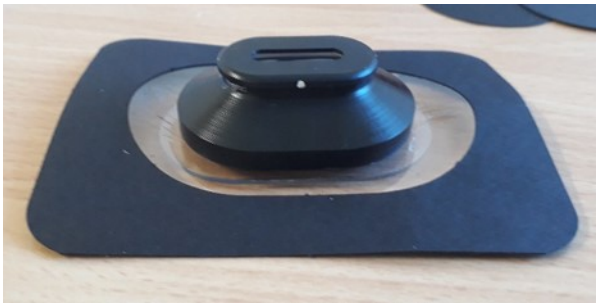

a.

b.

Figure 3.1 a. HFU Probe b. Probe Cap Showing the Slot Which has a Plastic Lining

3.1.2 Image Analysis and Outcome Measures

Incorporated DermaScan software (DScan version 3 application software for Windows, advanced configuration) produces images in 'A' and 'B' mode. 'A' mode produces a graph of reflected sound across the image, showing the amplitude of sound echoes (**Figure 3.2**) and is useful for skin thickness measures (Waller & Maibach, 2005). B mode is a cross section of that amplitude graph, showing areas of brightness or intensity of reflected sound (**Figure 3.3**) which is commonly used for assessments of echogenicity (Bagatin et al., 2013; Caetano et al., 2015; Gniadecka & Quistorff, 1996), although it may also be used to assess skin thickness (Alsing & Serup, 2020; Bagatin et al., 2013; Gniadecka & Jemec, 1998). Being a two-dimensional representation, B-mode is considered more reproducible (Waller & Maibach, 2005).

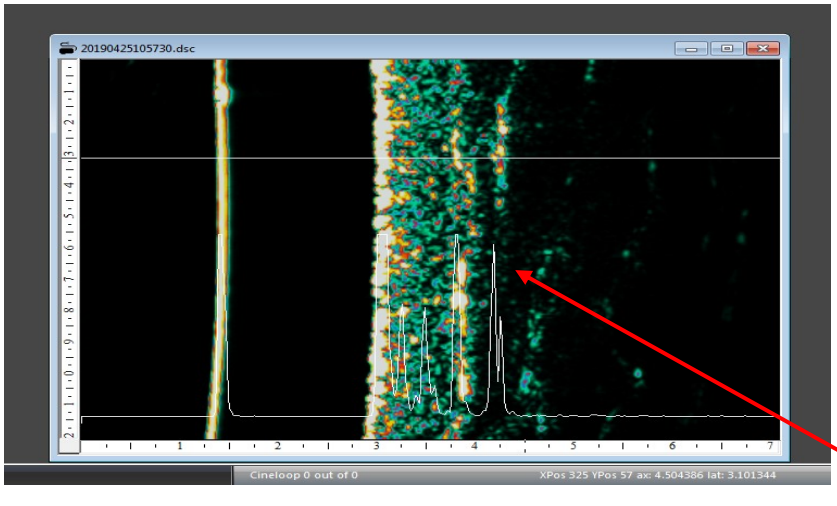


Figure 3.2 DermaScan C HFU Image Showing the A-Scan of Peaks of Intensity (arrowed)

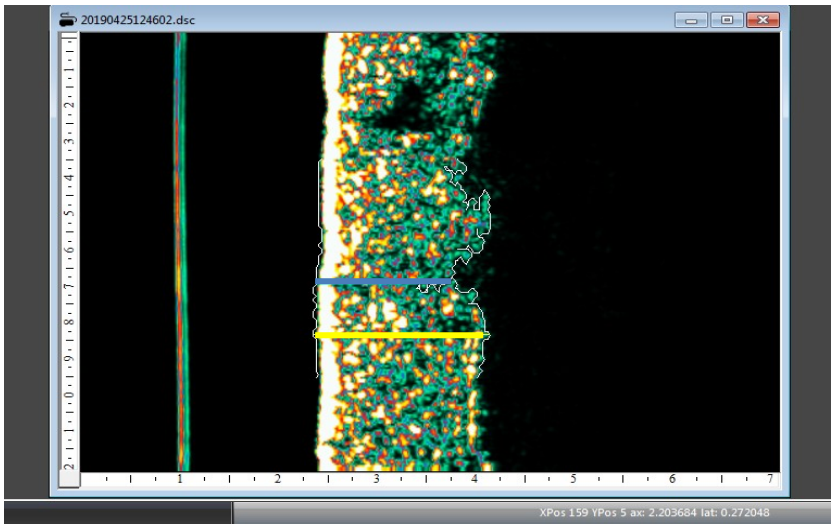


Figure 3.3 DermaScan C HFU Image in B Mode.

B mode is used for assessment of echogenicity. The blue and yellow lines demonstrate minimum and maximum total skin thickness.

3.1.2.1 Echogenicity

The smallest picture elements, known as pixels, are differentiated on screen by a colour spectrum (see **Figure 3.4**) in images produced by the DermaScan C, from black through green, blue, red and yellow to white, according to the level of intensity reflected at that point (Gniadecka & Jemec, 1998). Across this spectrum of intensity in the DermaScan C, numbers have been allocated from 0-255. The lower intensity or darker (black) end of this spectrum represents fluid (Gniadecka, 2001; Gniadecka, Gniadecki, et al., 1994; Gniadecka & Jemec, 1998; Schou et al., 2004) and is described as low echogenic pixels (LEP) (Alsing & Serup, 2020) and is represented on the spectrum from 0 to 30. LEP measured in the range of 0-30, accepted as representative of fluid, (Seidenari, 2006) may be measured within the dermis.

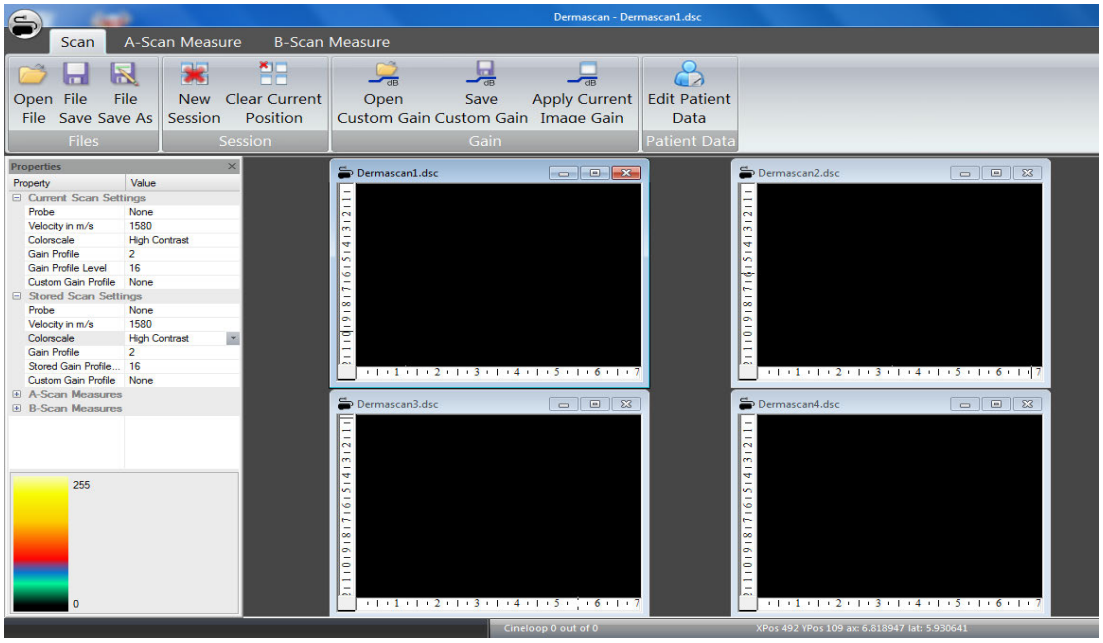


Figure 3.4 DermaScan C Screen Shot.

Left side: 0-255 assigned colours. Right side: Four screens ready for scanning.

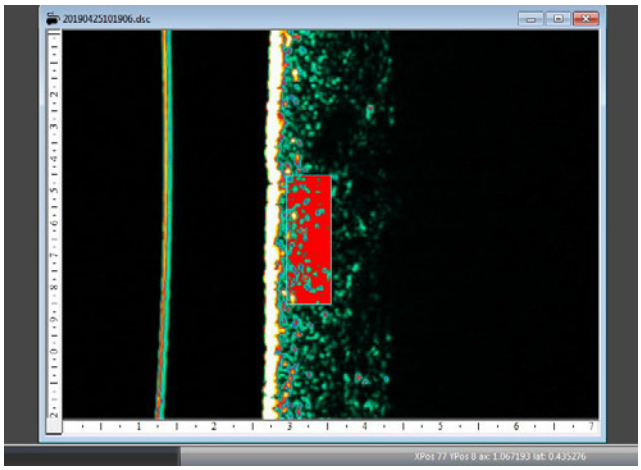


Figure 3.5 DermaScan C Image

The rectangular Region of Interest (ROI) with range of pixel intensity from 0-30 is highlighted in red.

Intensity or echogenicity is affected by individual characteristics, such as aging (de Rigal et al., 1989; Gniadecka, Serup, et al., 1994; Querleux et al., 2009) and type of tissue (dense collagen fibres versus high fluid content) (Gniadecka, Gniadecki, et al., 1994; Gniadecka & Quistorff, 1996; Lucas et al., 2014; Schmid-Wendtner & Burgdorf, 2005; Seidenari, 2006). As well, device characteristics such as the gain setting, which increases the intensity of the image for clearer viewing (Bagatin et al., 2013; Seidenari, 2006) can alter the measurement of dermal thickness and echogenicity. These characteristics, by their effect on echogenicity, can therefore cause variation

in measures of dermal thickness and relative fluid content within an HFU image (Jemec et al., 2000; Serup et al., 2006) and decrease reliability for comparison of images.

3.1.3 Study One: Reliability Study Method in NLO

The aim of this study was to identify a method that resulted in high intra-rater reliability and repeatability for both image capture and measurement of dermal echogenicity. The site of image capture was standardised and a variety of images with different gain settings were taken and then measured, to determine the most reliable and repeatable method for the main study.

Ethical approval was granted for the reliability study by the Royal Children's Hospital (RCH) Human Research Ethics Committee (HREC) (HREC/16/RCHM/136). Local governance approval (SSA/16/RCHM/ 142; local reference 36273) for RCH and MCRI (Murdoch Children's Research Institute) was granted at the same time.

3.1.3.1 Participants

Ten healthy NLO volunteer participants (eight females and two males, aged 17 to 54) provided one lower limb each. Participants were sourced from among friends and colleagues with no lymphoedema (NLO), who provided verbal and written consent.

3.1.3.2 Measurement sites

Measurement sites were marked on the dorsal foot, posterior calf and posterior thigh of the lower limb as described in **Table 3.1**. A tape measure attached to a Jobst measuring board, according to clinical guidelines (Australasian Lymphology Association, 2004), a water-based body-marking pencil and a cardboard template (approximately 6x3cm, just large enough for the head of the HFU probe) were used to outline the measurement site. Participants lay supine on a plinth while repeated images were taken on the foot and turned to prone for images taken on the posterior calf and thigh. A pillow supported the limb during measurements.

Table 3.1 Lower Limb Measurement Sites

Table 3.1 Lower Limb Measurement Sites	
Measurement Site (Measures taken at site)	**Level marked**
Dorsum of foot measurement site (HFU, Ind, circ)	Between the second and third metatarsals, just proximal to the metatarsophalangeal joint. Circumference level was marked on the lateral foot, with the foot on a Jobst measuring board, then marked medially.
Posterior calf measurement site (HFU, Ind, circ)	A point half-way from the lateral knee joint line to the level of the least ankle was marked medially and laterally with participant in supine. When prone, and using the tape from medial to lateral, the mid-point across the posterior calf was marked and outlined by the template.
Knee joint line	Lateral knee joint line. Marked to enable measurement from knee to least ankle, so midpoint of calf could be positioned.
Posterior thigh measurement site (HFU, Ind, circ)	A point half-way from upper thigh to knee joint line was marked medially and laterally with participant in supine. When prone, and using the tape from medial to lateral, the midpoint across the posterior thigh was marked and outlined by the template.
Upper thigh line (circ)	Level just distal to greater trochanter at maximum girth of thigh. Marked laterally to enable measurement from top of thigh to lateral knee joint line, so midpoint of thigh could be established laterally.

*HFU=High Frequency Ultrasound; Ind= Indurometer; circ= circumference

3.1.3.3 HFU Image Capture

Water-based gel (Dane-Gel R1, Rohde Produits, Holte, Denmark) was applied to the slot in the head of the probe to maintain contact with the skin. A smear of gel was added to the skin as per manufacturer's instructions (Cortex Technology, 2014) and care taken to ensure there were no air bubbles within the gel which would cast 'shadows' on the image. The probe was held parallel to the skin (see **Figure 3.6**) and images were taken with several different gains, to establish the clearest image. After four images were captured and saved, gel was reapplied to the probe and a repeat set of four taken. Three sets of four were captured at each site for each participant. Images were taken using a range of gain settings within a set of four and then repeated in the next set of four, to investigate inter-session reliability (removal of probe, reapplication of gel, and recapture).

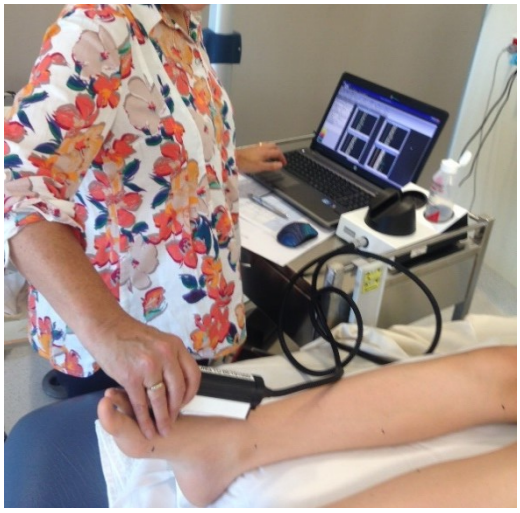


Figure 3.6 Holding the HFU Probe Over the Dorsum of the Foot.

3.1.3.4 Image Measurement

Images with a gain setting of mode one level 13 on the DermaScan C were used for low echogenic pixels (LEP) analysis, in accordance with advice from the manufacturers (P. Holm Pedersen, personal communication, May 2019). A long thin rectangular standardised region of interest (ROI) was established (Shape 1 of area: 6.894712mm^2), which stretched from the top to the bottom of the screen along the whole image. The ROI was positioned consistently just below the entrance epidermal echo, just brushing the border of the epidermis, and aligned centrally (Derraik et al., 2014) within the length of the image (12.1mm). The area of LEP was then selected for measurement by setting the range to 0-30 to highlight that area (Seidenari & Di Nardo, 1992; Serup et al., 2006) within the rectangle (see **Figure 3.5,** showing a smaller ROI). Four measures were generated by the DermaScan software: area (in mm^2 and in pixels), the total intensity (%) and total intensity within range (%).

3.1.4 Statistical Analysis - Reliability

Reliability was determined by intraclass correlation coefficient analysis for each site. The intra-class correlation co-efficient (ICC), used to assess test-retest reliability, combines both correlation and agreement (Koo & Li, 2016; Portney & Watkins, 2015). ICC may be calculated several ways depending on which error components are included, and whether there are fixed or random effects of the independent variables (Portney & Watkins, 2015, p. 590; Weir, 2005). For this assessment, the third form of ICC was used, according to McGraw and Wong (1996) (McGraw & Wong, 1996), which is a mixed effects model, where the subjects are random and number of trials or the rater (in this instance) is fixed. Single measures were used to investigate their relationship

to each other, with the aim of determining the degree of difference between them (McGraw & Wong, 1996). For a clinical tool, variation in repeated measures must be as close as possible to zero: hence, measures were investigated for absolute agreement, rather than consistency. In summary, SPSS version 25 (IBM Corp, 2017) was used to calculate ICC, with the three items of repeated measurement as the levels of independent variable, using 2-way model mixed effects, single score, and absolute agreement, denoted ICC $_{(3, 1)}$.

Both the ICC and the 95% confidence intervals were considered in interpretation of reliability scores. ICC values over 0.90 are the preferred option for clinical measures, indicating excellent reliability (repeatability) whilst values above 0.75 indicate good reliability and those between 0.50 and 0.75, moderate reliability (Portney & Watkins, 2015, p. 595),(Koo & Li, 2016). In a previous study on HFU measurement of dermal thickness in lymphoedema, scores above 0.80 were accepted, indicating good reliability (Dylke et al., 2018). However, given the current study aimed to investigate clinical measures, the standard of above 0.90 for excellent reliability was applied. Where the confidence interval extended below 0.75, even with a higher value ICC, the reliability was rated as a range indicating the lower limit (for example, an ICC of 0.92, where the lower limit of CI extended from 0.70, would be rated as good to excellent, not excellent). Scores between 0.50 – 0.75 (rated as moderate reliability) could indicate the need to use repeated measures to generate a mean value for clinical situations.

3.1.5 NLO Reliability Results

LEP intra-rater reliability was investigated applying the method described above to measure LEP on ten randomly chosen images from different sites, then repeating the measurements two more times with approximately two hours between each set of measurements so there was no chance of memory carryover.

Intraclass correlation coefficients (ICC) for intra-rater reliability were excellent, and, although the lower limit extended below 0.9, the confidence intervals were narrow (see **Table 3.2**).

Table 3.2 Non-Lymphoedema Intra-Rater Reliability of Image Analysis for LEP Measurement

Initial methodology of image analysis taking three repeated sets of measures, using images taken from a mix of lower limb sites in healthy people without lymphedema.

Table 3.2 Non-Lymphoedema Intra-Rater Reliability of Image Analysis for LEP Measurement					
Measure	N	ICC*	95% Confidence Interval		Result**
			Lower	Upper	
Segmented Area	10	.991	.943	.998	Excellent
Total Intensity	10	.993	.966	.998	Excellent
Total Intensity Within Range	10	.989	.925	.998	Excellent

* ICC = Intraclass Correlation Coefficient

** Reliability rating based on Poor: ICC <0.5; Moderate: 0.5-0.75; Good 0.75-0.90 and Excellent >0.90

LEP inter-session reliability was investigated by applying the method above to measure LEP on three images that were taken within five minutes of each other, with the same HFU gain setting but with the head of the probe lifted and gel re-applied in between each image capture.

Inter-session reliability was lower, rating good (see **Table 3.3**).

Table 3.3 Non-Lymphoedema Inter-session Reliability of LEP Measurement in Repeated Image Capture

Initial methodology of image capture, taking three repeated images at the same site, with five minutes between each image capture.

Table 3.3 Non-Lymphoedema Inter-session Reliability of LEP Measurement in Repeated Image Capture					
Measure	N	ICC*	95% Confidence Interval		Result**
			Lower	Upper	
Segmented Area	57	.867	.804	.914	Good
Total Intensity	57	.890	.836	.930	Good
Total Intensity Within Range	57	.727	.614	.817	Moderate

* ICC = Intraclass Correlation Coefficient

** Reliability rating based on Poor: ICC <0.5; Moderate: 0.5-0.75; Good 0.75-0.90 and Excellent >0.90

3.1.6 HFU Methodology Modifications Following Study One

3.1.6.1 Image Capture Modifications

Further refinements to the methodology of image capture were made to increase site consistency following Study One, with the aim to increase inter-session reliability from 'moderate to good', to 'good to excellent'. A Fixomull adhesive template was used to mark the measurement sites (instead of body pencil) and marks were made on the screen monitor of the HFU (Serup et al., 2006) to assist in keeping the probe perpendicular to the skin (by ensuring the image was in vertical alignment with the marks on the screen monitor). This visual check also served to highlight when gel thickness varied: the additional smear of gel on the skin was omitted and extra attention paid to ensuring no excess gel remained on the surface of the probe beyond the gel space. Gain setting mode two level 16 had shown most consistency for good image intensity in healthy NLO participants but gain mode one was advised for assessment of fluid. Uncertain if this would be the same in participants with primary lymphoedema (PLO), several gains (mode one, level 13 and 16, mode two, levels 16 and 19) were initially used in Study Two for image capture and reliability reassessment, to find the gain providing the clearest image for both populations.

3.1.6.2 Image Measurement Modifications

For echogenicity measures, the smaller ROI (shape three: 2.287931mm^2) was used, centred on 6.5 on the vertical scale (see **Figure 3.5**). Low echogenic pixels (LEP) segmented within the ROI were obtained by the DermaScan software as previously described.

3.1.7 Study Two: Reliability Study in People with Primary Lymphoedema

3.1.7.1 Participants and method

The first ten participants with primary lymphoedema (five each female and male, aged three to forty years) in the main (SkiPL) study provided images taken on their affected lower limb at the posterior calf and dorsum of the foot for this reliability study.

LEP intra-rater reliability was investigated applying the method described above, to measure LEP in the smaller ROI on ten randomly chosen images from different sites. Measures were repeated two more times with approximately two hours between each set of measurements, as in Study One, so there was no chance of memory carryover.

LEP inter-session reliability was investigated by applying the modified method described above to capture three images with the same HFU gain setting (mode one level 13), appropriate for

echogenicity evaluation. Images were taken within five minutes of each other but with the head of the probe lifted and gel re-applied in between each image capture.

3.1.7.2 Results

With the modifications described, the intra-rater reliability and inter-session reliability improved compared with that in Study One with NLO (see **Tables 3.4 and 3.5**). Each site was then assessed separately. Intra-rater reliability (**Table 3.6)** remained high: the measure of LEP (segmented area of low echogenic pixels) for both calf and foot were excellent. Inter-session reliability (**Table 3.7**) showed good to excellent reliability for three repeated images captured in the foot. Segmented area in the calf was lower although rated 'good', but with a wider confidence interval that extended from moderate to excellent (CI: 0.551-0.903).

Table 3.4 Study One and Two Intra-Rater Reliability of Image Analysis for LEP Measurement

Comparison of outcomes from amended methodology of primary lymphoedema (PLO) with initial methodology in non-lymphedema (NLO) population, using images taken from both lower limb sites

Table 3.4 Intra-Rater Reliability of Image analysis for LEP Measurement										
A. NLO Reliability Pilot						B. PLO Reliability Pilot				
Measure	N	ICC	95% CI		Result*	N	ICC	95% CI		Result*
			Lower	Upper				Lower	Upper	
Segmented Area	10	.991	.943	.998	Excellent	10	.999	.998	1.000	Excellent
Total Intensity	10	.993	.966	.998	Excellent	10	1.000	.999	1.000	Excellent
Total Intensity Within Range	10	.989	.925	.998	Excellent	10	.997	.991	.999	Excellent

ICC = Intraclass Correlation Coefficient; CI = Confidence Interval

* Reliability rating based on Poor: ICC <0.5; Moderate: 0.5-0.75; Good 0.75-0.90 and Excellent >0.90

Table 3.5 Primary Lymphoedema Inter-Session Reliability.

Repeated images were taken from two lymphoedema participants utilising two sites (dorsum foot and calf).

Table 3.5 PLO Inter-Session Reliability					
Measure	N	ICC	95% CI		Result*
			Lower	Upper	
Segmented Area	10	.917	.786	.977	Good to excellent
Total Intensity	10	.892	.693	.970	Moderate to good
Total Intensity Within Range	10	.916	.783	.976	Good to excellent

ICC = Intraclass Correlation Coefficient; CI = Confidence Interval

* Reliability rating based on Poor: ICC <0.5; Moderate: 0.5-0.75; Good 0.75-0.90 and Excellent >0.90

Table 3.6 Primary Lymphoedema Intra-Rater Reliability Specific to Site

Table 3.6 PLO Intra-Rater Reliability Image Analysis by Site						
Measure	Site	N	ICC	95% CI		Result*
				Lower	Upper	
Segmented Area	Calf	10	.992	.977	.998	Excellent
	Foot	10	.999	.998	1.000	Excellent
Total Intensity	Calf	10	.997	.992	.999	Excellent
	Foot	10	1.000	1.000	1.000	Excellent
Total Intensity in Range	Calf	10	.989	.968	.997	Excellent
	Foot	10	.999	.996	1.000	Excellent

ICC = Intraclass Correlation Coefficient; CI = Confidence Interval

* Reliability rating based on Poor: ICC <0.5; Moderate: 0.5-0.75; Good 0.75-0.90 and Excellent >0.90

Table 3.7 Primary Lymphoedema Inter-Session Reliability Specific to Site

Table 3.7 PLO Reliability Inter-Session Reliability by Site						
Measure	Site	N	ICC	95% CI		Result *
				Lower	Upper	
Segmented Area	Calf	16	.767	.551	.903	Good
	Foot	16	.887	.765	.955	Good
Total Intensity	Calf	16	.765	.540	.902	Good
	Foot	16	.872	.737	.949	Good
Total Intensity in Range	Calf	16	.616	.332	.828	Moderate
	Foot	16	.811	.623	.922	Good

ICC = Intraclass Correlation Coefficient; CI = Confidence Interval

* Reliability rating based on Poor: ICC <0.5; Moderate: 0.5-0.75; Good 0.75-0.90 and Excellent >0.90

3.1.8 Discussion and Implications

This study has established a method of HFU image capture and echogenicity measurement in both healthy people and those with primary lymphoedema that results in reliable measurement of LEP within and between sessions by the investigator. Future HFU clinical and research studies can confidently use this method to determine individual reliability.

The aim of this study was to identify a method and outcome measure that could accommodate both groups. The method involved finding a gain setting appropriate for both PLO and NLO, to be consistent across groups. The range of echogenicity across both groups is wide, with the dermis in affected limbs of PLO participants having low echogenicity. Healthy skin in the feet showed high echogenicity, as previously reported (Olsen et al., 1995; Seidenari et al., 2000). The gain setting was chosen to be high enough for image evaluation in PLO participants but not too high for NLO participants.

For measurement, consideration was initially given to using a ratio of the segmented LEP to the total ROI intensity (Tsukahara et al., 2001; Veen et al., 2001) as the outcome measure. However, the intensity in the total area of the ROI could vary due to previously mentioned individual characteristics such as age and tissue condition such as fibrosis as occurs in lymphoedema. These individual factors could vary to a greater extent than would LEP, affecting the denominator of such a ratio. The difference in thickness of the dermis between people with and without lymphoedema (Naouri et al., 2010) precluded measurement of LEP in the total dermis. The use of low echogenicity measures (LEP) taken from a standardised area within the dermis (ROI) limited the intensity variation between individuals, due to other causes such as photo-damage, that could arise if using ratios, and therefore allowed comparisons between sites and individuals.

Based on these reliability studies, a segmented area of LEP within a standardised region of interest (ROI) in the dermis was chosen to investigate differences between those with and without primary lymphoedema for this research.

3.1.9 Limitations

These reliability outcomes pertain to the specific population and the DermaScan C HFU used in this study; the small number of participants in this study limits conclusions to the population assessed and the one operator. The balance between the burden on participants (Shoukri et al., 2004) for repeated imaging and sample size for a repeatability study was in part addressed by taking

triplicate images (Watson & Petrie, 2010) on the same day.

3.2 Intra-Rater Reliability for Circumferences

Measurement of limb circumference is a clinical method of tracking change in lymphoedema, standardised to be repeatable (Australasian Lymphology Association, 2004), but it was important to establish intra-rater reliability as before and after measures were planned for the intervention study investigating compression.

3.2.1 Method

Healthy people were recruited from among friends and colleagues without lymphoedema (NLO) to test the intra-rater reliability of the investigator in conducting circumferential measures. Participants lay supine on a plinth. Sites measured on the calf and foot are described in full in the measurement protocol for the HFU study (**Section 3.1.3.1 & Table 3.1**). Measurement sites were marked with reference to a tape measure attached to the footboard of a Jobst measuring board and circumferences were taken with a spring-tape measure, both in accordance with ALA guidelines for measurement (Australasian Lymphology Association, 2004). Marks were removed prior to the second set of measures. Circumferences were taken three times, successively measuring different points before taking repeated measures, so that previous measures at any one point were not recalled. The standard error of measurement and minimum detectable difference (MDD) was calculated according to the formula

MDD= 1.96xSEMx√2 (Portney & Watkins, 2015, pp. 645-646),

where SEM is the standard error of measurement. SEM is calculated by

SEM=SD√(1-ICC) (Portney & Watkins, 2015, pp. 608-609).

3.2.2 Results

Participants Six people without lymphoedema (three each female and male, aged 15 to 32 years) provided one or two lower limbs for measurement, resulting in ten sets of repeated circumferential measures at the two sites (foot and calf, as described in **Section 3.1.3.1**).

Excellent intra-rater reliability was shown in lower limb circumferential measurement in healthy people ($ICC_{(3,1)}$ 0.984 and 0.997 for foot and calf measures respectively) (see **Table 3.8**). The SEM was 0.1cm at both the foot and the calf, and the MDD 0.3cm at the calf and 0.2cm at the foot.

Table 3.8 Non-Lymphoedema Intra-Rater Reliability of Circumferential Measures

Table 3.8 NLO Intra-Rater Reliability of Circumferential Measures					
Measure site	N	ICC	95% CI		Result*
			Lower	Upper	
Foot	10	.984	.955	.996	Excellent
Calf	10	.997	.993	.999	Excellent

ICC = Intraclass Correlation Coefficient; CI = Confidence Interval

* Reliability rating based on Poor: ICC <0.5; Moderate: 0.5-0.75; Good 0.75-0.90 and Excellent >0.90

3.3 Indurometer Reliability

The Indurometer provides a measure of tissue compressibility, an indication of tissue resistance.

3.3.1 Equipment

Figure 3.7 The Indurometer

The Indurometer (Model BME 1563G) is an electronic hand-held instrument (Flinders Biomedical Engineering (BME), Flinders Medical Centre Bedford Park, South Australia 5042). The Indurometer (see **Figure 3.7**) has a plunger (of one-centimetre diameter) which protrudes through the disc onto the skin when downward pressure is applied by the researcher, until a force of 200g is reached. A beep is heard, and a reading of resistance is produced, measured in induration units (IU), which are equivalent to the distance travelled into the skin using a force of 200g, in increments of 0.01mm, on a scale up to 15 (Flinders University Biomedical Engineering, 2013; Pallotta et al., 2011). High tissue compressibility is indicated by a high reading, as the plunger moves further into the soft tissues.

This study sought to establish the reliability of the investigator to use the Indurometer in the foot and posterior calf of people with and without primary lymphoedema.

3.3.2 Method

Friends and colleagues without lymphoedema (NLO) were recruited for an investigation of intra-rater reliability of indurometry on the posterior calf and foot. Participants lay supine on a plinth, with a pillow under the leg for measures taken on the dorsum of the foot. Three repeated measures were taken at each site, alternating sides to allow for tissue recovery in between measurements. Measures were taken successively on alternate feet, until three were recorded for

each foot prior to turning prone, with a pillow under the leg, for measures to be recorded alternately on each posterior calf (site positions described in **Table 3.1**).

3.3.3 Results

Participants Five people provided ten sets of repeated measures for the Indurometer taken bilaterally (four male and one female, aged from 23 to 32 years).

Intra-rater reliability The intra-rater reliability of this researcher in using the Indurometer was excellent in the calf, based on the ICC $_{(3,1)}$. The foot, however, was lower (ICC $_{(3,1)}$: .662) with wide confidence intervals (**Table 3.9**).

Table 3.9 Non-Lymphoedema Intra-Rater Reliability of the Indurometer

Table 3.9 NLO Intra-Rater Reliability of the Indurometer					
Site	N	ICC	95% CI		Result*
			Lower	Upper	
Foot	10	.662	.309	.891	Moderate
Calf	10	.910	.763	.975	Good - Excellent

ICC = Intraclass Correlation Coefficient; CI = Confidence Interval
* Reliability rating based on Poor: ICC <0.5; Moderate: 0.5-0.75; Good 0.75-0.90 and Excellent >0.90

3.3.4 Implications and Modifications

Lower than acceptable intra-rater reliability led to the use of repeated measures of IU on all NLO and PLO participants in the main study. Modifications were made to the positioning of participants, by moving the pillow to support the sole of the foot, to minimise foot movement during measurement. Further reliability analysis was planned, to inform the analysis required for the main study.

3.3.5 Results of Intra-Rater Reliability for Indurometry in the Main Study

3.3.5.1 Results of Preliminary Investigation of Intra-Rater Reliability in PLO

The first eleven primary lymphoedema (PLO) participants in the main study provided IU measures for preliminary investigation. The intra-rater reliability improved, with a good ICC (over 0.75), but wide confidence intervals ranged from moderate to excellent for both the foot and the calf (ICC $_{(3,1)}$ 0.873 (CI:0.696-0.961) and ICC $_{(3,1)}$ 0.888 (CI:0.710-0.966) respectively). Capture of three repeated measures for each site was continued, and a mean value calculated as the outcome measure for each site.

3.3.5.2 Results of Intra-Rater Reliability in All Matched Pairs of NLO and PLO

Confirmation of reliability was carried out on completion of data collection in the main study. NLO and PLO pairs were matched for ethnicity, age and gender. For the healthy participants (NLO), one lower limb was designated 'affected' and the other 'unaffected' in accordance with the affected and unaffected limbs in their matched PLO participant. Participants included five male and fifteen female matched PLO and NLO pairs, aged three to forty years.

Acceptable reliability was found in both PLO and NLO at all sites (see **Table 3.10**); with reliability now being excellent for both feet in NLO and the affected foot in PLO. Reliability in the calf varied between sides in NLO, but remained acceptably good in both PLO and NLO, reaching excellent in PLO.

Table 3.10 Intra-Rater Reliability of the Indurometer in the SkiPL Study

Table 3.10 Intra-Rater Reliability of the Indurometer in the SkiPL Study (all participants)					
	Site	N	ICC	95% CI	
				Lower	Upper
PLO	Affected foot	16	.919	.828	.968
	Unaffected foot	15 ǂ	.865	.715	.948
	Affected calf	16	.929	.815	.974
	Unaffected calf	16	.921	.828	.969
NLO	Affected foot	16	.936	.862	.975
	Unaffected foot	16	.902	.794	.961
	Affected calf	16	.856	.702	.942
	Unaffected calf	16	.939	.846	.978

ǂ One measure missing due time for one participant
ICC = Intraclass Correlation Coefficient
*Reliability rating based on Poor: ICC <0.5; Moderate: 0.5-0.75; Good 0.75-0.90 and Excellent >0.90

The outcomes of all reliability studies indicate that the data collected and the LEP measurements by the investigator in this project were reliable for the observational studies, and repeatable for the intervention study.

CHAPTER 4
METHODS

This chapter describes the methodology— recruitment, equipment used, assessment procedures, intervention, data management and the statistical analysis—used to answer the research questions outlined in Chapter 1, in the study Skin in Primary Lymphoedema (SkiPL).

4.1 Ethics

Ethical approval was provided by the Human Research Ethics Committee (HREC) at the Royal Children's Hospital (RCH) Melbourne nationally for RCH Melbourne, Victoria, and The Sydney Children's Hospitals Network (incorporating The Children's Hospital at Westmead, and Sydney Children's Hospital, NSW) (National ethics approval HREC/16/RCHM/136) on 16th December 2016. Local governance approval (SSA/16/RCHM/ 142; local reference 36273) for RCH and MCRI (Murdoch Children's Research Institute) was granted at the same time.

Separate ethical approval for the same protocol was granted by Mercy Health HREC, Melbourne (R16-67) on February 14th, 2017. Board approval from Mt Wilga Private Rehabilitation Hospital was also received for that protocol in 2017. (All approval letters are attached in **Appendix E**).

The approved protocol with amendment descriptions is provided in **Appendix F**. Amendments related to recruitment procedures. An additional letter was added to the recruitment procedure to trace people with primary lymphoedema if their last contact with a lymphoedema service was greater than two years and the number of data collection sites were increased to facilitate recruitment of people without primary lymphoedema.

4.2 Trial Registration on ANZCTR

This study was registered with the Australia New Zealand Clinical Trial Registry (ANZCTR) on February 2nd, 2018, with number: ACTRN12618000162213. Web address of trial: http://www.ANZCTR.org.au/ACTRN12618000162213.aspx

4.3 Recruitment

4.3.1 Primary Lymphoedema Participants (PLO)

Participants in Victoria with primary lymphoedema (PLO) were recruited through public

lymphoedema services at Mercy Health and The Royal Children's Hospital (RCH) in Melbourne. Potential participants clinically diagnosed with primary lymphoedema were identified by staff of lymphoedema services at Mercy Health and the Children's Private Medical Group, and via medical records at RCH in Melbourne. The electronic attendance system and data base at each site were checked for the status and most recent contact of those identified. Letters of invitation (see **Appendices G.1** and **G.2**) with the study information statement (see **Appendix H**) were posted out to those who had been in recent contact with the hospital or lymphoedema service. Tracing letters were sent to those who had not been in contact for more than two years. Those who did not respond to initial contact after two weeks were followed up by phone to check their interest in participating.

Private therapists working in Victoria were advised of the study in an email (see **Appendix G.3**), circulated by the secretary of the Lymphoedema Practitioners Education Group of Victoria. The email invited private therapists to provide study information and contact details to potential participants.

Participants in NSW, recruited through Mt Wilga Private Rehabilitation Hospital Sydney, were invited by hospital staff if they were known to have primary lymphoedema, and were currently receiving or had in the past received services from the Lymphoedema Clinic. All potential participants replied directly to the primary investigator by phone or email.

To facilitate participation by people with no lymphoedema, three further suburban sites (Surrey Hills Medical Centre, Vermont Health and Lifestyle, and Victorian Lymphoedema Practice) were approved for recruitment in Victoria (in protocol Version 8).

4.3.2 Inclusion and Exclusion Criteria

Potential participants with primary lymphoedema were phoned to confirm their eligibility using a screening questionnaire (**Appendix J**) of inclusion and exclusion criteria. Inclusion criteria were a diagnosis of primary lymphoedema from a qualified lymphoedema therapist or doctor, and aged between three and forty years. Exclusion criteria included: pregnancy, due to the possibility of additional swelling of venous origin (Rasmussen et al., 2020); any skin condition such as dermatitis or eczema, due to their inflammatory effect on the dermis; uncontrolled cardiac, embolic, or thrombotic conditions, due to the risk of applying compression in such conditions, as well as the difference in the distribution of dermal fluid in swelling of venous origin; connective tissue

conditions such as Marfan's Disease or inflammatory or infective conditions such as rheumatoid arthritis or cellulitis, due to their effect on the fluid and collagen content of the dermis; and active metastatic disease, due to safety concerns (contraindication for intermittent pneumatic compression) and possible effect on the dermis. The presence of a cardiac pacemaker excluded any participant from bioimpedance (ImpediMed Limited, 2016) but allowed inclusion for other outcome measures. Once eligibility was confirmed, people with primary lymphoedema were sent the information statement and consent form and were allotted an attendance time and date. Participants needed to attend once only. Written consent was provided by mail prior, or on the day of attendance.

4.3.3 Participants Without Primary Lymphoedema (NLO)

The number of participants with primary lymphoedema was expected to be small, due to the low prevalence of primary lymphoedema, so case control matching by age, gender, and ethnicity with people without lymphoedema (NLO) was undertaken. Participants with primary lymphoedema were asked to invite a friend of the same age, ethnicity and gender with no lymphoedema to participate in the study as a 'buddy'. If a PLO participant did not provide a buddy, the sourcing of NLO participants matched by age, gender and ethnicity was undertaken by word of mouth through colleagues, friends, and church networks of the primary investigator.

Potential NLO participants who responded by phone or email to the primary investigator were screened for absence of lymphoedema, and to confirm matching for gender, ethnicity, and age within a year of the matched PLO participant. The same exclusion criteria were applied as for the PLO participants. On receiving the full study information statement, NLO participants were given the opportunity to confirm or decline their interest in participating. Again, written consent was provided by mail prior or on the day of attendance.

4.3.4 Participant Home Preparation

The presence of hair can affect adhesion of electrodes and clarity of ultrasound images. As well, the presence of gels, creams or soap can affect skin measures, particularly the MoistureMeter. Therefore, hirsute participants were asked to shave the testing and electrode sites the day prior to attendance using the lymphoedema shaving protocol (National Breast and Ovarian Cancer Centre, 2013).

On the day of attendance, participants were advised not to apply moisturiser to the skin, and for

two hours prior to attendance, not to exercise, or drink caffeine (coffee or tea, sports drinks such as Red Bull or cola), and for 12 hours prior, not to drink alcohol (if applicable). Participants were advised to wear light, loose-fitting clothing and bring something to do while lying down, such as a book, an iPod with music to listen to or an iPad with a movie to watch. Parents of children were reminded to bring snacks, favourite toys, or an iPad.

4.4 Procedure for Assessment at Study Visit and Outcome Measures

On arrival, participants were asked to visit the bathroom to establish baseline hydration status for bioimpedance measures, according to manufacturer's instructions (ImpediMed Limited, 2016). Height, taken using a stadiometer and weight, taken without shoes on a portable scale (Centres for Disease Control, 2011), were recorded for all participants. PLO participants then removed any compression garments. Stage of lymphoedema was noted by visual assessment of the skin. The presence of pitting denoted early stage II and skin changes such as thickening or early fibrotic changes such as papillomatosis denoted late stage II; loss of pitting with marked skin changes denoted stage III. All participants rested in a supine position on an examination couch with a pillow under their head for 20 minutes prior to baseline measurements. During this resting time, they completed the Attendance Questionnaire (**Appendix K**), relating to matters such as history of cellulitis and measurement sites were marked on both legs (**Section 4.5.1**).

Participants with primary lymphoedema (PLO) were asked to nominate which side was worse affected, as IPC was to be applied to that limb. If participants with bilateral PLO found it difficult to nominate which side was worse than the other, the side with the largest foot by circumference at the dorsal foot site was chosen. IPC was applied in those with no lymphoedema (NLO) on the side corresponding to their matched PLO participant. Measures were taken on both lower limbs: monitoring the untreated limb provided a control comparison for orthostatic effects (Kushner et al., 1996).

4.4.1 Sites for Outcome Measures

To standardise measurement sites for HFU image capture, MoistureMeterD Compact (MMDC) and the Indurometer an adhesive template was applied following circumferential measurements (see **Table 4.1**). Measurement sites were marked medially and laterally with a water-soluble skin pencil, standardised along each side of the limb using the distance from the foot plate of a Jobst measuring board (see **Figure 4.1**) as per ALA measurement guidelines (Australasian Lymphology Association, 2004).

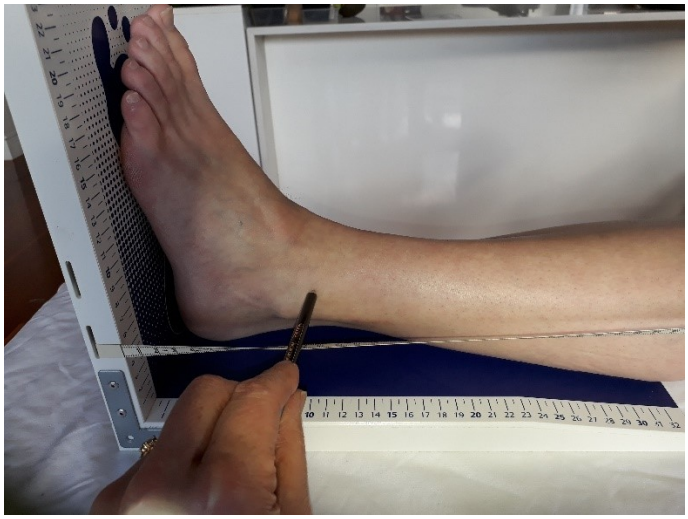


Figure 4.1 Marking Leg on Jobst Measuring Board

4.4.2 Circumferences

Circumference measurements were taken at 1) the dorsum of the foot, and 2) the posterior calf (described in full in **Table 4.1**). One plastic tape measure was used for all circumferential measurements. Reliability was assessed at these same sites, as reported in **Chapter 3, Section 3.2**.

Table 4.1 Measurement Sites and Levels for Circumferences

Table 4.1 Measurement Sites and Levels for Circumferences	
Measurement Site	**Marking of site**
Dorsum of foot * situated between the second and third metatarsals, proximal to the metatarsophalangeal joint (MTP).	The circumference level was marked on the lateral edge of the foot, just proximal to the MTP joint. This level was noted against the Jobst board, then also marked medially.
Least ankle line situated at the point of least circumference on the leg.	The minimum ankle circumference line was marked on the affected leg laterally with reference to the Jobst board (to enable leg length measures and positioning of posterior calf measurement site). Distance from base of foot was noted and both the medial side and contralateral leg were marked at the same level.
Posterior calf measurement site * Situated half-way from the knee joint line to the least ankle line, at the mid-point across the posterior calf.	In supine, a tape measure was used to find the point half-way from the knee joint line to the least ankle, which was then marked medially and laterally. On turning prone, the mid-point across the posterior calf site was marked.
Knee joint line	The knee joint line was located by manual palpation and marked laterally. A bioimpedance electrode was later placed at this lateral knee line.

***** Measurement sites for MMDC, Indurometer and HFU. Circumferences were also recorded at these levels.

4.4.3 MoistureMeterD Compact: Percent Water Content

The MoistureMeterD Compact (MMDC; Delfin Technologies Ltd Kuopio Finland) is a small hand-held instrument with a head of 20mm which is held against the skin (see **Figure 4.2**). The MMDC measures the tissue dielectric constant and displays it as a percentage water content (PWC): the higher the reading, the higher the percent water in the tissue. Correct and consistent application of the MMDC is facilitated by a pressure sensor which indicates high, low, or ideal skin contact.

As per manufacturer's directions the MMDC was rested on the skin for approximately five seconds at each measurement site, and measures with ideal skin pressure were recorded both manually and electronically. Three measures were taken immediately one after the other at each site, with

the participant in supine, beginning on the dorsum of the left foot, then the right foot. The participant then turned to prone. The adhesive template was then applied with reference to the marks on the posterior calf made during the marking procedure (**Table 4.1**) and measures were taken on the posterior calf.

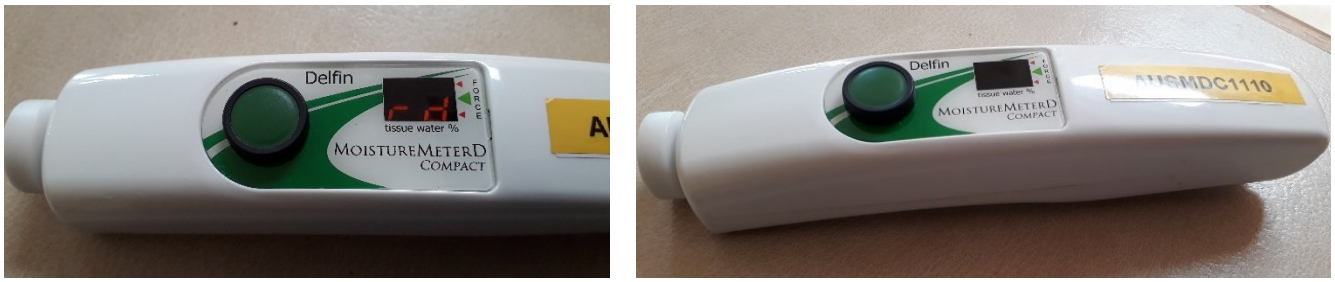


Figure 4.2 The MoistureMeterD Compact

Left: PWC screen displays 'ready'. Right: whole unit. During operation, the circular head at the left end is lightly rested on the tissues.

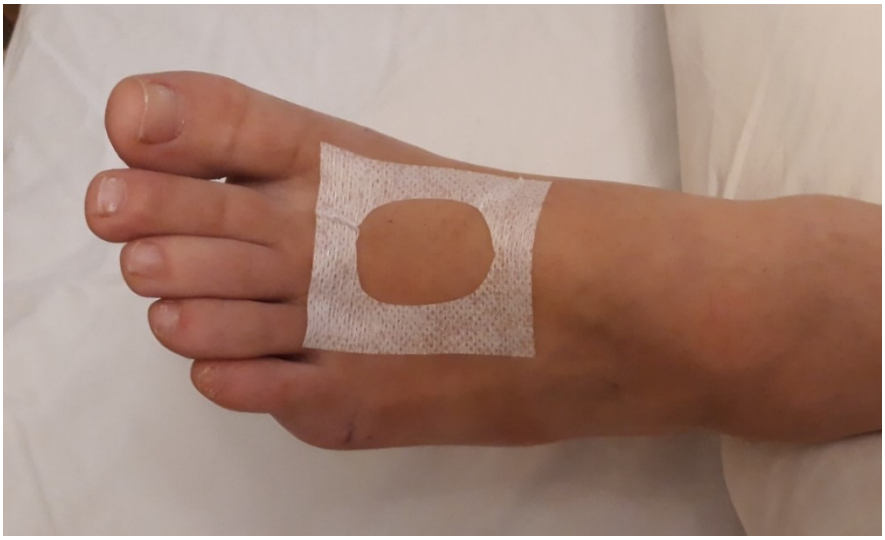


Figure 4.3 Position of Template on the Dorsum of the Foot

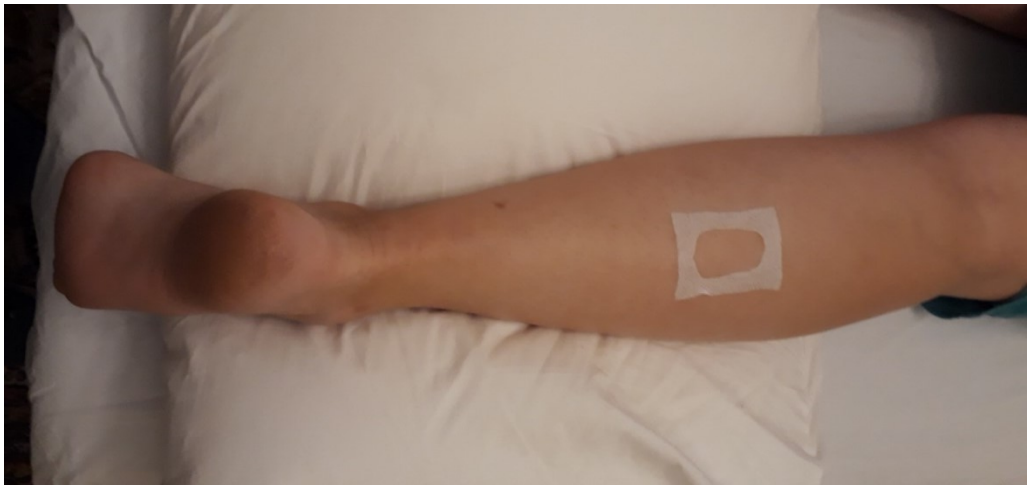


Figure 4.4 Posterior Calf Template

4.4.4 High Frequency Ultrasound: Low Echogenic Pixels (LEP)

The DermaScan C high frequency ultrasound (Cortex Technology, Hadsund, Denmark) was used to assess dermal fluid content (Cortex Technology, 2014). The gel used to maintain contact between the probe and the skin, Dane-Gel R1 (Rohde Produits, Denmark), was supplied with the DermaScan C. Distilled water was used in the probe as per manufacturer's instructions and was replaced after each image capture session. Image analysis software within the DermaScan C was used to identify the region of interest, within which the dermal fluid content was measured. Area measures representative of low echogenicity in pixels (LEP) were extracted for analysis. One image of the same gain setting was captured at each site. Full methodology and reliability for image capture and measurement is described in the HFU reliability study (see **Chapter 3, Sections 3.1.3.3, 3.1.3.4** and **3.1.6;** also (Phillips et al., 2020) in **Appendix C**).

Water-based gel was applied to the slot (see **Chapter 3**, **Figure 3.1a and b**) in the head of the high frequency ultrasound (HFU) probe. The probe was placed on the skin in the centre of the adhesive template at measurement sites. (Alignment of the entrance echo with marks on the ultrasound screen monitor ensured the probe was held perpendicular to the skin. Images were captured on the posterior calf measurement site (described in **Table 4.1**) on both legs before the participant turned supine for images to be taken on the dorsum of the foot. For the foot image capture, a pillow was situated under the leg so that the foot was as horizontal as possible (**Figure 4.4**). Gel was replaced after each time the probe left the skin. If image clarity was not acceptable due to the presence of bubbles or flaws causing 'shadowing', the gel was replaced in the probe, and a repeat set of images was captured.

4.4.5 Indurometry : Induration Units

The Indurometer (Model BME 1563G, see **Figure 4.5**) is a hand-held instrument (Flinders Biomedical Engineering (BME), Flinders Medical Centre, South Australia) which rests on the skin and, following downward pressure equivalent to 200g, produces a measure of tissue resistance in 'induration units' (IU). A higher reading indicates greater compressibility and less tissue resistance, while a lower reading indicates less compressibility and greater tissue resistance. At the beginning of each day, the Indurometer was calibrated using the manufacturer-supplied 200g weight, in accordance with manufacturer's instructions (Flinders University Biomedical Engineering, 2013). (See also **Chapter 3, Section 3.3** Indurometer Reliability.)

Figure 4.5 The Indurometer

With the participant prone and a pillow under their leg three measures were taken alternately on the left and right posterior calf. The Indurometer was applied to each site alternating between sides for each reading until three measures had been taken for each site. The participant then turned to supine and three measurements were taken alternately on the dorsum of the feet. A pillow was rested against the sole of the foot to support the foot during the downward pressure of the Indurometer (see **Chapter 3, section 3.2.2**). Measures were recorded manually and later copied to an Excel spreadsheet.

4.4.6 Bioimpedance and Electrode Placement

The SFB7 (Impedimed Limited, Unit 1 50 Parker Court, Pinkenba Qld 4008 Australia) was used in bioimpedance spectroscopy (BIS) mode to measure tissue bioimpedance in whole lower limbs and lower limb segments.

4.4.6.1 Participant preparation

Participants removed any jewellery from within the area to be measured (Ward, 2015) and during measurement, lay supine with legs apart.

4.4.6.2 Analysis of bioimpedance measurements

The distinctive shape of the Cole plot indicates a valid measure has been captured (ImpediMed Limited, 2016) and this was inspected at the time of each measurement. If the Cole plot indicated a measure that was not valid, the participant's position and the electrodes were checked for adherence and the measurement repeated.

The resistance ratio of extracellular fluid to intracellular fluid (R_i/R_0) was the outcome of interest. Both R_0 and R_{inf} are produced in the SFB7 (ImpediMed Limited, 2016) and internal software calculations within the SFB7 provide the resistance (R_i) representative of intracellular fluid (ICF), using the formula:

$R_i = (R_0 \times R_{inf})/(R_0 - R_{inf})$ (Steele et al., 2018).

The SFB7 was calibrated each day by attaching electrode leads to a calibration cell and calibrated according to manufacturer's instructions (ImpediMed Limited, 2016).

4.4.6.3 Electrode Placement for Limb Segments

The skin was cleaned with alcohol wipes prior to electrode placement (ImpediMed Limited, 2016; Ward, Winall, et al., 2011) of Impedimed single tab gel-based electrodes, which should be used within an hour of application (ImpediMed Limited, 2016). Electrode placement was developed in discussion with an international expert in bioimpedance spectroscopy and consultant to Impedimed Ltd (L. Ward, personal communication, July 20, 2018).

Drive electrodes were placed on the foot on the distal phalanx of the third toe (affected side; black lead), and the wrist joint line of the contralateral hand (red) in accordance with manufacturer's instructions (ImpediMed Limited, 2016) (see **Figure 4.6**).

Measurement (sense) electrodes (blue and yellow) were placed at the boundaries of each segment and ensuring there was five centimetres between electrodes:

Foot segment: Electrodes were placed on the metatarsophalangeal joint line (blue; over the 3^{rd}-4^{th} interosseous spaces) and the anterior ankle joint line (yellow)

Leg segment: Electrodes were placed on the anterior ankle joint line (blue) and the lateral knee joint line (yellow)

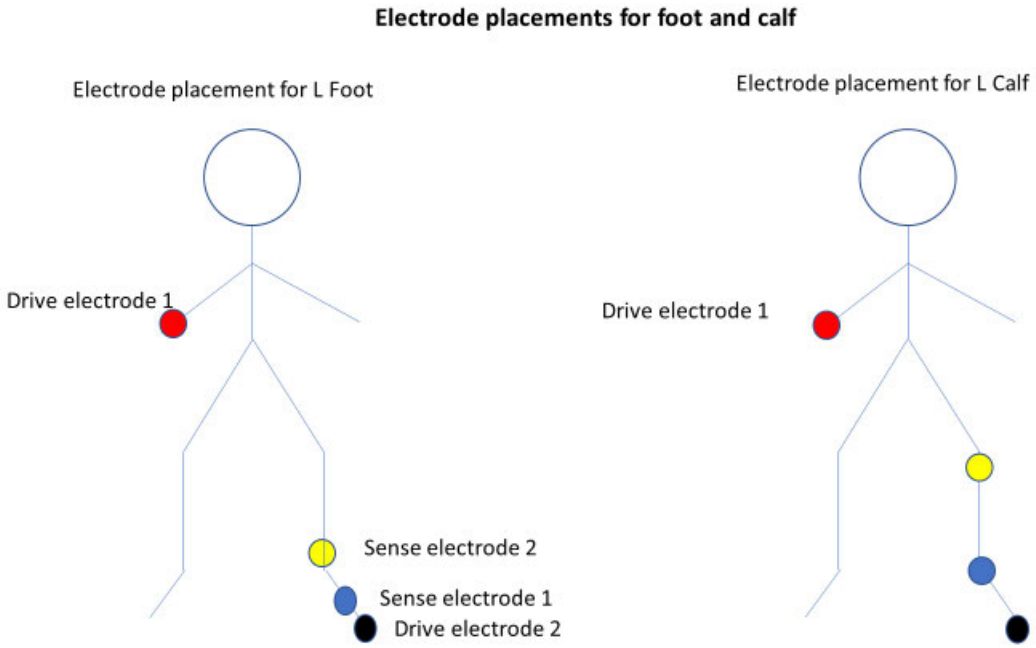


Figure 4.6 Electrode Placements for Foot and Calf Segment Measurement of Bioimpedance

4.4.6.4 Electrode Placement for Whole Limb Measure

Drive electrodes were placed as above: on the distal phalanx of the third toe (affected side) (black lead), and the wrist joint line of the contralateral hand (red).

Measurement electrodes were placed on the affected leg, anterior ankle joint line (blue lead) and contralateral ankle joint line (yellow lead) in accordance with principles of equipotential (Cornish, Eles, et al., 2000; Cornish et al., 1999) **(Figure 4.7)**.

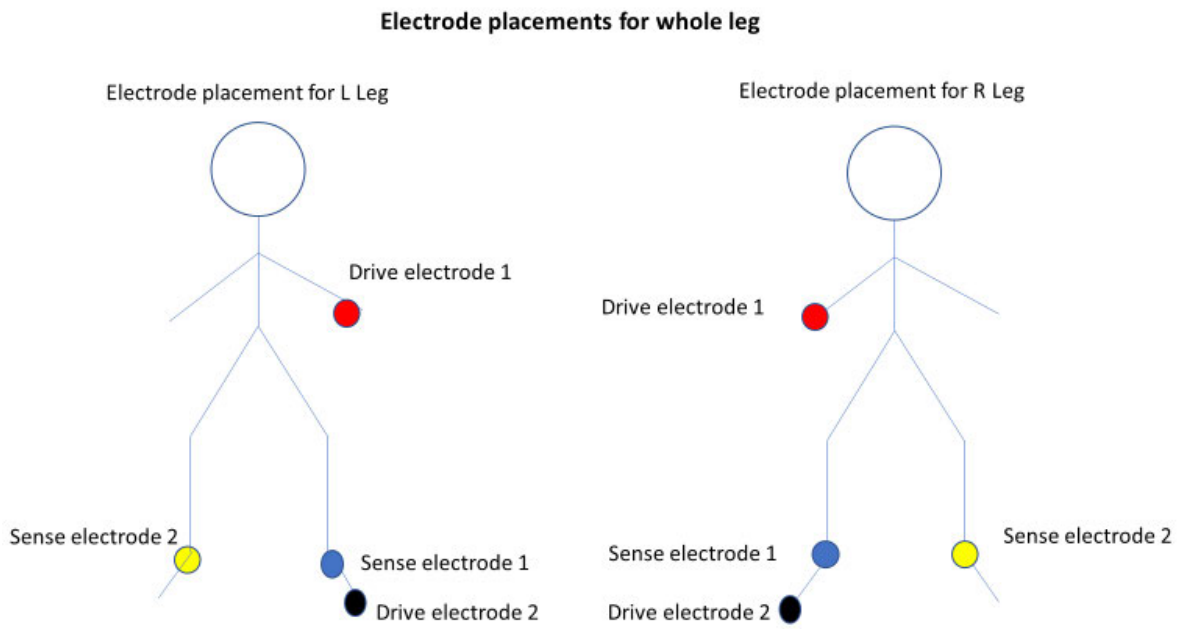


Figure 4.7 Electrode Placements for Whole Lower Limb Measurement of Bioimpedance

The distal segment on the affected side (the foot) was recorded prior to moving leads to measure the leg, then the full limb was recorded before measurement of the contralateral foot, leg segments and whole limb. Electrode positions were mirrored on the opposite side for the contralateral leg. The device was set to record three repeated measures. The Cole plot of each third measure was checked for shape indicating an acceptable measure as per manufacturer's instructions (ImpediMed Limited, 2016) before moving to the next site; measures were repeated if the Cole plot was an unacceptable shape. R_0 and R_{inf} (resistances at frequency approximating zero and infinity) were recorded manually as a back-up of the data. Electrodes were left in place during intermittent pneumatic compression and then bioimpedance measures were repeated immediately after. Electrodes were then removed.

4.4.7 Order of Measurements

A specific order of measurements was followed, so that no measurement should potentially influence another taken at the same anatomical site. For example, the water-based gel of the HFU had potential to influence the percent water content of the MMDC, or the pressure from the Indurometer to influence the distribution of fluid in the dermis in HFU images. The order before intermittent pneumatic compression (beginning in supine) was:

1. Circumferences of foot and calf, then the template was applied to the foot.

2. MoistureMeterD Compact on the dorsum of the foot.
3. **Turn to prone**: template applied to calf site; MoistureMeterD Compact on posterior calf.
4. HFU image capture on posterior calf
5. Indurometer on posterior calf
6. **Turn to supine**: HFU image capture on dorsum of foot.
7. Indurometer on dorsum of foot
8. Electrode sites cleaned, then electrodes were applied. Bioimpedance of all segments, beginning with the affected foot, then the leg, then the whole limb, followed by the contralateral foot, leg, and whole limb.

Following intermittent pneumatic compression:

1. Bioimpedance measures were taken first, to minimise time the electrodes were on the skin (ImpediMed Limited, 2016) and followed the same order as prior to IPC: beginning with the foot, then the leg, then the whole leg, followed by the contralateral foot, leg, and whole leg. The electrodes were then removed.
2. MoistureMeterD Compact on dorsum of foot.
3. **Turn to prone**: MoistureMeterD Compact on posterior calf.
4. HFU image capture on posterior calf
5. Indurometer on posterior calf
6. **Turn to supine**: HFU image capture on dorsum of foot.
7. Indurometer on dorsum of foot
8. Templates were removed before circumferences of foot and calf were taken.

4.5 Intervention: Intermittent Pneumatic Compression (IPC)

The same IPC unit, the LX9 supplied by Medi-Rent Pty Ltd, Matraville NSW 2036 and four chamber inflatable leg sleeve were used for all IPC applications. No pressure setting assessments or

Figure 4.8 Inflatable Four Chamber Leg Sleeve for the LX9 IPC Unit.

(Photo used with permission of Medi-Rent.) Note a single leg sleeve may be applied.

calibration procedures were advised by the manufacturers.

4.5.1 Side of treatment

If bilateral PLO was present, participants were asked to nominate which leg was the more affected, and the intervention was applied to that leg. This side was called the treated side and the lesser affected side called the untreated side. In those with unilateral lymphoedema, the side with lymphoedema was the treated side. The side treated in NLO was matched to their PLO counterpart's treated side.

After baseline measures, participants were positioned supine with a pillow longitudinally under the treated limb for application of intermittent pneumatic compression (IPC). As per standard IPC protocol, lymphatic drainage techniques were applied to all participants (NLO and PLO) (deep breathing, nodal massage over inguinal nodes) (Queensland Health, 2014). A participant-specific loose cotton liner was applied to the treated limb for hygiene purposes, over which the inflatable sleeve was applied. A rigid footplate was inserted into the inflatable sleeve under the sole of the foot, as per manufacturer's instructions.

4.5.2 Dosage

The pressure and time for treatment was chosen based on a systematic review of IPC dosage in lymphoedema which provided limited information on specifically applicable dosage (Phillips & Gordon, 2019) (see **Appendix B**); consequently, a conservative approach was taken in applying the lower end of the scale for the younger age group. Dosage was adapted for young adults by applying 10mmHg less pressure than in adults (Hassall et al., 2001; McLeod et al., 1991) (see **Table 4.2**). The LX9 enabled the application of compression to only the distal three sleeve chambers for shorter limbs.

The 50-minute treatment consisted of IPC Mode A (pre-clearance treatment cycle of the proximal before distal segment of the limb), before IPC Mode B (treatment cycle beginning distally, inflating successive chambers proximally along the limb until all were inflated, before all simultaneously deflating) (International Lymphoedema Framework, 2006; Queensland Health, 2014). The sixth setting for cycle timing of inflation and deflation on the LX9 was used, as it had the least deflation time, so provided the most continuous compression.

Table 4.2 Intermittent Pneumatic Compression Dosage

Table 4.2 Intermittent Pneumatic Compression Dosage		
Age group	**Pre-clearance**	**Treatment**
Adults 19-40 years*	10 minutes 30 mmHg	60 mmHg 40 minutes
Young adults 11- 18 years**	10 minutes 30 mmHg	50 mmHg, 40 minutes

* Phillips & Gordon (2019).
** Hassall et al (2001); McLeod et al (1991).

All participants were monitored for comfort during IPC application. If IPC became uncomfortable for any participant at any time during the intervention, the device was paused. The limb was checked for signs of injury or excessive pressure, especially around the foot; the footplate was checked and the IPC restarted with monitoring, in discussion with the participant.

Following IPC, all measures were repeated in the order specified in **Section 4.4.7**.

4.6 Data Management

4.6.1 Accuracy Checking and Missing Data

All data measures were twice copied onto Excel spreadsheets which were compared for accuracy. Errors identified were corrected against original data records. Data evaluation prior to analysis included screening for missing data, and outlier data that might indicate an error was checked against raw data. Missing data was examined and reported in the results of each analysis. Any data points missing for one participant (whether due to equipment malfunction or contra-indication) resulted in removal of the same data point for their matched pair. This ensured that all analyses included only matched pairs. Sensitivity analyses were carried out by re-running analyses on all available data to investigate the effect of the removal of non-matched data. There was no difference in significant outcomes using all available data compared to those where matches had been removed.

4.6.2 Data Management Within the PLO group

The measures of the untreated (less affected) limb were taken at baseline, as a control to measure the potential effect of lying supine. However, as the PLO group included people with both unilateral and bilateral lymphoedema, there was potential for differences at baseline. The PLO group was sub-divided into bilateral (biPLO) and unilateral (uniPLO) and the difference between

groups was investigated on each side for each outcome measure. For outcome measures where significant difference was found between the two sub-groups, PLO were divided into uniPLO and biPLO for comparison against NLO.

4.6.3 HFU Data Management

The segmented area measure of low echogenic pixels was the sole measure extracted from HFU images, as per the procedure in **Chapter 3** (see **Section 3.1.6 Image Capture Modifications**)**.** Measures were extracted from a single image following the high reliability established by the reliability study (**Chapter 3**).

4.6.4 Bioimpedance Data Management

Raw bioimpedance measures were uploaded from the SFB7 using Bioimp software (Impedimed Ltd) into an Excel spreadsheet. Values of R_0 and R_i from each set of three raw measures were used to calculate three ratios of R_i/R_0 and the mean of the three measures was used for within group and between group comparisons of limb segments.

4.6.5 MoistureMeterD Compact and Indurometer Data Management

The mean of three measures for each of the Indurometer and the MoistureMeterD Compact (MMDC) was used for analyses. The MMDC required calibration in August 2019, approximately halfway through data collection. The calibration certificate (**see Appendix D)** reported a pre-calibration variation of 4% in the ethanol/water concentration test. Hence, data under 44 PWC that were measured prior to calibration were adjusted down by 4% (multiplied by 0.96), in consultation with the manufacturers (J. Pärnänen, Delfin Technologies, personal communication October 12, 2020) and the local engineer who carried out the calibration.

4.7 Statistical Analysis

All analyses were carried out using IBM SPSS version 25 (IBM Corp, 2017). Normality was assessed by scrutinising skewness and kurtosis, the Shapiro Wilkes statistic and noting outliers. Descriptive data of central tendency and spread was extracted. The raw data of outliers were investigated for error.

4.7.1 Group Information

4.7.1.1 Confirming the Effectiveness of Matching.

Participants in the two groups (PLO and NLO) were matched on gender, ethnicity, and age.

However, given the small number of characteristics on which the two groups were matched, analysis of differences was carried out between the two groups, rather than of paired participants. Investigations for normality informed the choice of test of difference between groups. The Mann-Whitney U Test was used to confirm the efficacy of matching based on age, expected to be non-significant and to investigate between group differences in BMI.

4.7.1.2 Verification of Lymphoedema Status

Thresholds for lymphoedema were used to verify lymphoedema status. To confirm the lymphoedema status of PLO, the group was divided into uniPLO (unilateral PLO) and biPLO (bilateral PLO) for comparison against appropriate bioimpedance thresholds for lymphoedema. The threshold for unilateral lower limb lymphoedema, established using R_0 inter-leg ratios (and based on the mean plus 3SD), is 1.144 for male and 1.167 for female (Ward, Dylke, et al., 2011b). Calculating the mean less 3SD produces a range of (.844, 1.144) for males and (.831, 1.167) for females; unilateral PLO were compared against this range (see **Table 4.3**.) Inter-leg ratios of the affected to unaffected limbs were calculated for comparison against corresponding normative ECF/ICF (R_i/R_0) data (Steele et al., 2018) for NLO and ECF (R_0) for unilateral PLO (Ward, Dylke, et al., 2011b).

Inter-leg ratios in bilateral lower limb lymphoedema provide little information; there is no published threshold for establishing lymphoedema in bilateral lower limb lymphoedema using inter-leg bioimpedance ratios. To establish the presence of lymphoedema in a bilateral lower limb lymphoedema population, the R_i/R_0 ratio, which can be used for comparisons of differently sized limbs (Cornish et al., 2002; Dylke & Ward, 2020), was compared in the limb of interest between PLO and NLO. The difference between NLO and PLO groups was investigated by mixed ANOVA, using the full leg R_i/R_0 for the more affected side in biPLO, the affected side in uniPLO and the side matched to their PLO counterpart in NLO, within factor of side and between factor of LO group. (The use of an arm-to-ipsilateral leg ratio for establishing lymphoedema in bilateral lower limb lymphoedema was published (Steele et al., 2018, 2019), after the methodology and data collection for this study.)

In confirming the non-lymphoedema status of NLO, full limb impedance measures provided the mean inter-leg R_i/R_0 ratio which was compared to the mean inter-leg R_i/R_0 ratio in normative data. The mean (SD) normative inter-lower limb R_i/R_0 ratio of 1.024 (0.183), established in the healthy

population, used the ratio of dominant lower limb: nondominant lower limb (Steele et al., 2018). However, as dominance has been found to have little effect in the lower limbs (Ward, Dylke, et al., 2011b), the inter-limb ratio of affected to non-affected lower limb was compared, using the side in NLO that was matched to their PLO counterpart as the 'affected' lower limb.

Table 4.3 Normative Inter-Leg Ratio and Threshold for Unilateral Lower Limb Lymphoedema

Table 4.3 Normative Inter-Leg Ratio and Threshold for Unilateral Lower Limb Lymphoedema in Males and Females		
	Mean (SD)	**Range** (Within 3SD)
Normative Inter-leg ratio (R_i/R_0) *	1.024 (0.183)	0.910, 1.138
Inter-leg (R_0) ratio for establishing unilateral lower limb LO: Male **	0.994 (0.050)	0.844, 1.144
Inter-leg (R_0) ratio for establishing unilateral lower limb LO: Female **	0.999 (0.056)	0.831, 1.167

* Steele et al (2018) ** Ward et al (2011b)

4.7.1.3 Investigating Differences Between Bilateral PLO and Unilateral PLO

Within PLO group characteristics were explored between those with unilateral and bilateral PLO using a mixed ANOVA with within factors of side and leg-part and a between factor of lymphoedema status (unilateral or bilateral).

4.7.1.4 Demographics

The independent T-test was used to investigate between group baseline differences in mean BMI. PLO participants were further described by their mean duration of lymphoedema, and the side 'affected' by lymphoedema.

4.7.2 Fluid Distribution and Tissue Resistance of People With and Without Primary Lymphoedema

The between group (PLO and NLO) differences were analysed for LEP, IU, and PWC measures for each site in the affected, less affected, and unaffected limbs. The difference within each group between sites and sides was also investigated. These comparisons were made using the mixed

ANOVA, within factors of site and side, and a between factor of group (PLO or NLO). Comparisons between three groups (NLO, uniPLO and biPLO) were made using univariate ANOVA. Analysis of the whole limb ECF/ICF (R_i/R_0) was calculated in a mixed ANOVA separate from the ECF/ICF analysis of the foot and leg. Residuals were investigated for normality. As there are no non-parametric versions of the mixed ANOVA, limitations of any violations of normality are discussed in the results.

Multiple comparisons and small sample. The alpha level for statistical significance was set at 0.05. Given the small sample size and high number of comparisons planned, the Bonferroni adjustment was used a priori in all analyses using mixed or univariate ANOVA. Where the sample was divided into sub-groups of even smaller size, Tukey's adjustment in post hoc analysis was applied (Field, 2018) in univariate ANOVA. No adjustment was made for multiple comparisons: the more conservative Bonferroni adjustment was used in view of the small sample size and significance was discussed with reference to clinical meaning (Feise, 2002).

Clinical significance was applied to interpret the clinical impact of statistically significant results. In measures where the reliability was known, the minimal detectable difference (MDD) was calculated by the formula

MDD= 1.96xSEMx√2 (Portney & Watkins, 2015, pp. 645-646), where SEM is the standard error of measurement. SEM is calculated by

SEM=SD√(1-ICC) (Portney & Watkins, 2015, pp. 608-609). The $ICC_{(3,1)}$ for each measure was sourced from the reliability studies of circumferential measurement, indurometry and HFU. The $ICC_{(3,1)}$ for PWC was calculated from the final data set, as three repeated measures were recorded.

Clinical significance was deemed present where a difference was found beyond the threshold of the MDD, where MDD was available. Further impact of the difference was considered from the perspective of a therapist and person with lymphoedema, for example, where there was also a skin change that may be assessed clinically such as Stemmer's sign (Goss & Greene, 2019) or that could make a difference to a person with lymphoedema.

4.7.3 Convergent Validity

The relationships between echogenicity, extracellular fluid, percent water content and induration were explored visually by scatter plot. Outliers were investigated for data error. Baseline outcome

measures in both groups were investigated for the strength and direction of their relationship with each other using Spearman Rank Order correlation (rho) with 95% confidence intervals for correlation generated by bootstrapping in IBM SPSS. In acknowledgement of the small sample, resulting from the recruitment challenges of a rare condition, and the challenges of normality in a small sample size, Spearman's rho was chosen as the conservative co-efficient of correlation (Field, 2018, p. 344). Based on the interpretation used in Portney and Watkins (Portney & Watkins, 2015, p. 525), strength of association was rated as little or no relationship for rho values of 0.00 to 0.25; fair, 0.25 to 0.5; moderate to good, 0.50 to 0.75; and good to excellent relationship for values above 0.75.

4.7.4 Analysis of Response to Compression

The response to compression within each group was analysed by mixed ANOVA using a between factor of lymphoedema group (PLO/NLO) and within factors of side (treated and untreated) and time (before and after IPC). Analyses were calculated separately for the foot and leg.

The difference between groups was investigated for both their response to compression (treated side) and resting supine (untreated side). Three groups (NLO, unilateral and bilateral PLO) were compared for changes in echogenicity and two groups (NLO and PLO) for changes in clinical tools (bioimpedance, percent water content, circumferences and indurometry), using univariate ANOVA. Results were taken from Tukey's adjustment in post-hoc analysis given the smaller sample size that resulted from sub-division of PLO, resulting in samples of seven or eight (Field, 2018, p. 657).

CHAPTER 5 RESULTS

This chapter contains the results of recruitment, confirms the lymphoedema status of participants with (PLO) and without (NLO) primary lymphoedema and answers the core research questions:

1. What are the differences in fluid distribution and tissue resistance between people with and without primary lymphoedema?
2. Is there a difference in the response to compression in fluid distribution or tissue resistance between people with and without primary lymphoedema?
3. Is there convergent validity between any clinical measures of fluid and tissue resistance?

5.1 Results of Recruitment

Invitations or contact tracing letters were sent to 136 potential PLO participants, comprising 19 sourced from RCH, 88 from Mercy Health, eight from private therapists in Victoria, and 21 from Mt Wilga Private Rehabilitation Hospital, Sydney. Thirty-five people responded to the invitation and were sent further information. Of 29 screened (see **Figure 5.1**), one was excluded for Ehlers Danlos and local psoriasis, two for co-morbidities affecting pain perception, thermoregulation, or ability to communicate (intellectual disability), and one was of an ethnicity that could alter skin measures related to increased pigmentation. One presented with upper limb primary lymphoedema, and three were under the age of eleven. Due to links with The Royal Children's Hospital, Melbourne, it was expected that more children with primary lymphoedema would be recruited. However, only three prepubertal children were enrolled, and, due to concerns about skewing the data, these three were excluded from analysis for this thesis. One with upper limb lymphoedema was excluded for similar reasons, as the total sample size was too small for stratification. A case study of the matched upper limb pair was presented at the ALA Conference in May 2022 and results of the prepubertal matched pairs are planned for publication as a case series. Four people withdrew between the screening process and participation, leaving sixteen PLO participants. Two people without lymphoedema (NLO) participated as the outcome of a PLO buddy invitation; all others were sourced by word of mouth and through colleagues. Of the 21 NLO screened, two were excluded due to ethnicity-related skin pigmentation, one for having a tattoo on the foot over the measurement site and two participants withdrew (due to time availability).

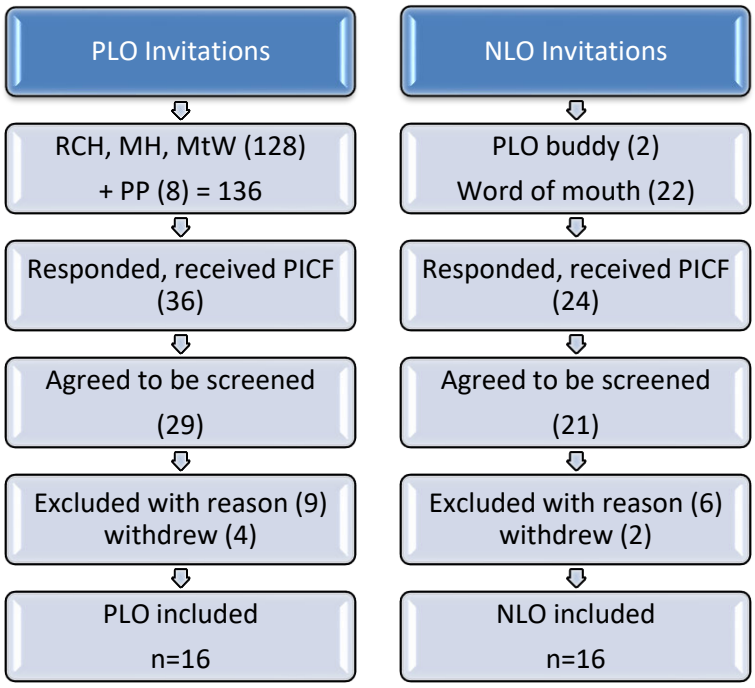


Figure 5.1 Recruitment Flow Diagram

RCH=Royal Children's Hospital; MH= Mercy Health; MtW= Mt Wilga Private Rehabilitation Hospital; PP= private lymphoedema practices

5.1.1 Characteristics of PLO and NLO

PLO participants Sixteen PLO were recruited, with median age 24 years: three males (11, 12 and 16 years) and thirteen females (13–40 years). Their Body Mass Index (BMI) ranged from 17 to 42, with a median of 24.2 (IQR 6.7) and they had lymphoedema for a mean duration of 12 years (13 months to 27 years). For four of the 16 PLO, the age of onset was at birth, although two of the four were not formally diagnosed until later in childhood. Unilateral lymphoedema (uniPLO) was present in eight participants (four on the left and four on the right), whereas eight were bilaterally affected (biPLO). Six each of uniPLO and biPLO were late onset. Fifteen PLO were early stage II by ISL lymphoedema staging, with one PLO late stage II (small trace of papillomatosis on two toes) and all were Caucasian.

NLO participants Matched NLO participants had a lower BMI (range 16 to 30, median of 22.4 (IQR 4.8)) than PLO participants, however, the difference was not significant (p=0.287). Hence PLO and NLO groups were similar in age, gender, ethnicity, and BMI at baseline.

5.2 Verification of lymphoedema status

5.2.1 Lymphoedema Status of NLO

The mean (SD) inter-leg ECF/ICF ratio for NLO participants of 1.002 (.038) was within one standard

deviation of the expected normative mean and range values for healthy people (1.024 (SD 0.183); 0.841, 1.207). This confirmed that all NLO participants did not have lymphoedema.

5.2.2 Lymphoedema Status of PLO

PLO participants were grouped by the presence of unilateral (uniPLO) or bilateral (biPLO) lymphoedema for comparison against appropriate bioimpedance thresholds to confirm lymphoedema status. This subdivision resulted in extremely small numbers for comparison, into six female and two male participants in the uniPLO sub-group and only one male and seven female participants in the biPLO sub-group. The mean (SD) inter-leg ECF (R_0) ratio of female uniPLO participants lay outside the range of expected normative inter-leg ECF ratios, confirming the presence of lymphoedema in the female uniPLO sub-group. There was a small overlap of the male uniPLO inter-leg ECF (R_0) ratio with the expected normative range (see **Table 5.1**).

For the bilateral PLO group (biPLO), full leg impedance measures of R_0 and R_i/R_0 for the most affected side were compared to available normal mean values for healthy people. Both male and female biPLO participants overlapped with the range of normative values of R_0 (see **Table 5.2**). A normative range for R_i/R_0 was not available, but a normative mean R_i/R_0 was calculated from published data for comparison with biPLO R_i/R_0.

The overlap of bioimpedance values for PLO participants with normative ranges is likely due to the measurement protocol to establish lymphoedema using bioimpedance excluding the foot. In this study, the clearest distinction between groups was at the foot. The distribution of fluid in PLO compared to NLO in ECF/ICF (R_i/R_0), used for all subsequent comparisons, is demonstrated in **Figure 5.2**.

The impedance values ECF/ICF (R_i/R_0) for the most affected limb in the entire PLO group compared against those of the matched limb of NLO were significantly higher in PLO than NLO (p=.006) (see **Table 5.3**).

Table 5.1 Comparison of Normative and UniPLO Inter-Leg Bioimpedance Ratios

Table 5.1 Comparison of Normative and UniPLO Inter-Leg Bioimpedance R_0 Ratios			
UniPLO Mean (SD)		**Normative Range** (3SD around mean)	
Female n=6	Male n=2	Female	Male
0.791 (0.187)	0.855 (0.023)	0.831, 1.167	0.844, 1.144

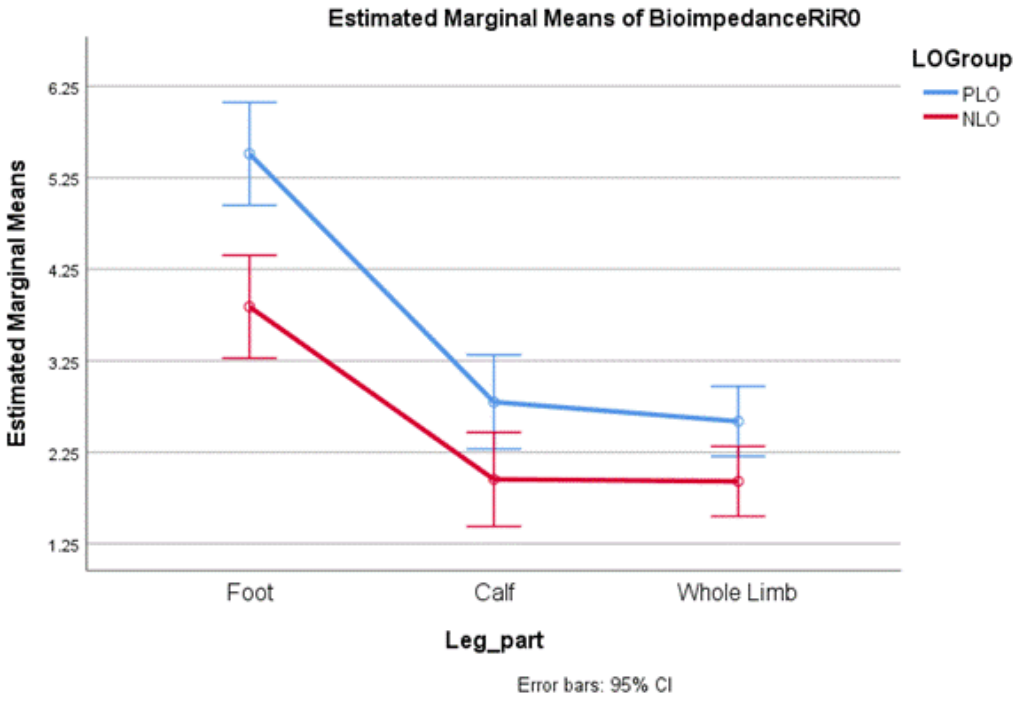


Figure 5.2 Lower Limb ECF/ICF in PLO Compared to NLO

Table 5.2 Comparison of BiPLO Against Expected Normative Values

Table 5.2 Comparison of BiPLO Against Normative Lower Limb Values				
Group	**Normative Limb** Mean (SD) Range*		**BiPLO Limb** (7F, 1M)	
	Male	Female	Male n=1 Mean	Female n=7 Mean (SD)
R_0	270.7 (39.2) 153.1, 388.3	301.0 (39.9) 181.3, 420.7	204.0	275.6 (56.0)
R_i/R_0	2.298**	2.426**	3.089	3.063 (1.750)

* Range within 3 SD either side of mean

** Calculated from Ward et al (2011b), using mean R_i and R_0 values for dominant leg.

Table 5.3 Comparison of PLO Most Affected Side With NLO and Normative Impedance Values

Table 5.3 Comparison of PLO Against NLO and Normative Lower Limb Impedance Values Ri/R0						
Normative Limb Mean		**PLO (n=13)**		**NLO (n=13)**		**PLO - NLO**
Male	**Female**	**Mean (SD)**	**95% CI**	**Mean (SD)**	**95% CI**	**Mean Difference (95% CI) p value***
2.298**	2.426**	3.151 (1.484)	2.582, 3.720	1.971 (.334)	1.402, 2.540	1.179 (.375, 1.984) 0.006*

*Significant p-value <0.05

** Calculated from Ward et al (2011b), using mean R_i and R_0 values for dominant leg.

5.3 Baseline Differences Between PLO and NLO

This section provides the results for the first research question: What are the differences in fluid distribution and tissue resistance between people with and without primary lymphoedema.

Significant baseline differences in LEP between the affected limbs of participants with uniPLO and biPLO supported sub-grouping for comparison with NLO (see **Appendix L.2**). For all other measures, no significant differences in baseline measures of affected limbs between uniPLO and biPLO were found. Hence for all analyses of these measures (ECF/ICF, PWC, IU), uniPLO and biPLO were grouped together for comparison with NLO.

For between group comparisons with NLO, only the affected side of uniPLO and the more affected side of biPLO were compared with NLO.

5.3.1 Baseline Differences in LEP Between UniPLO, BiPLO and NLO

Both uniPLO and biPLO had significantly more LEP in the foot than NLO, indicating higher dermal fluid in the foot of both uniPLO and biPLO than NLO (see **Table 5.4**).

The difference in mean LEP foot values between biPLO and NLO (LEP 993) was greater than the standard error of measurement (SEM 532) but was not greater than the minimum detectable difference (MDD: 1476). As well, there was a similar difference in mean LEP values between uniPLO and NLO (770 LEP). When considering the MDD, and the small samples size, some caution is cast over conclusions about the clinical relevance of these results.

Table 5.4 Baseline Differences in Low Echogenic Pixels.

Bilateral (more affected side) and unilateral PLO (affected side) compared with NLO

Table 5.4 Baseline Differences in Low Echogenic Pixels (LEP) [p values given for comparisons with NLO]			
Group (n)	**Bilateral (7)**	**NLO (15)**	**Unilateral (8)**
	Mean (SD) p value *	**Mean (SD)**	**Mean (SD) p value***
Foot	2863 (173) <0.001*	1870 (580)	2640 (329) 0.002*
Posterior Calf	1841 (421) 0.063	1335 (557)	1410 (260) 0.929

* Significant p value <.05 compared to NLO. Tukey's adjustment, post hoc tests.

5.3.2 Baseline Differences in ECF/ICF, PWC and IU Between PLO and NLO

Significantly higher PWC and ECF/ICF were identified in the feet of PLO when compared to NLO. As well higher PWC was present in the posterior calf and higher leg ECF/ICF in the PLO group when compared to the NLO group. There was no significant difference between NLO and PLO groups in IU (see **Table 5.5**).

Table 5.5 Baseline Differences Between PLO and NLO in ECF/ICF, PWC, IU and Circumference

Table 5.5 Baseline Differences Between PLO and NLO in ECF/ICF, PWC, IU and Circumference					
Group	**PLO**		**NLO**		
	Mean (SD)	**95% CI**	**Mean (SD)**	**95% CI**	**Mean Difference (95% CI) p value***
ECF/ICF (R_i/R_0) (n=13)					
Foot	6.114 (1.349)	5.468, 6.760	3.702 (0.854)	3.056, 4.349	2.412 (1.498, 3.326) <0.001*
Leg	3.096 (1.477)	2.480, 3.712	1.948 (.367)	1.333, 2.564	1.148 (0.277, 2.019) 0.012*
Whole Limb	3.151 (1.484)	2.582, 3.720	1.971 (0.334)	1.402, 2.540	1.179 (0.375, 1.984) 0.006*
PWC (n=16)					
Foot	44.4 (10.3)	39.8, 49.0	33.5 (7.5)	28.9, 38.1	10.9 (4.3, 17.4) 0.002*
Posterior Calf	46.7 (9.4)	42.5, 51.0	33.4 (7.0)	29.1, 37.6	13.4 (7.4, 19.4) <0.001*
IU (n=16)					
Foot	2.8 (1.0)	2.4, 3.3	2.6 (0.9)	2.1, 3.2	0.3 (−0.4, 0.9) 0.444
Posterior Calf	3.5 (0.7)	3.2, 3.8	3.7 (0.5)	3.4, 4.0	−0.2 (−0.6, 0.3) 0.425
Circumference (cm) (n=16)					
Foot	22.7 (1.7)	22.0, 23.4	21.2 (0.9)	21.5, 22.9	1.5 (0.5, 2.5) 0.005*
Calf	36.0 (5.4)	33.8, 38.2	33.7 (2.9)	31.5, 35.9	2.3 (−0.9, 5.4) 0.153

* Significant p value <.05.
** Minimum detectable difference at the calf was 0.2cm based on the NLO pilot group. There was variation in presence of lymphoedema in the calf of those with bilateral lymphoedema.

5.4 The Effect of Compression in PLO and NLO

This section presents the results of the second research question: Does compression change fluid distribution or tissue resistance in PLO or in NLO and if so, is there a difference in response to

compression between PLO and NLO?

The results from fifteen matched pairs were available for post-compression analysis, given the withdrawal of one participant from IPC. Seven pairs had IPC on the left and eight on the right. Fourteen pairs were analysed for circumferential measures (due to one missing foot measure). Twelve pairs for the foot and fourteen pairs for the leg were analysed for ECF/ICF (bioimpedance was contraindicated for one participant and two faulty foot measures were excluded). Fourteen pairs for the foot and thirteen for the posterior calf were analysed for LEP (one participant had no images and one posterior calf image was missing due to equipment fault). The untreated lower limb in both PLO and NLO was also investigated for change, as a control for the effect of supine lying during treatment time.

5.4.1 Response to Compression in NLO

In the treated limb of NLO, significant decreases in leg and whole limb ECF/ICF, and foot and calf circumference were identified after compression (see **Table 5.6**). However, a similar significant decrease in calf circumference also occurred in the untreated limb, possibly indicating that the circumferential decrease was due to positioning rather than IPC. The change in the treated foot did not exceed the standard error of measurement (SEM: 0.1cm for both foot and calf) and was unlikely to be clinically meaningful.

Table 5.6 Differences in All Measures Following IPC in NLO on Both Sides

Table 5.6 Differences in All Measures Following IPC in NLO										
	Treated Side (IPC)					Untreated Side				
	Pre		Post			Pre		Post		
Site (n)	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean difference (95% CI) p value *	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean difference (95% CI) p value *
ECF/ICF (R_i/R_0)										
Foot (12)	3.733 (.884)	3.028, 4.437	3.524 (1.039)	2.750, 4.298	0.208 (−0.110, 0.527) 0.189	3.982 (1.102)	3.334, 4.629	3.837 (1.004)	3.190, 4.484	0.145 (−0.073, 0.363) 0.181
Leg (14)	1.975 (.369)	1.247, 2.703	1.834 (.338)	1.136, 2.533	0.141 (0.045, 0.236) 0.005*	1.976 (.417)	1.219, 2.734	1.903 (.357)	1.203, 2.603	0.074 (−0.034, 0.182) 0.173
Whole Limb (15)	1.975 (.336)	1.410, 2.539	1.856 (.310)	1.307, 2.405	0.118 (0.027, 0.210) 0.013*	1.986 (.398)	1.504, 2.468	1.935 (.374)	1.412, 2.458	0.051 (−0.018, 0.120) 0.141
LEP										
Foot (14)	1901 (589)	1646, 2157	1912 (399)	1713, 2111	−11 (−248, 226) 0.925	1944 (412)	1667, 2221	1907 (558)	1596, 2218	37 (−173, 247) 0.719
Calf (13)	1345 (491)	1085, 1605	1276 (443)	994, 1557	69 (−163, 301) 0.544	1411 (462)	1123, 1700	1225 (342)	969, 1480	187 (−89, 463) 0.175
PWC										
Foot (15)	33.4 (7.8)	28.5, 38.3	33.2 (6.9)	28.7, 37.6	0.2 (−1.9, 2.4) 0.820	33.8 (7.1)	30.6, 37.1	32.8 (5.9)	29.6, 36.1	1.0 (−1.2, 3.2) 0.362
Calf (15)	33.9 (7.0)	29.4, 38.3	32.8 (6.8)	28.3, 37.2	1.1 (−.6, 2.7) 0.187	34.1 (7.8)	30.0, 38.3	32.7 (7.6)	28.7, 36.8	1.4 (−.5, 3.3) 0.140
IU										
Foot (15)	2.65 (0.91)	2.16, 3.14	2.73 (0.83)	2.24, 3.23	−0.09 (−0.32, 0.14) 0.434	2.72 (0.74)	2.19, 3.24	2.67 (0.78)	2.17, 3.18	0.04 (−0.24, 0.32) 0.760
Calf (15)	3.71 (0.49)	3.4, 4.0	3.71 (0.49)	3.4, 4.0	−.0 (−0.2, 0.2) 0.978	3.96 (0.55)	3.62, 4.30	3.91 (0.50)	3.56, 4.27	0.04 (−0.15, 0.24) 0.641

* Significant p value <.05

Table 5.6 (continued) Differences in All Measures Following IPC in NLO										
	Treated Side (IPC)					Untreated Side				
	Pre		Post			Pre		Post		
Site (n)	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean difference (95% CI) p value *	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean difference (95% CI) p value *
Circumference (cm)										
Foot (14)	21.3 (0.8)	20.6, 22.0	21.2 (0.8)	20.6, 22.0	0.1 (.03, 0.25) 0.018*	21.3 (1.1)	20.6, 22.0	21.2 (1.0)	20.5, 21.9	0.1 (−0.06, 0.20) 0.261
Calf (14)	34.1 (3.0)	31.6, 36.5	33.7 (2.9)	31.3, 36.2	0.3 (0.19, 0.47) <0.001*	34.3 (3.0)	32.3, 36.2	34.0 (3.0)	32.1, 35.9	0.3 (0.14, 0.48) <0.001*

* Significant p value <.05

5.4.2 Response to Compression in PLO

Due to baseline differences in posterior calf echogenicity (LEP) between uniPLO and biPLO , the response to compression for this outcome was analysed separately. For all other measures, there were no significant baseline differences between uniPLO and biPLO and the results are presented for the whole PLO group.

5.4.2.1 Echogenicity: UniPLO and BiPLO Response to Compression

Neither uniPLO nor biPLO showed any significant change in LEP in response to IPC in the foot or the posterior calf (see **Appendix L.3**).

5.4.2.2 Clinical Measures: All PLO Response to Compression

In the PLO limb treated with IPC, a significant decrease was seen in leg bioimpedance, PWC and circumference after application of IPC (see **Table 5.7**).

A significant decrease in calf circumference of the same magnitude after application of IPC also occurred in the untreated limb, as was observed in the NLO group.

The decrease in calf circumference of the treated limb of PLO (0.4 cm p<0.001) following IPC was also clinically meaningful, as the minimum detectable difference in the calf was 0.3cm (SEM 0.1cm). However, the circumference of the untreated calf decreased by a mean 0.6cm, so the clinically meaningful change on the treated side following IPC cannot be attributed to IPC alone. As in NLO, this may indicate a change due to positioning rather than IPC.

There was a further global effect on the whole untreated limb in PLO. The bioimpedance ratio of ECF/ICF for the whole limb significantly increased on the untreated side. The magnitude of changes in bioimpedance have indeterminate clinical significance, given the paucity of information regarding leg segments, and the increase was so small that it is unlikely to be clinically meaningful. However, an increase was not expected and raises the possibility that the contralateral limb was impacted by IPC, based on the uncertain and variable drainage pathways in primary lymphoedema. No increase in circumference or discomfort were noted in the untreated lower limb of PLO, as this was monitored for adverse reactions.

There was a statistically significant mean value decrease in PWC at the posterior calf of 2.3, which was greater than the SEM (1.0). However, the minimum detectable difference in PWC at the calf in PLO was 2.7, indicating the decrease of 2.3 was not clinically meaningful.

However, both ECF/ICF and PWC are measures of fluid across more than just the dermis. The statistically significant reduction in PWC at the posterior calf, taken together with the significant reduction in ECF/ICF seen in the same segment, the leg, suggests that there was a clinically meaningful change in fluid distribution in response to IPC in the leg of PLO.

Table 5.7 Differences in ECF/ICF, PWC, IU and Circumference Following IPC in PLO

Table 5.7 Differences in ECF/ICF, PWC, IU and Circumference Following IPC in PLO										
	Treated Side (IPC)					Untreated Side				
	Pre		Post			Pre		Post		
Site (n)	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean difference (95% CI) p value *	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean difference (95% CI) p value *
	ECF/ICF (R_i/R_0)									
Foot (12) [1]	6.126 (1.409)	5.422, 6.830	5.933 (1.505)	5.158, 6.707	0.193 (−0.125, 0.512) 0.221	4.896 (1.062)	4.248, 5.544	5.012 (1.152)	4.365, 5.659	−0.116 (−0.334, 0.102) 0.282
Leg (14) [2]	3.291 (1.838)	2.562, 4.019	3.178 (1.767)	2.479, 3.877	0.113 (0.018, 0.208) 0.022*	2.985 (1.905)	2.227, 3.743	2.924 (1.767)	2.224, 3.625	0.061 (−0.047, 0.169) 0.259
Whole limb	2.926 (1.354)	2.361, 3.491	2.857 (1.320)	2.308, 3.406	0.069 (−0.022, 0.161) 0.131	2.547 (1.122)	2.065, 3.029	2.619 (1.237)	2.096, 3.142	−0.072 (−0.141, −0.003) 0.040*

Table 5.7 (continued) Differences in Clinical Measures Following IPC in PLO										
	Treated Side (IPC)					Untreated Side				
	Pre		Post			Pre		Post		
Site (n)	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean difference (95% CI) p value *	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean difference (95% CI) p value *
	PWC									
Foot (15)	44.7 (10.6)	39.8, 49.6	46.0 (9.6)	41.6, 50.5	−1.37 (−3.50, 0.77) 0.200	38.7 (5.1)	35.4, 41.9	39.6 (6.3)	36.3, 42.8	−0.9 (−3.1, 1.3) 0.411
Calf (15)	46.5 (9.6)	42.0, 50.9	44.1 (9.8)	39.7, 48.6	2.3 (0.7, 4.0) 0.007*	43.0 (7.8)	38.9, 47.2	41.7 (7.8)	37.6, 45.7	1.4 (−0.5, 3.3) 0.147
	IU									
Foot (15)	2.8	2.3, 3.2	2.7	2.2, 3.2	0.01 (−0.22, 0.24) 0.930	2.8	2.3, 3.3	2.7	2.2, 3.2	0.10 (−0.18, 0.38) 0.466
Calf (15)	3.5	3.2, 3.8	3.5	3.2, 3.9	−.02 (−.22, .18) 0.853	3.6	3.2, 3.9	3.5	3.1, 3.9	.06 (−0.14, 0.25) 0.558
	Circumference (cm)									
Foot (14)	22.9 (1.6)	22.3, 23.6	22.9 (1.6)	22.2, 23.5	0.1 (−0.1, 0.2) 0.296	22.3 (1.6)	21.6, 23.0	22.3 (1.5)	21.6, 23.0	0 (−0.1, 0.1) 1.000
Calf (14)	36.5 (5.6)	34.0, 39.0	36.1 (5.6)	33.7, 38.5	0.4 (0.2, 0.5) <0.001*	34.7 (4.0)	32.8, 36.6	34.2 (3.9)	32.2, 36.1	0.6 (0.4, 0.7) <0.001*

*p value significant at <.05

5.4.3 Differences Between PLO and NLO in Response to Compression

No significant difference in LEP was found in response to IPC when comparing the treated limb of NLO, uniPLO and biPLO groups (see **Table 5.8**).

Table 5.8 Response to Compression in LEP of NLO Compared with UniPLO and BiPLO

Table 5.8 Response to Compression in LEP of NLO Compared with UniPLO and BiPLO			
Site (n in respective groups)	**Treated Side (IPC)**		
	Mean reduction / increase ** (SD)	**Mean reduction / increase ** (SD) p value compared to NLO**	**Mean reduction (SD) p value compared to NLO**
HFU	**NLO**	**uniPLO**	**biPLO**
Foot (14, 7, 7)	−11 (567) **	-69 (263) ** 0.955	84 (168) 0.886
Calf (13, 6, 7)	95 (394)	66 (271) 0.987	83 (527) 0.998

** Indicates an increase in LEP post compression.

* Significant p-value (<0.05) compared to NLO

No significant difference occurred on any measure (percent water content, tissue resistance or bioimpedance) at any site when the NLO and PLO groups were compared after IPC (see **Table 5.9**).

Table 5.9 Response to Compression in PLO Compared with NLO

Table 5.9 Response to Compression in PLO Compared with NLO			
	PLO	**NLO**	
ECF (R_i/R_0)			
Site (n)	Mean reduction (SD)	Mean reduction (SD)	Mean difference (95% CI) p value*
Foot (12)	0.193 (0.675)	0.208 (0.334)	0.015 (−0.436, 0.466) 0.946
Leg (14)	0.054 (0.184)	0.120 (0.076)	0.066 (−0.053, 0.185) 0.265
PWC			
	PLO	**NLO**	Mean difference (95% CI) p value
Foot (15)	−1.4 (4.9) ↑	0.2 (2.9)	1.6 (−1.4, 4.6) 0.285
Calf (15)	2.3 (2.9)	1.1 (3.3)	−1.3 (−3.6, 1.1) 0.279
IU			
	PLO	**NLO**	Mean difference (95% CI) p value
Foot (15)	0.01 (0.46)	−0.09 (0.41) ↑	−0.10 (−0.43, 0.23) 0.538
Calf (15)	−0.02 (0.45) ↑	−0.00 (0.28) ↑	0.02 (−0.26, 0.30) 0.911

Table 5.9 (continued) Response to Compression in PLO Compared with NLO			
Circumferences (cm)			
	PLO Mean reduction (SD)	**NLO** Mean reduction (SD)	Mean difference (95% CI) p value
Foot (14)	0.1 (0.2)	0.1 (0.2)	0.1 (−0.1, 0.2) 0.310
Calf (14)	0.4 (0.3)	0.3 (0.2)	−0.1 (−0.3, 0.1) 0.558

* Significant p-value (<0.05)

5.5 Convergent Validity Between Measures of Fluid Distribution and Tissue Resistance

Convergent validity was explored between clinical measures, to answer the third research question: Is there convergent validity between clinical measures of fluid distribution and tissue resistance in people with and without primary lymphoedema?

When all data was analysed together (NLO and PLO) for correlation with LEP, there was a moderately good statistically significant correlation between LEP and R_i/R_0 in the foot on the affected side (**Table 5.10**). PWC showed a fair correlation with LEP at the calf, with confidence intervals that crossed zero. Significant correlations were found only on the affected side, so further analysis of each group separately was undertaken. No correlations were found between any baseline clinical measures at any site, within the NLO group

Table 5.10 Convergent Validity of LEP With Each Clinical Measure Using All Data

Table 5.10 Convergent Validity of LEP With Each Clinical Measure All Data (NLO and PLO together) Spearman's Rho (95% CI) two-tailed p value			
LEP with:	**PWC (n=30)**	**R_i/R_0 (Calf n=28; Foot n= 24)**	**IU (n=30)**
Affected side: Foot	.312 (−.042, .620) .093	.550 (.207, .771) .005*	.219 (−.192, .593) .244
Affected side: Calf	.373 (−.030, .683) .042*	.296 (−.120,.627) .126	−.134 (−.491, .278) .479
Unaffected side: Foot	.326 (−.043, .625) .079	.073 (−.409, .516) .734	−.020 (−.414, .373) .915
Unaffected side: Calf	.286 (−.122, .617) .125	.098 (−.365, 490) .619	−.346 (−.646, .023) .061

In the foot of PLO, there was a statistically significant moderate negative correlation between IU and PWC of r=−.511 (−.890, .049) p=.043 (see **Table 5.11**). This could indicate that high local tissue PWC in the foot, was convergently identified by low IU, indicating greater tissue resistance; however, given the confidence intervals cross zero, it was unlikely to represent a significant correlation.

As well, in PLO, convergence was indicated by moderate to good correlation between ECF/ICF in the leg and PWC at the posterior calf of r=.600 (.060, .904) p=0.018 although confidence intervals produced by bootstrapping were wide. This indicates point measures of PWC are convergent with the segmental measure of ECF/ICF in the leg.

Also at the posterior calf of PLO, IU showed significant negative convergence with LEP of r=−.539 (-.840, .009) p=0.038), with similarly wide confidence intervals crossing zero. Clinically this indicates that greater fluid in the dermis (as denoted by LEP), is convergent with low IU indicating higher tissue resistance. However, wide confidence intervals, crossing zero in IU associations, indicate more investigation is needed.

Table 5.11 Convergent Validity of Clinical Measures of Fluid Distribution and Tissue Resistance

Table 5.11 Convergent Validity of Clinical Measures of Fluid Distribution and Tissue Resistance
Spearman's Rho (95% CI)

	NLO Foot				PLO Foot			
Measure (n)	**LEP**	**PWC (15)**	**ECF/ICF (12)** Ri/R0	**IU (15)**	**LEP**	**PWC (15)**	**ECF/ICF (12)** Ri/R0	**IU (15)**
LEP	1.0	.011 (−.596, .607) .970	.147 (−.676, .704) .649	.450 (−.153, .852) .092	1.0	−.238 (−.762, .401) .393	−.476 (−.893, .152) .118	−.155 (−.727, .464) .580
PWC		1.0	.132 (−.479, .697) .668	.036 (−.500, .527) .894		1.0	.124 (−.522, .668) .687	−.511 (−.890, .049) .043*
ECF			1.0	−.302 (−.770, .311) .316			1.0	.118 (−.522, .686) .700
IND				1.0				1.0
	NLO Leg				**PLO Leg**			
	LEP	**PWC**	**ECF/ICF (14)** Ri/R0	**IU**	**LEP**	**PWC**	**ECF/ICF (14)** Ri/R0	**IU**
LEP	1.0	.370 (−.185, .788) .174	.048 (−.557, .646) .869	.233 (−.357, .786) .404	1.0	.236 (−.336, .702) .398	.323 (−.339, .803) .260	−.539 (−.840, .009) .038*
PWC		1.0	−.425 (−.701, .026) .114	.234 (−.313, .621) .384		1.0	.600 (.060, .904) .018*	−.435 (−.794, .115) .092
ECF			1.0	−.425 (−.840, .209) .114			1.0	−.250 (−.690, .305) .369
IND				1.0				1.0

*P value significant at 0.05, 2-tailed

CHAPTER 6
DISCUSSION: DIFFERENCES BETWEEN PEOPLE WITH AND WITHOUT PRIMARY LYMPHOEDEMA

6.1 The Dermis in Primary Lymphoedema

This is the first description of fluid in the dermis of the foot and leg specifically in primary lymphoedema. High fluid levels and low dermal echogenicity in people with primary lymphoedema compared to people without lymphoedema are consistent with low dermal echogenicity seen in lymphoedema of unspecified cause (Gniadecka, 1996; Naouri et al., 2010). High frequency ultrasound reliably demonstrated dermal fluid differences between people with and without primary lymphoedema and could be of further value if it could distinguish between mild and more severe changes in the dermis, as a monitor for increasing severity or progression of lymphoedema.

The influence of gravity (Baish et al., 2022; Mellor et al., 2011) over time is consistent with the distal high LEP in the foot of both uniPLO and biPLO in this study. The presence of higher dermal fluid distally in the lower limb has previously been demonstrated in the leg compared to the thigh in lymphoedema of unspecified cause (Naouri et al., 2010), and appears to be demonstrated here in the foot relative to the leg in primary lymphoedema. However, it must be recalled that distal fluid accumulation is a feature in some forms of primary lymphoedema (Mortimer, 2010; Sarica et al., 2019), consistent with functional abnormalities of lymph transport which have been demonstrated in the foot and the leg in those forms of primary lymphoedema (Sarica et al., 2019).

Different forms of vessel anomaly may affect the dermis in different types of primary lymphoedema, given the many different phenotypes of bilateral and unilateral primary lymphoedema (Gordon et al., 2020), with effects on the lymphatic system which are not yet all known. Differences in lymphatic vessel function, such as delayed fluid uptake in the foot and valve incompetence in the leg, are demonstrated by different patterns of dermal backflow in Milroy's disease (onset at birth) and in Lymphoedema Distichiasis (late onset) respectively (Sarica et al., 2019). Although lymphatic vessel function was unknown in this study, potentially such differing vessel abnormalities may underlie differences in fluid distribution seen between biPLO and uniPLO.

There is no previous literature comparing the more affected lower limb of bilateral primary lymphoedema with the affected lower limb of unilateral primary lymphoedema. In a post hoc analysis in this study, LEP in the dermis of the posterior calf was significantly higher in the most affected biPLO limb than the affected uniPLO limb. This may indicate that the dermal changes in biPLO are more progressed than uniPLO, but requires study in a larger sample size to investigate such concepts as progression.

Progression per se is beyond the scope of the cross-sectional study in this thesis, but is discussed as it pertains to severity. Progression may potentially occur in two different ways: progression within a limb or progression from one limb to two. The majority of the current cohort of PLO were described as early stage II, eight having bilateral and eight unilateral lymphoedema. Distally, both uniPLO and biPLO showed high LEP in the dermis of the foot compared to NLO. As dermal backflow increases in lymphoedema over time (Yamamoto, Narushima, et al., 2011), the first possibility is that progression could occur from distal to proximal up the leg, and that the dermis of the leg is not yet affected in uniPLO in this relatively young cohort, but could become affected over time. At the foot, the similarity between uniPLO and biPLO in dermal echogenicity indicated that the dermis of the foot was equally affected in both. The presence of fluid in the foot of both uniPLO and biPLO, but greater in the leg segment only in biPLO, supports the theory of distal to proximal progression, starting from the distal effect reported in early primary lymphoedema (Sarica et al., 2019).

In the second instance, progression can involve a previously unaffected limb (Burnand et al., 2012; Peters & Mortimer, 2021) and result in progression from unilateral to bilateral lymphoedema, as has been reported in unilateral late-onset primary lymphoedema (Gordon et al., 2020). Unilateral and bilateral forms of primary lymphoedema remain separate entities within the phenotypic algorithm for primary lymphoedema (Gordon et al., 2021). However, based on the report by Gordon et al (2020), progression may be possible in the majority of PLO in this study, as 12 of 16 PLO were late onset. At the time of the study, seven of the 12 were unilateral.

The foot has been of interest due to the early manifestation there of primary lymphoedema. In addition, the foot is subject to the accumulation of fluid under the effect of gravity (Baish et al., 2022; Mellor et al., 2011) and dermal backflow increases with longer duration of lymphoedema (Yamamoto, Narushima, et al., 2011). Methodology developed in this study detecting high dermal

fluid in primary lymphoedema using HFU presents the opportunity to study the change over time in dermal fluid in primary lymphoedema, particularly in the foot, as well as the effect of treatment to reduce dermal fluid. Further investigation is needed to determine if HFU monitoring of the unaffected foot in uniPLO is able to identify early changes indicating progression of primary lymphoedema. Such a progression could involve either proximal spread from the foot into the leg within a limb already affected, or progression into a limb previously unaffected, i.e. from unilateral to bilateral. Duration of lymphoedema in biPLO (24 to 327 months) and uniPLO (13 to 234 months), was similar in this study of only 16 PLO. A larger sample of people with primary lymphoedema, with genetic or accurate phenotypic profiling, is required to understand different lymphatic vessel abnormalities affecting fluid distribution in the dermis in primary lymphoedema. Progression, age and duration remain factors to consider in future research, due to the association of dermal backflow patterns with age of onset (Yamamoto et al., 2015).

6.2 Fluid Measures in The Foot: Baseline Comparison of PLO to NLO

The significantly higher ECF/ICF in the affected foot in PLO than NLO is consistent with previous studies which established higher extracellular fluid in limbs with lymphoedema of mixed cause, compared to those without lymphoedema (Steele et al., 2019). This study has confirmed the similarly elevated levels of ECF/ICF in primary lymphoedema compared to those without lymphoedema.

The difference in ECF/ICF between PLO and NLO at the foot (2.412), more than double the difference seen in the leg segment (1.148), appears to indicate that the foot in primary lymphoedema has particularly high ECF/ICF. Previous studies of ECF/ICF in the leg segment reported a difference of 1.8 between lymphoedema of mixed cause and control groups (Suehiro, Morikage, Yamashita, Harada, Ueda, et al., 2016). However, there is a vast difference in anatomical structure and composition between the leg and the foot which may influence ECF/ICF measures in these segments; as well, the cohort in the latter study was predominantly secondary lymphoedema, so provides no comparison with primary lymphoedema. Further research in the normal population is required to establish a threshold of ECF/ICF in the foot segment for lymphoedema.

This is the first report of PWC on the dorsum of the foot in primary lymphoedema. A previous report of PWC on the dorsum of the foot investigated secondary lymphoedema and included the

foot as one of five sites across the lower limb, which were aggregated to provide a mean PWC value of the lower limb for analysis (Yu et al., 2019), and so findings are not comparable.

The high PWC, ECF/ICF and LEP measures in the foot in this study are consistent with the distal effect of primary lymphoedema seen lymphoscintigraphically, and with clinical observations of swelling on the dorsum of the foot commonly seen in young people with primary lymphoedema (Sarica et al., 2019). The large proportion of the primary lymphoedema phenotypes that have late onset in the St George's algorithm (Gordon et al., 2020) was reflected in the majority of PLO having late onset in this study. It is interesting to speculate whether early detection and intervention might be possible in late onset primary lymphoedema and might prevent some of the dermal changes seen in the foot over time.

6.3 Fluid measures in The Leg: Baseline Comparison of PLO to NLO

The leg may vary in size substantially due to the relative size of the calf muscle, limiting the information regarding fluid distribution that may be provided by circumferential measures. This appeared to be the case in the large but statistically insignificant difference in calf circumference between NLO and PLO.

The measures of ECF/ICF, PWC and LEP, discussed below, provide information specific to fluid with increasing tissue specificity; ECF/ICF indicates fluid throughout the tissues in a limb segment, PWC provides a point measure dependent on fluid in the dermis and upper subcutaneous tissue, whereas LEP is specific to the dermis.

The presence of increased fluid in the lower limb has previously been established in lymphoedema of mixed cause using bioimpedance (Steele et al., 2019; Ward, Winall, et al., 2011) and in secondary lymphoedema using ECF and PWC, where five sites from foot to thigh were combined to provide an average lower limb measure (Yu et al., 2019). Increased fluid compared to the healthy lower limb is now confirmed specifically in the leg in primary lymphoedema in this study, as was evident in the statistically significant higher measures of ECF/ICF and PWC in PLO than NLO. Considered together with the lack of difference in the dermis (LEP) between NLO and either uniPLO or biPLO at the posterior calf, it appears that fluid accumulation in the leg in this cohort of primary lymphoedema occurred to a greater extent in the subcutaneous tissues, as measured by ECF/ICF, than in the dermis. This finding in the leg in primary lymphoedema is consistent with

previous knowledge of fluid accumulation in lymphoedema affecting subcutaneous tissues more than the dermis, assessed by echogenicity in lower limb lymphoedema of unknown cause (Iker et al., 2019) as well as in secondary upper lymphoedema (Mellor et al., 2004).

However, the clinical implications of the small yet significant difference between NLO and PLO in ECF/ICF at the leg (1.148, p=0.012) are uncertain, given the paucity of bioimpedance data in segments of the lower limb. Suehiro et al (2016) have previously shown a significantly higher ECF/ICF (Ri/R0) of 1.8 ($p<0.05$) in the leg of mainly secondary (four of 48 were primary) lymphoedema compared to healthy legs from an older age group (31 to 88 years) (Suehiro, Morikage, Yamashita, Harada, Ueda, et al., 2016). The current findings now demonstrate a difference of similar magnitude in primary lymphoedema. However, the median age in this study was 24 years whereas the latter study compared normal limbs of median age 35 with lymphoedema of median age 70; comparisons of ECF/ICF between groups of different ages are limited, due to the increased ECF/ICF associated with older age (Ward, Winall, et al., 2011). As well, the difference in ECF/ICF between groups in the leg in the current study may have been blurred by the variable presence of lymphoedema in the leg segment of the affected limb in biPLO. (In some biPLO, the calf circumference of the more affected limb, which was based on the size of the foot, was less than that of the less affected limb.) Investigation of the anatomical segment from ankle to knee in a larger young healthy cohort could provide greater certainty of the significance of the current findings in lower leg segments in primary lymphoedema.

6.4 Comparison of the Foot to the Leg in Fluid Measures

The findings of this study, demonstrating that only the deeper tissue had high fluid accumulation in the leg in PLO whereas both dermis and deeper tissue were affected in the foot, are consistent with what is known lymphoscintigraphically of fluid in the dermis in specific forms of primary lymphoedema. The genotype of this PLO cohort was unknown but the presence of high dermal fluid in the foot is consistent with reduced lymph uptake or functional aplasia of the initial lymph vessels in the foot in Milroy's Disease (Sarica et al., 2019). In addition, the finding of high dermal and deeper tissue fluid in the foot also appears consistent with increased intra-lymphatic pressure associated with valve incompetence in the leg in Lymphoedema Distichiasis (Mellor et al., 2011; Sarica et al., 2019).

Fluid accumulation in both subcutaneous tissues and the dermis has been observed in the leg on

ICG lymphography in Lymphoedema Distichiasis (Sarica et al., 2019), but whether the dermis of the leg becomes affected by fluid accumulation over time following fluid accumulation in the foot, is unknown. This study of early stage PLO demonstrated that, in the leg, only the deeper tissue, not the dermis, had high fluid accumulation. There are few studies measuring fluid accumulation in the dermis in any type of lymphoedema. The possibility of progression in primary lymphoedema, of greater fluid accumulation in the foot prior to that in the leg, contrasts to that in secondary lymphoedema, in which a proximal to distal progression is reported (Yamamoto, Matsuda, et al., 2011) as dermal backflow increases over time (Yamamoto, Narushima, et al., 2011).

Possible distal to proximal progression in primary lymphoedema also contrasts with the proximal to distal fluid redistribution in the lower limb during the day under the influence of gravity (Taniguchi et al., 2021), which affects all lower limbs. The issue of progression over time in primary lymphoedema is unclear. If both the dermis and subcutaneous tissue become affected proximally in the leg in later stages of primary lymphoedema, this might account for the similar clinical presentation of both primary and secondary chronic lymphoedema. Future research may determine if dermal fluid accumulation begins in the foot and progresses to the dermis of the leg over time in primary lymphoedema. If so, the use of dermal fluid measures such as LEP could underpin the monitoring of dermal fluid and development of interventions to address dermal fluid.

Within each group, PWC measures were of similar magnitude at the foot and posterior calf, in contrast to the distribution of measures of LEP (dermal fluid) and ECF/ICF (segmental fluid), which appeared to be of greater magnitude distally. This difference may be due to the fluid which is available to be measured by PWC—which is free and bound water molecules (Mayrovitz, 2015)—in contrast to that measured by LEP and ECF/ICF, which are both measures not specific to water.

Variation in ECF/ICF between foot and leg could be expected, based on their relative difference in size, shape and composition. The high distal fluid distribution seen in this study was consistent with that seen in the leg relative to the thigh in mainly secondary lymphoedema, which increased with gravity over time (Suehiro, Morikage, Yamashita, Harada, Ueda, et al., 2016). Although literature on ECF/ICF in thigh and leg segments of the leg is emerging in secondary lymphoedema (Suehiro, Morikage, Yamashita, Harada, Ueda, et al., 2016) and healthy limbs (Taniguchi et al.,

2021), none to date have analysed the foot segment and none in primary lymphoedema.

6.5 Clinical Implications of Bioimpedance Measurement in Primary Lymphoedema

Establishing the lymphoedema status of each group at baseline using bioimpedance demonstrated an overlap of the range around the mean of the PLO sub-groups with the range around the published normative mean. One could infer from the overlapping ranges that there was not a clear difference established by bioimpedance between NLO and PLO. However, the bioimpedance method to establish this difference excluded a key segment for primary lymphoedema: the foot.

The feet in PLO were demonstrably the most affected by lymphoedema, showing greater disparity in ECF/ICF with NLO at the foot than the leg. Yet the feet are not included in the standardised measurement of full limb bioimpedance on which the normative mean and thresholds for lymphoedema are based. Standard placement for electrodes in the lower limb typically measures from the ankle to the groin (ImpediMed Limited, 2016; Steele et al., 2018). Based on the results of this study, the foot could be considered for inclusion in assessments of early stage primary lymphoedema. Future research to develop normative bioimpedance values in the foot, leg and thigh segments could form the foundation for developing a threshold against which primary lymphoedema could be tested.

Bioimpedance protocols to detect lymphoedema were mostly developed within those with secondary lymphoedema, detecting early fluid accumulation post-surgery and changes with treatment (Cornish et al., 2001; Cornish et al., 1998). Lower limb testing of bioimpedance protocols has likely involved both primary and secondary lymphoedema (Steele et al., 2019; Ward, Winall, et al., 2011). Where participants with primary lymphoedema were included, the advanced age of the cohort (Ward, Winall, et al., 2011) may feasibly have compensated for the lack of measurement of the foot. Over time, there is little difference in the clinical presentation between established or chronic primary and secondary lymphoedema, perhaps due to the effect of gravity over time seen even in the healthy lower limb (Taniguchi et al., 2021). Assessment of the thigh and leg is appropriate in a secondary population, in which progression of lymphoedema occurs from proximal to distal in the lower limb (Yamamoto, Matsuda, et al., 2011). However, the exclusion of the foot may render this bioimpedance test invalid from appropriate measurement of early stage primary lymphoedema. Based on the fluid distribution in limbs of PLO in this study cohort, in

which a greater difference in ECF/ICF between NLO and PLO was seen in the foot than the full limb (significance unknown), standard bioimpedance measurement protocols may need amendment to include measurement of the foot in primary lymphoedema. Evidence regarding the distal development of primary lymphoedema, from the foot and leg (Sarica et al., 2019), in contrast to the early proximal accumulation in the thigh in secondary lymphoedema (Yamamoto, Matsuda, et al., 2011), supports the concept that current bioimpedance protocols for measurement of the full limb may be inappropriate for assessment of early stage primary lymphoedema. Segmental measures have the potential for valid assessment of primary lymphoedema. However, segmental comparisons are limited until the range within the normal population for the foot and leg segments are known and a threshold for lymphoedema in each segment established.

A further clinical implication of the fluid distribution in lower limbs with primary lymphoedema extends to the use of arm-to-leg bioimpedance (ECF) ratios, which are advocated to detect bilateral lower limb lymphoedema (Steele et al., 2019). It is questionable whether an arm to full leg ratio would be appropriate to assess ISL stage I and early II primary lymphoedema, due to the foot being excluded from the full leg measurement. Two modifications are suggested for use of this ratio in early primary lymphoedema. Firstly, the foot should ideally be incorporated in the lower limb bioimpedance measurement. Secondly, arm to leg ratios should be taken on both sides even in unilateral presentations, regardless of the site of overt swelling clinically, due to the uncertain nature of fluid distribution in the seemingly unaffected lower limb in unilateral primary lymphoedema. Such ratios could inform the relative status of the unilateral and bilateral sub-groups in primary lymphoedema, particularly regarding risk of progression to a previously non-symptomatic limb. In support of this, there was significantly higher ECF/ICF in PLO than NLO, on both sides, despite half of the PLO group having clinically unilateral lymphoedema (mean difference between NLO and PLO (less affected limb of biPLO and the unaffected limb of uniPLO) was 0.678 (0.073, 1.298) p=.030). Further implications of the differences between PLO and NLO in the foot and leg remain to be determined when more is known about segmental bioimpedance in the lower limbs.

Primary lymphoedema commonly presents early in life, and a size discrepancy is generally the first sign noticed, either by parents during a child's infancy or by an adolescent, becoming aware of body changes. By the time alteration in limb size is evident, dermal fluid accumulation with potential for subsequent skin changes will have begun. Possible future research could investigate

early detection of lymphoedema in late onset primary lymphoedema, such as Meige's disease, which develops at puberty and is predominantly below knee (Mortimer, 2010), and the relative importance of using foot and leg segmental bioimpedance measure. Interventional screening such as this could be intrusive and disruptive for a child or teenager yet may have a place for those wishing to allay fears based on family history, or be proactive in minimising potential lymphoedema. Particularly, those with late onset unilateral primary lymphoedema may be interested to screen for early signs of fluid accumulation in a seemingly 'unaffected' limb.

6.6 Tissue Resistance (IU)

In this study, fluid measures were more useful than IU for distinguishing differences between primary lymphoedema and healthy limbs. The clinical expectation is for lower tissue resistance where there is increased fluid accumulation (Sano et al., 2019). Conversely, tissue resistance may be greater in PLO than in NLO due to fluid accumulation with its associated potential for tissue fibrosis even early in lymphoedema (Hara et al., 2016; Herrada et al., 2019; Rockson et al., 2019). However, IU did not reflect fluid distribution despite significantly increased fluid measures of LEP, ECF/ICF and PWC in the underlying tissues in PLO.

The lack of difference in IU between NLO and PLO at both the foot and the posterior calf may indicate that the potential effect of early fibrosis from fluid accumulation was insignificant in this cohort of mainly early stage II PLO. It is clear from the staging of lymphoedema (ISL) that fluid accumulation is present in the early stages of lymphoedema, whereas tissue changes resulting in fibrosis and tissue hardening becomes more evident in the later stages. The results of this study confirm the staging in PLO participants, of whom 15 of 16 were early stage II, in finding no difference in IU, and significantly greater fluid measures of ECF/ICF, PWC, LEP when comparing PLO to NLO.

The early presence of primary lymphoedema in the foot (Sarica et al., 2019) may be a risk for early fibrosis; possibly, the prevalence of late onset or shorter duration in this PLO cohort impacted IU. However, study numbers in this cohort were too small to investigate the effects of duration of primary lymphoedema on IU. In a longitudinal study with higher numbers of people with primary lymphoedema, the investigation of factors such as stage of lymphoedema and time since diagnosis (or duration) may illuminate the relationship between fluid distribution and tissue resistance as stage increases.

It is possible that underlying structures may have had an influence on indurometry (Douglass, Graves, & Gordon, 2017) in both the foot and the leg. Measurement sites were chosen to avoid the hard anatomical structures of bone and tendons in the foot. However, it is possible that the relative lack of subcutaneous tissue in the feet and the tension in the short inter-osseous muscles underlying the measurement site may have influenced measures in both groups. There have been no previous reports of indurometry in the foot for comparison. In the leg, efforts were made to limit the effect of the underlying calf muscle by positioning the foot so that the calf was in a position of low tension, but despite this, the force with which the Indurometer was applied (equating to 200g) may have resulted in a depth of penetration potentially affected by underlying muscle tension.

In contrast to this study's results, an investigation into tissue resistance in secondary lower limb lymphoedema showed that the skin was significantly stiffer than control skin ($p<0.05$) even in ISL stage I (Sun et al., 2017). However, the two study populations are not comparable, due to the possible effect on the skin of radiation or chemotherapy in secondary lymphoedema, with resultant inflammatory changes that are not present in primary lymphoedema. Furthermore, in the latter study a Skin Fibrometer of 2.5mm diameter indented the skin with a force of 0.16N, equating to approximately 16g, in contrast to the force applied by 200g with the Indurometer.

6.7 Convergent Validity Among Clinical Measures

The investigation of convergent validity brought some unexpected results, as correlation between measures varied with site and between groups.

6.7.1 Fluid Measures

The moderate to strong correlation ($r=0.60$, $p=0.18$) between PWC and ECF/ICF in the leg in the current study shows the relationship in predominantly early stage II primary lymphoedema for the first time.

The strength of this relationship is consistent with the strong correlation ($r=0.62$) between PWC and excess ECF reported previously in a mixed lymphoedema population (Yu et al., 2019). However, the latter results were found in a population of both upper and lower limb secondary lymphoedema, outcomes from five sites along the limb were pooled for a mean limb value for analysis, and the percent excess ECF of the affected limb relative to the unaffected were analysed,

so results are not comparable with this study.

6.7.2 Tissue Resistance

The moderate inverse relationship between LEP and IU in the leg segment suggests that the more fluid in the dermis, the less the Indurometer could press into the tissues, in support of the observation that increased fluid content provides resistance (Belgrado et al., 2010). However, there was no such relationship seen between LEP and IU in the foot, where there was even higher LEP. Considering that there was no difference between PLO and NLO in either LEP or IU at the posterior calf, it appears that conversely, low LEP was associated with high IU, or softer tissue. IU was generally higher, indicating less tissue resistance, at the posterior calf than at the foot. This indicates that in the leg, it is likely that there was lower tension in the tissues (higher IU) associated with low LEP in the dermis.

At the foot, the moderate inverse relationship between PWC and IU ($r = -0.511$, $p=0.043$) appears to indicate that the fluid component of oedema in the foot resides to a greater degree in the subcutaneous tissue than in the dermis and contributed to stiffer tissues. This was corroborated by the high ECF/ICF measures throughout the segment compared to NLO. The correlation of IU with LEP (leg) and PWC (foot) suggests that tissue resistance measured by IU was associated with the superficial fluid measures, but the association varied with site. The potential influence of different anatomical sites and their underlying tissue on IU may explain the variation in correlation of IU with different fluid measures dependent on site. Regardless, the wide confidence intervals crossing zero that were found in correlations involving IU mean that further investigation is required to confirm any such associations.

In a recent study, a strong correlation of skin stiffness with lymphoedema stage was found across the lower limb in secondary lymphoedema (Sun et al., 2017). No such investigations of staging were possible in this small PLO cohort of nearly all the same fluid-dominated early stage lymphoedema. The latter study also reported significantly higher skin stiffness at all stages of lymphoedema than in healthy limbs. However, results from a cohort of secondary lymphoedema, with measures from the thigh and leg being combined for an average limb measure, are not comparable with individual site measures from primary lymphoedema. Notably, more than half of lymphoedema participants in the latter study had advanced stage (late stage II or stage III) lymphoedema, in contrast to the early ISL stage II of PLO participants in the current study.

In summary, the small numbers of this study limit the conclusions that may be drawn from correlation analysis. The combined group of NLO and PLO together provided a larger sample size more suitable for analysis of correlation, in which only one statistically significant moderate correlation was found, that of ECF/ICF with LEP at the affected foot. Given there is a degree of uncertainty regarding measures of bioimpedance in the foot until further testing can be carried out, this relationship requires further investigation.

6.8 Conclusion

The dermis in primary lymphoedema—unaffected by radiotherapy or other secondary influence—showed distal sites to be more affected by fluid accumulation than proximal ones. This is consistent with current understanding of areas affected in common forms of primary lymphoedema (Mellor et al., 2011; Sarica et al., 2019). Clinically, high dermal fluid measures in the PLO foot indicate the need for treatment to reduce fluid in the dermis before chronic pathological and fibrotic changes occur. The finding of significantly higher measures of ECF/ICF and PWC in PLO compared to NLO is consistent with the predominance of fluid in the earlier stages of lymphoedema, demonstrated here in the distal segments of the lower limb in early stage II primary lymphoedema.

The lack of significant difference between NLO and PLO in IU suggests that fluid measures predominated over IU in this cohort of PLO, consistent with their early ISL stage II. Despite a moderate to good correlation in PLO between PWC and ECF/ICF in the leg, there was no correlation between the two measures in the foot, even though there were high measures of both PWC and ECF/ICF in the foot. The composition of the foot relative to the leg may contribute to differences in correlation but requires investigation of bioimpedance in the foot and leg segments of the healthy lower limb to clarify the relative influence of the anatomical structure of the foot i.e., relatively greater bone and less muscle on bioimpedance measures.

Convergent validity among measures differed in the leg (ECF/ICF with PWC; IU with LEP) compared with that in the foot (IU with PWC), demonstrating site-specific variation in tissue condition in primary lymphoedema. However, the sample size was small and significant correlations between measures had very wide confidence intervals, requiring confirmation in a larger sample.

These baseline tissue measures, along with the differences identified between PLO and NLO,

provided the foundation for investigation of the response of the tissues to compression in these groups, which will be discussed in the next chapter.

CHAPTER 7
DISCUSSION: RESPONSE TO COMPRESSION

This study presents the first report of the response to compression in primary lymphoedema. Despite baseline differences between NLO and PLO, there was no difference between these two groups in their response to compression in any measure. In this chapter, the effect of compression on fluid distribution in different leg segments is discussed with reference to each clinical measure in PLO and NLO. Previous investigations of compression in secondary lymphoedema provide contrast to the findings of this study of primary lymphoedema, highlighting the possible effects of primary lymphatic anomalies on the response in primary lymphoedema to this commonly used treatment.

7.1 Response to Compression in the Leg

From the lack of significant difference between PLO with NLO in the leg at baseline in both circumference and dermal LEP measures, it appeared that PLO had relatively little oedema in the leg at the posterior calf measurement site, despite PLO having significantly more ECF/ICF than NLO at baseline. NLO and PLO then responded in a similar manner to compression, both showing significant ECF/ICF reduction in the leg segment, which appears to indicate the deep effect of IPC in promoting venous return (Bickel et al., 2011; Gibbons et al., 2019; Koo et al., 2014).

The clinical import of the significant reduction in ECF/ICF following compression in the leg in NLO (0.141, p=0.005) is not clear. The decrease following compression seen in the whole treated limb in NLO (0.118, p=0.013), was of similar magnitude to that in the leg, which is a change of approximately 7%. In contrast, the reduction in the leg in PLO (0.113, p=0.022) equated to a 3% change and was not matched by a significant reduction in the full leg. The magnitude of both changes is dwarfed by the 44.9% variation in full limb bioimpedance (female Ri/R0 inter-leg ratios) found in the normal population over 18 months (Steele et al., 2018). However, even such small percentage changes as 7% or 3% could be clinically meaningful given the short time frame of this study (one to two hours compared to 18 months). ,

Comparison with the untreated limb, however, provided further relevant information. In the untreated limb of NLO, where no significant changes in ECF/ICF were seen over the same time, suggests that the change in the treated limb of NLO, was indeed due to compression.

Changes in the whole limb in ECF/ICF provide the context against which changes in the leg segment may be considered. No segmental measures were taken in the thigh, so conclusions about the relative contribution of the leg reduction to overall limb reduction are limited. However, the size of change in the NLO leg compared to that in the whole limb suggests that the leg contributed largely to the reduction in the whole limb. In contrast, the lack of significant change in the whole limb in PLO suggests that the statistically significant reduction in the leg was not sufficient to impact the whole limb and therefore was less clinically meaningful. The small change on the treated leg of PLO, however, contrasts with an unexpected statistically significant **increase** in whole limb ECF/ICF found in the **untreated** limb of PLO, which has clinical relevance. Although this change in PLO was small, there were no such changes in NLO and clinically, an increase in an untreated limb would not be expected, nor desired. Plausibly, this could indicate a shift in fluid from one limb towards the other, which could happen in the case of drainage pathways to the contralateral inguinal nodes in primary lymphoedema (Yamamoto, Narushima, et al., 2011). It is feasible that a small increase in fluid delivered to the contralateral inguinal nodes along such a pathway may be sufficient to cause transport overload on the contralateral side, which may already be abnormal (Aldrich et al., 2012; Bourgeois, 2021; de Almeida et al., 2017) or have 'latent lymphoedema' (Peters & Mortimer, 2021), even if swelling is not clinically manifest. In those with primary lymphoedema, drainage paths are unpredictable and frequently unknown, so an increase in ECF/ICF on the contralateral side raises questions over the suitability of applying compression by IPC to only one limb in primary lymphoedema, unless drainage pathways have been imaged and are known.

Changes in the untreated limb were relevant to post-treatment analysis in more than one measure. A significant decrease in circumference was also evident in the **untreated** leg in the PLO cohort, which was seen to reduce by an even greater margin than the **treated** limb. The effect of elevation alone may have been enough to bring about this small circumferential change on both lower limbs, consistent with findings in venous oedema (Xia et al., 2004). Given that elevation alone is advised as a management strategy for lymphoedema and that reduction was seen in the untreated leg, the circumferential reduction in the treated leg could not be attributed solely to the effect of compression. Reduction of the untreated leg demonstrates the benefit elevation contributes to the effect in the treated limb. Furthermore, the reduction in circumference was seen in the untreated limb of PLO while the measure of ECF/ICF throughout the limb increased.

This highlights the misleading information that comes from relying on a non-specific measure such as circumference to describe fluid changes in a limb with primary lymphoedema, as well as the importance of monitoring both lower limbs.

PWC was the only measure that showed significant change post-IPC in PLO that was not evident in NLO, as well as showing a baseline difference between PLO and NLO that was both statistically and clinically significant. As there was no difference detected between PLO and NLO in dermal LEP at baseline, and no change in dermal LEP in response to compression, one can only surmise that the MMDC penetrated to subcutaneous tissue in which there was significantly higher fluid, as indicated by ECF/ICF, in the leg of PLO compared to NLO.

There are no previous studies of the response to compression in primary lymphoedema. However, in a study with a protocol similar to that of the current study, Zaleska & Olszewski (2018) investigated the response to 45 to 60 minutes of pneumatic compression in lower limb lymphoedema, and found only insignificant reduction (3-5%) in PWC, in the 'upper parts of the limb' (Zaleska & Olszewski, 2018), presumably the thigh. There are two differential factors that prevent comparison to the current study. Firstly, the participants in the latter study had stage II and III 'post-inflammatory lymphoedema', described as lymphoedema secondary to soft tissue infection, likely to have considerably greater tissue fibrosis causing resistance to compression, than the early stage II participants in the current study. Secondly, the latter study applied pressure up to 120mmHg, nearly double that applied in this study, and although flow in subcutaneous tissues increased, the effect of such pressure on fluid uptake in the dermis is unknown. High pressure up to 88mmHg was suggested to impede drainage in chronic lower limb oedema of unreported cause (Partsch et al., 2011). In contrast, pressure applied at 60mmHg in this study may have been sufficient to prompt fluid movement in the subcutaneous tissues, deeper than the dermis, based on the reductions in PWC and ECF/ICF seen in PLO.

It was conjectured that differences in vessel functionality, seen in sub-groups of PLO of different genetic anomalies (Sarica et al., 2019), may affect the response to compression. However, the specific anomalies present in the PLO group were unknown, and the small numbers in this study prevented further analysis of uniPLO and biPLO. Future investigations of PLO subgroups based on genetic abnormality could provide more clinically relevant information. In the current study, as baseline investigations showed no significant difference between uniPLO and biPLO in PWC in

either the foot or the posterior calf, PLO were analysed as one group post-IPC for comparison with NLO.

7.2 Response to Compression in the Foot

The complete lack of response in the foot in the current study raises three possibilities 1) the application of pneumatic compression is not effective in the foot, or 2) the dosage of fifty minutes and 60mmHg compression is insufficient to bring about change, or 3) possible changes were not detected by the devices used. The latter option, that possible changes were not detected, is unlikely given the range of measurement methods used, with devices that detected small changes in the leg segment.

The remaining possibilities—lack of efficacy in the foot, or inadequate dosage—involve the application of IPC, which aims to be effective by increasing fluid flow (Zaleska & Olszewski, 2018). However, assessment of IPC efficacy is commonly based on limb dimensions such as circumference or volume and dimensions of the foot are not scrutinised. A systematic review of the literature searched for IPC studies producing significant limb size reduction to identify optimal treatment timing and pressure dosage. In most studies, compression was applied over more than one treatment session, demonstrating a cumulative effect in limb size reduction. Reduction was shown by volume (water displacement) of the lower limb, which included the foot, or circumference of the leg and thigh, which did not include the foot. Due to the irregular shape of the foot and lower repeatability for measuring dimensional change in the foot (Henschke et al., 2006), the foot may be excluded altogether from investigations into the effects of compression (Zaleska & Olszewski, 2018), or included within a mean measure for the entire limb (Hacard et al., 2014). Reduction in the foot is not included in limb volume calculations (Williams & Whitaker, 2015) and has been reported simply by being able to return to usual footwear (Williams, 2016). When volume calculations based on circumferences include the foot (Hacard et al., 2014), it is unclear how the shape of the foot is accommodated in calculations.

During application of IPC, it is difficult to ascertain where the optimal pressure is delivered along the length of an IPC pneumatic sleeve due to the many factors that affect pressure delivery (Zaleska & Olszewski, 2018; Zhao et al., 2019), and indeed, debate continues over what is ‘optimal pressure’. The foot varies in radius of curvature as well as tissue resistance; pressure applied by the pneumatic sleeve around the foot may have varied from dorsum to sole and around the ankle

due to its irregular shape. The IPC sleeve appears to apply pressure consistently across the dorsum of the foot, whereas the sole of the foot was supported by a hard plastic insert, designed to keep the width of the foot from being compressed and thus guiding the compressive force to be applied on the dorsum and sole of the foot. The sole was therefore protected by this 'foot plate' insert, which did not conform to the shape of the foot, with the result that the surface of the sole was 'distanced' from compression. If greater pressure resulted on the dorsum than the sole of the foot, it is possible that, in the presence of variable lymph drainage patterns (Shinaoka et al., 2020), an adverse gradient may impede lymph drainage, as it does in venous flows (Partsch & Mani, 2019), and may contribute to the lack of response to compression in the foot.

The mode or timing of IPC compression may also affect drainage from the foot. The IPC used in this study applied compression to the foot via a single chamber encompassing the foot, resulting in either pressure on or off (no peristaltic motion) and pressure was maintained for the entire duration of the cycle until all chambers were full before deflation. Possibly, the prolonged period of compression compared to a relatively short deflation time limited the time during which vessels may re-fill with lymph (Zaleska et al., 2013), impeding drainage. In addition, working on proximal areas is required prior to addressing distal areas when applying manual lymph drainage (McNeely et al., 2004) to 'clear' the fluid downstream and this concept was followed in the chosen mode of IPC application in this study. However, it is possible that there was not time for sufficient movement proximally to effect a change distally, which may also have contributed to the lack of change in the foot.

As well as the anatomical and device-based limitations of applying IPC, there is also the possibility in primary lymphoedema that poor uptake of fluid into the initial lymphatic vessels (Sarica et al., 2019) is a key factor affecting the response to compression in the foot. Lymph flow under IPC compression of the lower limb has been demonstrated by ICG fluoroscopy (Aldrich et al., 2016; Zaleska & Olszewski, 2018) which also reveals lymph movement under variable pressure delivered by MLD (Wigg & Cooper, 2017), but none of these studies examined the foot, nor primary lymphoedema specifically. Poor uptake of fluid in the initial lymphatic vessels of the foot in primary lymphoedema cannot be ignored as a key barrier to fluid reduction in the foot.

Lack of response to compression by IPC in the foot measured by any device suggests that alternative treatment strategies must be considered for reduction in this segment. Future research

could include increasing the time for proximal clearance from the leg and/or thigh in the assessment of the foot segment response. This could allow time for negative pressure generated by downstream lymph flow to assist in drawing fluid into the initial lymphatics (Jamalian et al., 2017; Sloas et al., 2016) particularly for those with primary lymphoedema, where there is delayed uptake in the foot. In addition, mapping by ICG could facilitate tailored MLD by identifying the direction of drainage pathways in the foot. Understanding of the dermal fluid response to pressure may be promoted by investigating pressure applied by MLD during which the dermis may be stretched and subjected to variable pressure.

The foot is rarely investigated as a segment by itself. The cumulative effects of CDT are seen in the reduction of the size of the foot over time and pathological changes in the dermis are seen to reduce but remain, even during long-term compression use (personal observation). Further research is required to elucidate the optimal form of compression or treatment for fluid reduction in the foot in primary lymphoedema.

7.3 Dermal Response to Compression

The lack of response to compression in the dermis, seen in both groups at both sites, suggests that compression applied by IPC was not effective in reducing dermal fluid, as measured by LEP. The incompetence of lymphangions and dysfunctional initial lymphatics in the foot in primary lymphoedema (Sarica et al., 2019) immediately present possible reasons for lack of response in PLO, but the absence of response in the dermis also in NLO invites further scrutiny.

A decrease in LEP (relative to total pixels) has previously been reported following five days of compression in a population with lipodermatosclerosis (Gniadecka et al., 1998). On the basis that all oedema is returned to central circulation by the lymphatic system (Mortimer & Rockson, 2014), all conditions might be expected to respond in a similar manner to compression. However, lipodermatosclerosis is a condition of chronic inflammation associated with venous deficiency and tightly bound down skin (Choonhakarn et al., 2016) and as well, is distinct from lymphoedema on ultrasound. All oedema causes hypoechogenicity in dermal ultrasound images, but the concentration of LEP within the upper papillary dermis in lipodermatosclerosis contrasts to the distribution of LEP throughout the dermis in lymphoedema (Gniadecka, 1996), presenting the potential for a different response to compression. In addition, the function of the initial lymphatics (Sarica et al., 2019), and the fibrotic state of lymphatic vessels (Barone et al., 2020) in primary

lymphoedema both affect fluid uptake and therefore most likely, the response of the dermis to compression as well.

A further contrast between the current study and that by Gniadecka et al (1998) is the potential effect of movement. In the study by Gniadecka et al, compression was applied using stockings and re-measured after five days. Movement (Fukushima et al., 2017), and changes of position, which occur during any study over more than one day, are known to assist in reducing lymphoedema. Increased lymph flow occurs due to the effect of both skeletal muscle contraction and increased lymphangion contraction in the upright position (Olszewski & Engeset, 1980; Scallan et al., 2016). Even the lift-and-pull effect of movement on the skin creates fluctuating pressure (Ikomi & Schmid-Schönbein, 1995) in the dermal interstitium, which facilitates fluid uptake into the initial lymphatics (Michel et al., 2020; Mukherjee et al., 2018). Such effects from movement were absent in the current study of response to IPC in the supine position.

It appears that the relevance of pressure fluctuations in the dermis, to fluid uptake by the initial lymphatic vessels, is overshadowed in current treatment regimens by the focus on exercise to promote fluid flow and compression for volume reduction. Gaps in the initial lymphatics, which increase fluid uptake when open (Breslin et al., 2018; Ikomi & Schmid-Schönbein, 1995; Mendoza & Schmid-Schonbein, 2003), are not evident in the resting state (Mendoza & Schmid-Schonbein, 2003; Trzewik et al., 2001), as in the current stationary supine study. Furthermore, pressures in the subcutaneous tissue are more like the sub-garment pressures than those in the dermis (Karakashian et al., 2019). Lack of dermal response in this study may indicate that fluctuating pressure in the dermal interstitium, necessary for the uptake of fluid into initial lymphatics (Ikomi & Schmid-Schönbein, 1995), was absent during one application of IPC.

One further physiological feature of lymphatic flow may have impacted fluid uptake during IPC: the effect of position on dermal fluid uptake in primary lymphoedema. Negative intraluminal pressure in lymphatic collecting vessels increases fluid flow rate (Ikomi et al., 1997) but lower contraction rates in supine produce lower fluid flow (Olszewski & Engeset, 1980), thereby reducing the negative ‘pull’ of fluid flow downstream. Therefore, the lower lymphangion contraction rate in the supine position could have potentially further adversely affected fluid uptake in this PLO group.

The requisite of pressure changes for fluid flow into an initial lymphatic capillary is consistent with

the findings of no decrease in dermal oedema following IPC applied in supine in the current study. No change in posture occurred, and pressure was moderately constant, particularly at the foot. Investigation into application of variable pressure to invoke fluid uptake in the dermis is a subject for future research.

7.4 Tissue Resistance

In this study, the lack of difference in IU between NLO and PLO, despite the stage II status of PLO, may have been the absence of an actual difference, or a limitation of indurometry due to the effect of underlying tissues. The equality between NLO and PLO in response to IPC argues that there was in fact no difference in superficial tissue stiffness between the two. However, the possible influence of underlying tissues on the Indurometer limits the conclusions that may be drawn about tissue resistance in response to compression.

A previous study has reported different response to a single application of IPC between the superficial tissue or skin resistance and deep tissue resistance (Zaleska & Olszewski, 2018). Direct comparison of results with this study is not useful, as tissue resistance was measured at different tissue depths and in different study populations ('post-inflammatory' lymphoedema of stage II and III in contrast to the current study of early stage II primary lymphoedema). By-products of inflammation are factors of potential influence on tissue stiffness, which reduce following compression (Brix, Apich, Ure, et al., 2020). However, there are several useful points that emerge from examining the response of the tissues in each study, which add to our understanding of the tissue response to compression.

Following one application of IPC at high (80-120mmHg) pressure, Zaleska et al (2018) found that superficial skin stiffness varied little, whereas subcutaneous tissue stiffness reduced, therefore deducing that fluid movement under the influence of IPC occurred in subcutaneous tissues rather than the dermis (Zaleska & Olszewski, 2018). This is consistent with the current findings in primary lymphoedema of decreased ECF/ICF throughout the leg segment, no change in dermal LEP, and no change in IU. It appears that the response of the dermis to compression applied by IPC remains unclear in both studies.

In the latter study by Zaleska and Olszewski (2018), both skin stiffness and deep tissue tonometry were determined by the force of tissue deformation in Newtons. Subcutaneous tissues were

observed to reduce in stiffness following IPC at all levels along the limb from ankle to groin, as measured by deep tonometry. Baseline deep tonometry at the calf was reported to be 1200g/cm^2 force to indent the tissues to 10mm depth with a 10mm diameter probe (Zaleska & Olszewski, 2018). In contrast, the Indurometer used in this study measures units of a standardised force equivalent to 200g, varying in penetration depth according to the tissue stiffness. This is a relatively superficial depth in comparison to the previously described deep tonometry, but even at this relatively superficial depth, it has been suggested that the Indurometer may be influenced by underlying structures (Douglass, Graves, & Gordon, 2017). Furthermore, the methods used by Zaleska et al (2018) included an invasive assessment of pressure, by the wick-in-needle technique to measure subcutaneous tissue pressure, which involves insertion of a needle into the tissue, and vasoconstrictors are injected to control bleeding at the needle tip. The effect of vasoconstrictors, as well as the invasive needle technique have the potential to alter tissue pressure, and provoke an inflammatory response.

Skin stiffness was measured using a Skin Fibrometer of 2.5 mm diameter and much less penetration of 1mm; significant change in response to compression was reported only at the ankle, an area which showed the greatest stiffness at baseline (Zaleska & Olszewski, 2018). The ankle potentially has higher dermal fluid than the posterior calf, given the gravitational distribution of fluid in the lower leg (Suehiro, Morikage, Yamashita, Harada, Ueda, et al., 2016) and the increased thickness of the dermis at the lower third of the leg (Suehiro et al., 2021). In addition, greater fibrosis might be expected in the latter cohort given their advanced staging, and the increased hardness that has been associated with increased dermal thickness at the lower inner leg (Suehiro et al., 2021).

In the current study, the lack of change in fluid in the dermis following compression, in the calf or the foot, may have influenced the response of tissue stiffness to compression. However, measures describing tissue resistance such as IU are influenced by several tissue layers and fail to describe the difference in physiology that causes increased resistance. Understanding the qualities of lymphoedematous tissues that contribute to its inherent resistance is limited by the ability to precisely measure and describe it, let alone its response to compression.

7.5 Conclusion

The lack of change in dermal oedema (LEP) suggests external IPC compression as applied in the

current study did not facilitate fluid uptake into the initial lymphatics in the dermis. Much is already known about the effect of external compression, which results in the reduction of limb circumference and volume (Damstra & Partsch, 2009; Gianesini et al., 2020) by reducing capillary filtrate (International Lymphoedema Framework, 2012) and intra-lumen lymphatic capillary pressure and diameter (Franzeck et al., 1997), increasing lymph flow (Adams et al., 2010; Kitayama et al., 2017; Zaleska & Olszewski, 2017). Ultimately, compression reduces subcutaneous tissue (J. H. Lee et al., 2013) and skin thickness (Hacard et al., 2014). But few studies have investigated fluid in the dermis in lymphoedema or its response to compression. Variation in pressure within the dermis is important to the uptake of interstitial fluid into the initial lymphatic (Michel et al., 2020; Mukherjee et al., 2018), the first step in reducing fluid within the dermis, yet it appears from this study of primary lymphoedema that conditions for uptake of fluid in the dermis were absent during the application of IPC in supine. Although compression acts to increase capillary uptake (Mortimer & Levick, 2004; Mosti & Cavezzi, 2019), this study has highlighted the lack of effect of compression as applied by IPC on the dermis in primary lymphoedema.

Further research is required to investigate optimal methods to reduce fluid in the dermis, and to determine the effect of fluctuating pressure in the dermis. Such investigations could include the effect of exercise, changing posture and manual lymph drainage on dermal fluid or emerging technologies such as negative pressure therapy (for example, suction devices that lift and move the skin). Further investigation of the dermis in each of these conditions could form the basis for treatment development to reduce fluid accumulation in the dermis and its subsequent pathological changes.

In addition, the lack of response to compression in the foot requires further investigation, particularly in primary lymphoedema. Factors for investigation include the length or style of compression application, including the amount and pattern of pressure applied by IPC. Varied drainage patterns from the foot in sub-types of primary lymphoedema may affect the response to compression. Sub-grouping cohorts of primary lymphoedema based on phenotype or genetic profile could allow investigation of the dermal fluid response to compression specific to the primary vessel anomaly which may affect fluid uptake in the dermis. Understanding the response of the dermis to compression according to the vessel anomaly in primary lymphoedema will form the foundation for targeted treatment.

7.6 Limitations

7.6.1 Participants

Participants were matched for age and gender, however, there a difference of two in median BMI between groups. Although not statistically significant, this could constitute a clinical difference between groups, as lymphoedema has a known association with increased subcutaneous fat deposition (Brorson et al., 2009). Fat deposition occurs in the subcutaneous tissue (Tashiro et al., 2017), not affecting the dermis. Bioimpedance measures penetrate throughout the limb, which may be influenced by BMI (Ward et al., 2000); however, the latter influence was associated with ethnic group which was constant in this cohort. More recently, BMI was reported to have no effect on ECF/ICF or TDC (Mayrovitz, Forbes, et al., 2020).

A difference between groups in history of cellulitis was also noted. Although numbers were too small to investigate its effect on the outcomes of this study statistically, the greater occurrence of cellulitis in those with primary lymphoedema is consistent with the increased incidence of cellulitis in those with lymphoedema cited in previous literature (Burian et al., 2021; Deng et al., 2015; Dupuy et al., 1999; Keeley, 2008; Vignes et al., 2007).

During data examination for normality, one NLO participant consistently presented as an outlier on LEP measures in the foot. This participant had a tattoo on the foot, not immediately under the HFU measurement site, but taken in consideration with the outlying LEP measures, it was deemed advisable to use the measures of an alternative matched NLO participant.

7.6.2 Lymphoedema Status of PLO Compared to NLO

That PLO participants were not screened genetically or investigated lymphoscintigraphically to understand underlying abnormalities which may have contributed to differences between or within this group is a limitation of this study. Instead, clinical measures were used to establish the state of each limb comparative to both its contralateral side and to the healthy group, which is the reality for many in clinical practice, where lymphoedema imaging is scant or unavailable.

Bioimpedance was used to investigate between group differences as it is used to detect lymphoedema in clinical practice (Koelmeyer et al., 2019). The bilateral presentation of primary lymphoedema in the lower limbs of approximately half of the present PLO study cohort, along with the potential for abnormal drainage in the contralateral ‘normal’ limb (Bourgeois, 2021; de

Almeida et al., 2017) of uniPLO, precluded the present study from investigations based on inter-lower limb ratios.

Investigation of lymphoedema status was undertaken within unilateral and bilateral sub-groups (uniPLO and biPLO) as those with uniPLO could be compared to the normative interleg impedance ratio (Steele et al., 2018).

The threshold for establishing bilateral lymphoedema requires an arm to leg ratio, and was published in 2019 (Steele et al., 2019), after data collection for this study had begun. To establish lymphoedema was present in the legs of those with biPLO, the mean ECF/ICF for the whole limb on each side was compared to normative values, which was limited by the exclusion of the foot. These limitations were addressed by comparing biPLO and NLO in each of the foot and leg segments and, although a difference in ECF/ICF was demonstrated between PLO and NLO in each segment statistically, these differences have yet to be tested in greater numbers to establish thresholds for lymphoedema in the foot and leg segments. The lack of previous published data also limited the conclusions that could be drawn regarding the clinical significance of the difference between PLO and NLO in the foot and leg.

7.6.3 Sample Size and Statistical Significance

These results must be understood within the limitations of a small sample, where the distribution of data in many variables was a moderate or poor representation of a normal distribution. Consequently, the results provide an indication of possible differences, to provide a base for further investigation in a larger sample.

The small sample size in this study was further reduced in comparisons of uni- and bilateral sub-groups of PLO. Results were taken from Tukey's adjustment in post-hoc analysis for the smaller samples when the PLO group was sub-divided, which resulted in samples of seven or eight (Field, 2018, p. 657) . In view of the multiple comparisons, the conservative Bonferroni adjustment was made in mixed ANOVA analyses, to avoid making a type I error (Feise, 2002).

Due to insufficient power (Pallant, 2016, p. 210) as well as the conservative adjustment undertaken (Bonferroni), the greater risk may be in making a type II error, finding no significance where there is one (Feise, 2002). Statistical significance, as indicated by p-value, is a combination of effect size and sample size: where sample size is small and effect size is large, p value will be

small (Kalinowski & Fidler, 2010). Consequently, given the small sample size here, where the effect size is small, the p-value will be large, indicating an insignificant result where possibly the effect though small, may be clinically meaningful. To ensure that an effect is recognised where there is one in the population (avoiding a type II error), generally the sample size is increased. However, the low prevalence of primary lymphoedema led to recruitment challenges with the resultant small sample. To compensate for these challenges to some extent, the clinical significance is discussed for results of interest (Feise, 2002).

7.6.3.1 Missing Measures

The removal of unmatched pairs resulted in even smaller sample sizes in some analyses, particularly uniPLO and biPLO groups. For example, one PLO refused compression, so no post-compression measures were analysed for their matched NLO participant. Sensitivity tests were run to determine the effect of removal of unmatched data on the significance of results, but found no difference in statistical significance, so results were as reported, with even pairs, unless otherwise reported.

7.6.4 Devices and Outcome Measures

There were limitations associated with each of the devices used in this study:

7.6.4.1 Bioimpedance

Standard bioimpedance measurement protocols for the whole limb (from ankle to groin) may have been inappropriate to investigate difference between groups or post intervention, given the distal (foot and leg) fluid distribution in limbs of PLO in this study cohort and the protocol for measuring whole limb bioimpedance (incorporating the leg and thigh). It is possible that whole limb bioimpedance measures taken from ankle to groin may not detect significant differences in ECF/ICF in PLO. For this reason, the segmental measurements of leg and foot were included, despite the paucity of literature describing this method. Further testing of electrode placement for foot bioimpedance is needed, along with the assumptions and modelling for foot bioimpedance and its role in discerning between groups. Measurement of the thigh segment may have provided further information regarding the fluid distribution in the lower limb of this group.

The resistivities of ECF and ICF are affected by electrolyte balance (Ward, Winall, et al., 2011). Use of the ECF/ICF ratio for comparisons, both between participants and following compression, relied on the assumption that the resistivity of these fluids remained constant over the study period

(four hours, whilst supine lying with restricted eating and drinking).

7.6.4.2 MoistureMeterD Compact

TDC has been shown to decrease with temperature (Mayrovitz, 2015), which may have caused difference due to seasonal variation. However, in the clinical setting, temperature was postulated to have little effect when 15 minutes is allowed for stabilisation following removal of garments, based on results in a healthy skin (Mayrovitz, Berdichevskiy, et al., 2020). In the present study, it is possible that there was a seasonal temperature difference between groups due to the time of year during which data was collected in each group, which consequently may have affected TDC. However, this effect was limited by data collection being undertaken in temperature-controlled clinical spaces. Furthermore, possible seasonal variation would not affect the response to compression, as measures post-compression were taken on the same day, in the same temperature-controlled environment.

As dermal thickness was not measured, the tissues which are measured by PWC are surmised from comparison to ECF/ICF and LEP and previous literature describing a thicker dermis in lymphoedema compared to healthy skin (Hacard et al., 2014; Idy-Peretti et al., 1998).

It is possible that the measurement site on the foot was influenced by underlying structures such as veins. Ideal placement for the MMDC is between the first and second metatarsals (Mayrovitz, 2019a), although good reliability is also reported at the mid-dorsum of the foot (Mayrovitz, 2015). In this study, the space between the second and third metatarsals, the second greatest space, was chosen as a flatter surface, to enable stable application of all devices, particularly the HFU and the Indurometer.

7.6.4.3 Indurometer

Factors affecting fluid balance such as hydration and menstrual cycle have been found to affect IU (Douglass et al., 2018) as well as bioimpedance (Brantlov et al., 2017a; Douglass et al., 2018): the absence of information regarding menstrual cycle was a limitation in the present study. Efforts to control hydration factors, in accordance with bioimpedance requirements, included a bathroom visit to void on arrival prior to data collection, as well as the request not to exercise vigorously prior to data collection, nor to drink alcohol for 12 hours and caffeine for four hours prior to attendance.

7.6.4.4 High Frequency Ultrasound

Diurnal fluid variations may have occurred between participants measured at different times of the day. Diurnal variation in echogenicity has been reported where changes in LEP were observed over 12 hours from morning to evening in both the elderly (aged 75-100) and the young (aged 17-27) (Gniadecka, Serup, et al., 1994). However, no change in echogenicity was seen in the latter study in those who remained supine over the same period, indicating that the change occurred due to gravitational stress. In this study, data collection occurred during supine lying, following approximately 30 minutes to allow for equilibrium, eliminating gravitational stress.

CHAPTER 8 CONCLUSION

8.1 Summary Overview: Contribution to Knowledge

This study demonstrates three original contributions to knowledge. High distal fluid accumulation was described by dermal and deeper tissue fluid measures in the foot and lower leg in young people with primary lymphoedema for the first time. This is consistent with lymphoscintigraphic descriptions of the distal fluid distribution in primary lymphoedema and indicates the need to address treatment to the dermis in the foot in primary lymphoedema. The second original contribution was the observed lack of response to compression in the dermis at any site. This raises important clinical questions about the effect of pressure applied by IPC on the initial lymphatics. Fluid uptake in the dermis is known to be influenced by variable pressure, which may not be optimally applied by IPC in supine. The third original contribution was the lack of response to IPC in all measures in the foot. These findings impact on clinical practice and warrant further investigation. If IPC is ineffective in changing fluid accumulation in the foot, alternative treatment strategies are required, and clinical practice adapted accordingly.

The first original contribution of this research was evident in the foot, which, with the distal leg, is a segment particularly affected in primary lymphoedema. The demonstration of higher dermal fluid in the foot in people with primary lymphoedema, compared with normal tissues, forms the basis for future investigation to address skin changes in this understudied segment. In contrast to the foot, the dermal fluid in the posterior calf of PLO was not significantly different to NLO, despite lymphoedema measures of local percent water content (PWC) and extracellular to intracellular fluid ratio (ECF/ICF), being elevated in PLO compared to NLO throughout the foot and leg. Clinically, high dermal fluid measures in the PLO foot are consistent with pathological changes in the dermis observed in primary lymphoedema in the foot. The finding of high fluid content in the dermis of PLO supports and confirms in clinical measures the effect of lymph vessel anomalies seen by lymphoscintigraphy in the distal leg of primary lymphoedema. This highlights the clinical need for treatment to address increased fluid in the dermis in the foot.

A second original contribution of this research demonstrated that applying pneumatic compression did not instigate measurable change in either fluid distribution or tissue resistance in the foot in people either with or without primary lymphoedema. This is a commonly used treatment and, subject to confirmation in further studies, has wide implications for treatment of

lymphoedema generally, especially for those with primary lymphoedema in whom the foot is particularly affected.

The third original contribution demonstrated no response in the dermis at any site following compression with IPC. Fluid accumulation in the dermis leads to superficial evidence of pathological tissue alteration, observed in the skin as lymphoedema progresses, and yet a key treatment strategy for lymphoedema, compression applied by IPC, has been demonstrated in a single dose to have little effect on the dermis in people with primary lymphoedema.

This study provides the first objective clinical evidence of fluid accumulated in the dermis in the foot in primary lymphoedema and furthermore, that it is not responsive to a standardised dose of a commonly used treatment strategy. This study sets the foundation for future research to confirm and widen understanding of the effect of compression on the dermis, particularly in the foot.

Further research is needed, both to confirm these initial findings and to investigate methods that will reduce fluid accumulation in the foot and in the dermis. Possible areas for investigation include 1) IPC effectiveness: sufficient dosage, effective transfer of pressure from the sleeve to the foot; 2) treatment of the dermis: effect of variation in dermal pressure on fluid uptake; and 3) the effect of compression on the dermis in primary lymphoedema with known specific drainage anomalies.

8.2 Implications for Clinical Practice

8.2.1 Primary Lymphoedema and the Foot

The foot is the site for assessment of early skin changes by the Stemmers test, which is an indirect indication of tissue thickening, but provides no understanding of the physiological change that has taken place in the dermis or below. This study has demonstrated high fluid content in the foot in primary lymphoedema, particularly in the dermis, and flags the need to address treatment to the foot to reduce dermal fluid.

8.2.2 Use of High Frequency Ultrasound

Staging of lymphoedema has been long been hampered by the lack of a quantitative measure to track the change in tissues from fluid to fibrosis. The use of high frequency ultrasound (HFU) in tandem with percent water content and bioimpedance has provided fluid measures in the dermis,

at a point and within the segment, offering some evidence towards understanding of fluid distribution in the distal lower limb segments in primary lymphoedema previously seen lymphoscintigraphically, and now demonstrated in clinical measures for the first time.

A reliable method for dermal fluid measurement using the DermaScan C high frequency ultrasound has been established.

8.2.3 Segmental Bioimpedance in the Lower Limb

This study has demonstrated the impact on clinical practice of the distal fluid distribution in primary lymphoedema when using bioimpedance. Fluid distribution in lower limb primary lymphoedema seen in the PLO of this study cohort contrasts to that reported of lower limb secondary lymphoedema, in which bioimpedance protocols have been developed. This has implications for the use of bioimpedance in primary lymphoedema. Evidence of fluid accumulation in the foot, found in the early stages of primary lymphoedema in this PLO cohort, indicate the importance of including the foot in bioimpedance measures for primary lymphoedema.

For the first time, segmental bioimpedance was measured in the foot as well as the leg, showing significantly increased bioimpedance measures of ECF/ICF in primary lymphoedema compared to those without lymphoedema. The foot is a segment which is seen clinically to have both overt swelling as well as skin thickening, which is palpated as tissue resistance; measurement of the foot by bioimpedance could be applied to broaden understanding in all types of lymphoedema. Further clinical significance of the greater fluid accumulation in primary lymphoedema remains to be determined when more is known about segmental bioimpedance of the foot and leg.

8.2.4 Compression

That none of the five measurement methods used in this study detected change following IPC in the foot indicates questionable efficacy of compression applied in this manner to the foot. The lack of a response to compression in the foot could be attributed to anomalous fluid uptake in the foot or vessel anomaly in the leg or thigh impairing drainage in primary lymphoedema. However, the absence of a response in NLO as well prompts further investigation of IPC applied to the foot. To my knowledge, previous literature on IPC has not reported its effect on fluid distribution specifically in primary lymphoedema, nor the foot in any population. Until IPC can be demonstrated to invoke change in the clinical measures of bioimpedance, MoistureMeter, and HFU at the foot, alternative treatment methods need to be considered to reduce fluid in the foot.

In addition, the lack of response to compression in the dermis at any site prompts further scrutiny. The mechanism of fluid uptake into the initial lymphatics requires variable pressure in the interstitium of the dermis; the lack of response to compression suggests that IPC is not providing optimal conditions for fluid uptake. Further, the lack of response in people with no lymphoedema suggests that the mode of compression was responsible. Few studies have investigated echogenicity in the dermis in lymphoedema and there is limited information on the expected echogenic response of the healthy dermis to compression. Previous clinical investigations of response to compression have investigated subcutaneous tissue not the dermis.

The impact of increased dermal fluid is seen in the pathological changes that appear in the dermis in later stage lymphoedema; effective treatment is required to reduce dermal fluid in early stage lymphoedema. The clinical impact of this study appears to indicate that IPC, one form of compression treatment, is not addressing dermal fluid. Furthermore, given the lack of correlation of dermal fluid measures with either segmental or point fluid measures, it appears that current clinical tools of bioimpedance and MoistureMeter do not assess change in the dermis. Consequently, it appears that current treatment methods are not assessed for their effect on dermal fluid.

The response of the untreated limb in PLO also invites further scrutiny. The small significant increase in ECF/ICF in the contralateral leg of PLO following compression suggests caution in applying pneumatic compression to one limb only in primary lymphoedema, as drainage paths are frequently unknown. Although this response to compression was small and requires investigation in greater numbers to confirm this risk, an increase in ECF/ICF on the contralateral side suggests therapists may need to consider applying pneumatic compression bilaterally in primary lymphoedema, unless drainage pathways have been imaged and are known.

8.3 Future Research

These findings form the basis for further investigation of the dermis in primary lymphoedema, and treatment methods to address fluid in the dermis. The response of the dermis to compression bears further investigation under differing conditions invoking variable pressure within the dermis to assess changes that may indicate improved fluid uptake into initial lymphatic vessels. Such investigations could involve different modes of compression or indeed, different modes of treatments applying variable pressure to the skin, such as manual lymphatic drainage or negative

pressure devices. Such research could include the effect of postural changes on the dermal fluid in the foot. Understanding the response of the normal dermis could form the foundation for investigation of treatment for the dermis in the primary lymphoedema. Such studies, addressing not only the response of the dermis, but the whole foot, are required to underpin improved treatment of the dermal changes seen in the foot in lymphoedema, which could be useful in other forms of chronic lymphoedema.

Future research in people with primary lymphoedema may benefit from baseline stratification either by genetic phenotyping, or by type of drainage fault or vessel anomaly delineated by ICG imaging and/or lymphoscintigraphy. Stratification would require large numbers of primary lymphoedema but could allow investigation of treatment specific to phenotype. Due to the relatively low prevalence of primary lymphoedema, this may involve cooperation between several large centres with access to imaging. The addition of imaging to illustrate anomalous drainage in different phenotypes of primary lymphoedema, together with measures of dermal fluid, could inform understanding of the effect of different forms of anomalous drainage on the dermis compared to the healthy dermis, and differences in its response to compression or other forms of treatment, particularly in the foot.

Stratifying lymphoedema by a consistent staging system with objective tissue measures could allow study of the tissues at different stages of fluid-to-fibrotic change and improve understanding of the effect of compression on the tissues at each stage. Comparison between the superficial Skin Fibrometer and the deeper penetrating Indurometer may further understanding of the tissue resistance specific to the dermis in isolation from underlying tissues. At this stage, it appears that fluid measures provide more appropriate measures for assessment of response to compression than indurometry.

A longitudinal study of duration from childhood through adolescence and into adulthood, along with the genetic makeup of the underlying cause, may provide information regarding the progressive nature of lymphoedema in the lower limb in different phenotypes of primary lymphoedema. Understanding the nature of the physiological deficit (for example valve dysfunction, vessel dysmorphia) within future studies on primary lymphoedema will enable study of treatment adaptation according to anomaly.

8.3.1 Detection of Fluid Accumulation in Primary Lymphoedema

Detection of fluid accumulation in the foot and leg has implications for potential early intervention in children and young adults, in whom primary lymphoedema develops in most cases, and who go on to live with lymphoedema for the longest time. If risk indicators such as family history are known, investigation of the foot or leg potentially may assist early identification of fluid accumulation in those who have no obvious swelling but may be susceptible to familial forms of late onset primary lymphoedema, which become overt in late childhood, adolescence or later.

The possibility of progression in primary lymphoedema (for instance, the foot being a precursor to leg involvement) is an area for investigation to identify whether different types of primary lymphoedema are more susceptible than others to progression, and over what period. If the foot is a precursor to leg involvement in primary lymphoedema, and oedema causes progressive damage, as described in ISL staging, the urgency to detect and intervene as early as possible in primary lymphoedema becomes clear, as is standard practice in secondary lymphoedema. Alternatively, unilateral primary lymphoedema may simply be demonstrating a less severe form of disease than bilateral. If so, current ISL staging fails to describe these differences in primary lymphoedema.

Future research to establish appropriate segmental testing protocols in bioimpedance could facilitate more accurate measurement of fluid accumulation in primary lymphoedema. Investigation of leg and foot segments in a large cohort of healthy lower limbs is needed to identify a protocol for measurement of impedance in the foot and leg and establish lymphoedema thresholds. Normative bioimpedance measures in the foot and leg segments could then be used to explore early identification of primary lymphoedema. Bioimpedance protocols that include measurement of the foot may also be useful in the management of other forms of lymphoedema, as distal spread occurs under the effect of gravity.

To accurately measure and identify group differences in primary lymphoedema, measurement protocols for the foot could be incorporated into future research. Previous studies have reported a proximal distribution of fluid in secondary lymphoedema, in contrast to the distal distribution of fluid in primary lymphoedema. Comparison of impedance values of stage I to early II lymphoedema in foot and leg segments in primary lymphoedema with those of similar stage of secondary lymphoedema, could guide the focus for treatment according to the relative

distribution of fluid in early stage primary compared to secondary lymphoedema.

Until appropriate bioimpedance protocols are established for segmental measures of fluid, PWC appears to provide a clinically useful measure of local fluid accumulation, capable of distinguishing between those with and without primary lymphoedema, in the foot and leg.

8.4 Perspective

There has been increased interest in tissue change in lymphoedema and its measurement over the seven years since this study's inception. Prior to that, the limitations of dimensional measures alone were beginning to be reported. Assessment of tissue change mostly relied on imaging equipment, available in large centres, but not available for the majority of lymphoedema therapists, many of whom are private therapy practices or rural and remote areas. Since this study began, tissues have been investigated using a range of devices in different populations of post-surgical or 'post-inflammatory' lymphoedema, with one also investigating response to one application of compression. As well as using volume or circumference to describe lymphoedema, previous studies have used bioimpedance to describe fluid distribution or combined dimensional measures with local tissue water, skin thickness, tonometry, bioimpedance and imaging to quantify the tissue changes on which staging of lymphoedema relies. All previous study samples consisted of secondary, mixed cause or mixed primary and secondary lymphoedema, or oedema following cellulitis. Investigations of the dermis and tissues in primary lymphoedema alone have not been reported.

Investigations of tissue properties in the lower limb have focused on the leg, excluding the foot. Although some HFU studies have described properties of the dorsum of the foot in healthy skin, those investigating lymphoedema included foot measures within mean lower limb measures. Primary lymphoedema has been described as more common distally, affecting the foot and below the knee frequently from birth onwards, so has an effect in the foot for potentially the longest time. It is in the foot that skin changes are noted, indicating the accumulation of fluid in the dermis, and this is used diagnostically for lymphoedema in Stemmer's sign. For this reason, it is important that studies address lymphoedema in the foot and its management, as well as tissue change exclusively in primary lymphoedema.

8.5 Concluding remarks

This study describes for the first time, the immediate response of the dermis and tissues of the foot in primary lymphoedema to compression. In addition, a reliable method was established using high frequency ultrasound for dermal investigation, as well as the first data comparing people with and without primary lymphoedema. Results indicate the utility of high frequency ultrasound for fluid measures specifically in the dermis, as well as percent water content (PWC) and extracellular fluid (ECF/ICF) measures in foot and leg segments for determining fluid-affected areas in lower limbs with primary lymphoedema.

Further work is required to isolate dermal tissue resistance from that of underlying tissues. Limitations have been demonstrated in primary lymphoedema in using lower limb bioimpedance measurement protocols, from which the foot, a key testing site for clinical signs indicative of lymphoedema, is omitted. Bioimpedance measurement of the foot as a segment requires further testing and protocols for detection of lymphoedema require adaptation to include the foot for use in early stage primary lymphoedema.

The clinical impact of this study extends to the potential for future early identification of primary lymphoedema using bioimpedance. Unlike secondary lymphoedema, which is predominantly identified following cancer treatment, there is no trigger to recognise primary lymphoedema. Early detection is absent in primary lymphoedema, which results in delayed diagnosis and treatment. Familial forms of primary lymphoedema, for which adolescents may be at risk, require a detection method appropriate to the fluid distribution in primary lymphoedema. Future research to established normative bioimpedance measures in the foot and leg segments could promote the development of protocols for early identification of late onset primary lymphoedema.

Finally, the lack of response of the foot to a single dose of compression highlights the need to investigate and treat each anatomical area independently. The lack of response of the dermis following IPC indicates that something other than a single dose of IPC compression plays a role in fluid uptake from the dermis. Such factors invite future investigation, which may include the role of variable pressure that arises in the dermis following changes in posture and exercise, or devices which stretch and move the skin. A ‘one size fits all’ approach to treatment, in which the global application of pneumatic compression to a limb is trusted to achieve changes in each leg segment, has been shown to have no effect in the foot in primary lymphoedema. The lack of response in the

foot to this commonly used treatment strategy requires further investigation to inform treatment decision-making for the therapist in clinical practice and address the accumulation of fluid in both the foot and the dermis.

The clinical importance of objectively quantifying the stage of lymphoedema led to this study, as the limitations of dimension alone were evident clinically as an insufficient basis for treatment decisions. Distal fluid accumulation observed in primary lymphoedema requires targeted treatment, particularly to address the dermis which was demonstrably unchanged by a common treatment modality. Future dermal investigations of primary lymphoedema could benefit by additional baseline stratification by imaging or genetic abnormality. Determination of the baseline vessel anomaly or underlying fluid transport fault in future investigations may progress understanding of the dermal response to treatment in primary lymphoedema.

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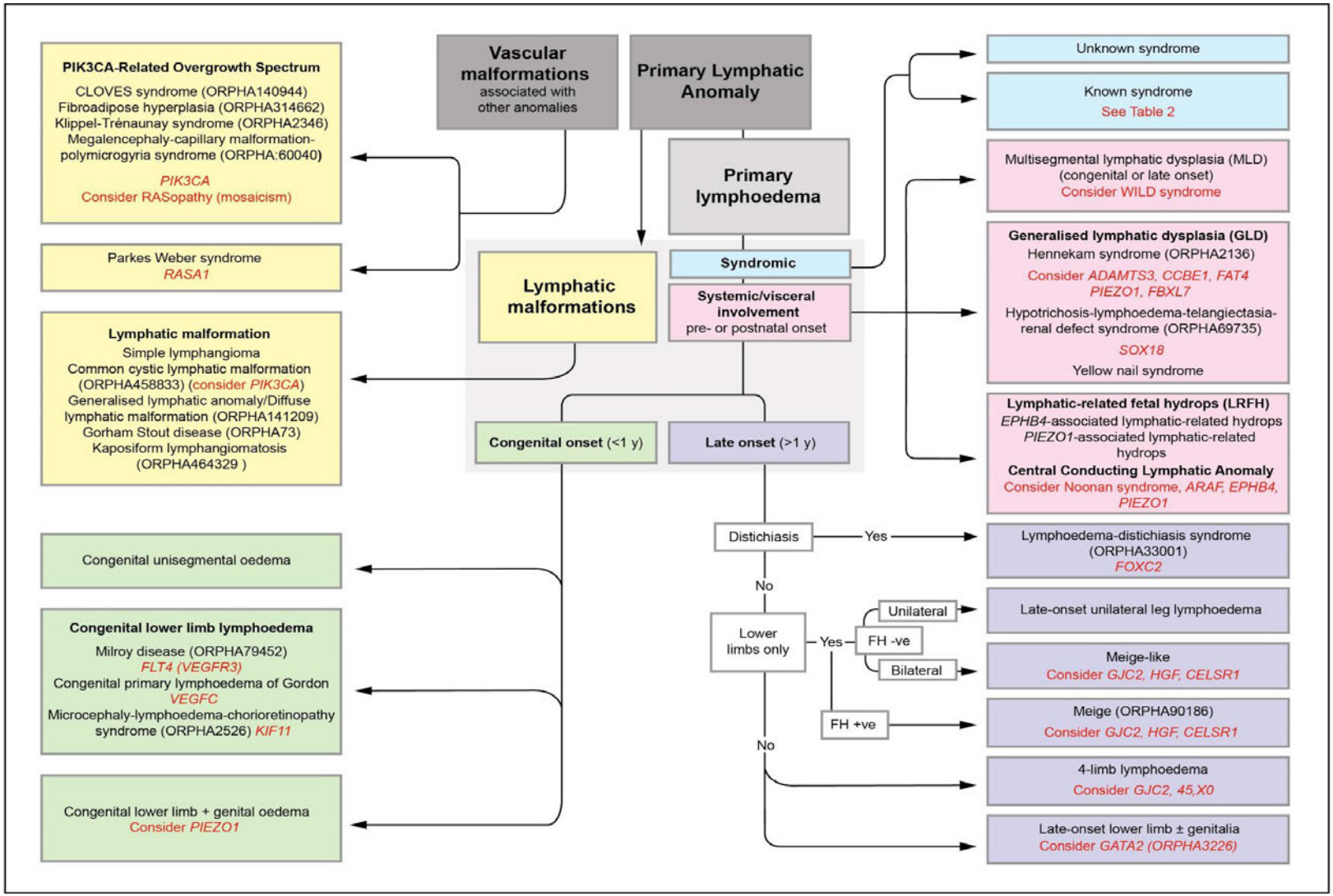
APPENDIX A
ST GEORGE'S CLASSIFICATION ALGORITHM OF PRIMARY LYMPHATIC ANOMALIES



St George's Classification Algorithm of Primary Lymphatic Anomalies
Gordon K, et al (2020). Image shared by St George's Lymphovascular Research Group under the CC BY-SA 4.0 International licence on Wikimedia Commons

Appendix B Systematic Review of Dosage for Intermittent Pneumatic Compression

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Intermittent Pneumatic Compression Dosage for Adults and Children with Lymphedema: A Systematic Review

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Abstract

Background: Pneumatic compression has been used for more than 40 years in the management of lymphedema (LE). Modes of application have evolved with little consensus regarding optimal treatment parameters or dosage. The aim of this systematic review was to report the evidence for dosage of intermittent pneumatic compression (IPC) for people with LE and, particularly, that for upper versus lower limbs or child versus adult dosage.
Methods: Medline, Embase, CINAHL, PubMed, and Scopus were searched with terms, including LE and IPC devices, with no restriction on time. Other materials searched included reference lists of included articles.
Study Selections: Systematic review registration: PROSPERO ID: CRD42017054338. Studies were assessed according to PRISMA (preferred reporting items for systematic reviews and meta-analyses) guidelines and were excluded if they were not in English, not human, had physiological outcomes, or studied IPC in combination with other therapies. Quality appraisal, using the McMaster University Critical Review Tool, was undertaken by two researchers with differences resolved by a third. One hundred twenty-two full-text studies were screened for eligibility.
Results: Sixteen met inclusion criteria for final analysis. Of these, four were reported separately due to concurrent use of compression garments during the study period. One randomized controlled trial met the requirements for a level II (National Health and Medical Research Council) rating; the remainder were level III-2 and below. Devices applying compression via multichamber sleeves were more commonly used in the past 20 years, with a trend toward lower pressures and shorter treatment times compared with earlier studies. Little evidence exists for application of specific dosage of IPC for children or a particular limb. New devices utilizing lower pressures support home use and self-management of LE.
Conclusion: Low-level evidence of moderate quality shows significant outcomes achieved with dosage times of 45–60 minutes, applying pressures between 30 and 60 mmHg in sequential IPC programs. Methodological limitations in most studies suggest caution in drawing conclusions.

Keywords: lymphedema, intermittent pneumatic compression, application, pressure

Introduction

Compression is the mainstay of lymphedema (LE) management. Intermittent pneumatic compression (IPC) is used as an adjunct treatment to compression garments (CG), bandages, and wraps; however, there is ongoing debate about optimal IPC dosage for the management of acute LE[1] and chronic LE.[2] Clinically, when CG are being prescribed, the level of compression is varied according to individual tolerance of pressure, upper limb (UL) or lower limb (LL), children or adult, and for early- or late-stage LE (according to the condition of skin and subcutaneous tissues). There are currently no guidelines to indicate how pneumatic compression dosage should be varied to optimize outcomes according to these factors.[3–5]

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Lymphoscintigraphy[6,7] has shown lymph movement in response to pneumatic compression with 50–125 mmHg pressure and near-infrared fluorescent lymphatic imaging (NIRFLI)[8] with low mean pressures (<15 mmHg). Fluid movement has been demonstrated after 3 hours,[6] 1 hour,[9] and even 1–3 minutes[10] of IPC application. Hence, both optimal pressure and duration of compression for lymphatic movement are unclear.

Historically, single-cell IPC sleeves[11] and application of constant pressure gave way to sleeves with multiple cells or chambers and the development of varied time cycles to prevent backflow of lymph and address patient discomfort.[12,13] More recently, IPC devices have been developed to replicate manual techniques of a therapist's hands, utilizing low pressure with short repetitive application moving progressively along a limb to simulate manual lymph drainage (MLD)[14] and sleeves that incorporate the root of the limb to clear the pathway for drainage.[14–16]

Varied application and dosage in IPC trials have resulted in a wide range of outcomes.[17] As well, IPC has mainly been investigated as an addition to standard decongestive treatment rather than in isolation, and hence, the effect of IPC alone remains unclear. In a recent systematic review of IPC for secondary UL LE, which included studies with contamination between interventions, only two of seven randomized controlled trials (RCTs) investigated IPC alone (both of which are included in this review). Understandably, the benefit of IPC was not clear from a meta-analysis of three RCTs where IPC was combined with complex decongestive therapy (CDT).[17]

An earlier review (2010), focusing on IPC for UL LE, found a lack of evidence for (1) the benefit of IPC over skin care alone; (2) the benefit of one IPC cycle type over another (e.g., intermittent vs. sequential); and (3) lack of agreement over pressure dosage.[18] Few reviews have investigated the effect of IPC in isolation from other management strategies and no reviews have previously investigated dosage specifically. To date, flow has been explored by both imaging (lymphoscintigraphy, NIRFLI) and invasive methods (needle-wick measures); flow has been demonstrated in the LL under both low[8] and high pressures,[19] and the occlusion pressure of superficial vessels in the UL has been shown to be relatively high.[20] However, what pressure is both comfortable for a limb and producing optimal flow (indicated by a reduction in limb size of importance to the patient) over what length of time?

Studies using a combination of reduction therapies provide no information regarding the optimal dose of IPC that is safe, comfortable, and effective. This systematic review aimed to (1) identify the literature with outcomes of IPC alone applied to lymphedematous limbs with or without maintenance CG use; (2) review the quality of the research; (3) consider objective limb reduction outcomes to identify dosage that was most effective with least adverse effects; and (4) identify evidence for dosage specific to age or limb.

Methods

This systematic review was registered with PROSPERO (ID: CRD42017054338) (https://www.crd.york.ac.uk/PROSPERO/) and followed the PRISMA (preferred reporting items for systematic reviews and meta-analyses) protocol.[21] Databases searched included Medline, Embase, CINAHL, PubMed, and Scopus until March 2018. Terms included LE and IPC devices and were limited to English:

1. lymphedema/or elephantiasis/or non-filarial lymphedema/
2. Intermittent Pneumatic Compression Devices/
3. (((intermittent or pneumatic or sequential or lympha-press or lympha-press) and (compression or pump* or massage* or hose or device)) or impulse or ArtAssist or Flexitouch or FLOWTRON or Plexipulse or (SC-2004 adj Sequential) or Walkcare).tw, kf, hw.
4. 1 and (2 or 3)
5. limit 4 to English language

Screening of articles was undertaken by two people (J.J.P., S.J.G.); any difference in inclusion was resolved by discussion with reference to a third researcher if necessary.

Study selection

Studies included were peer-reviewed studies of National Health and Medical Research Council (NHMRC) level III-3 or higher, with IPC being the intervention under investigation or comparator where IPC was applied in isolation from other therapies, or if they incorporated the use of CG between IPC treatments (in accordance with clinically accepted maintenance therapy for LE management[22]). Studies where CG were applied during the study period were assessed and reported separately.

Eligible studies provided objective limb reduction outcomes (such as limb volume or circumference) of greatest relevance and translation to practice for clinicians; those utilizing physiological or imaging outcomes, such as measures of lymph flow, were not included (e.g., Adams et al.[8] and Aldrich et al.[9]). Studies were also excluded if they were retrospective, expert opinion, provided incomplete or variable dosage parameters, or the study population was not human or did not have LE. Studies investigating constant pressure devices were excluded,[23] as they are no longer used in practice.

Quality assessment and data extraction

Each study was critically appraised by two of three assessors (J.J.P., RP/AB) using the McMaster University Critical Review Tool,[24] a generic validated quantitative appraisal tool. Differences in appraisal were resolved by discussion, and where there was an unresolved difference, a third assessor (S.J.G.) was consulted. Critical appraisal scores were categorized as poor (≤8); fair (9–10); good (11–12); very good (13–14); and excellent (15).[25]

Information relating to devices, dosages, and outcomes was extracted for all eligible studies (J.J.P.). Primary outcomes were limb volume or circumference; secondary outcomes included subjective response, skin or tissue assessment, or other objective assessments (e.g., bioimpedance). Dosage parameters of pressure, duration, cycle timing (inflation and deflation time, where available) were extracted, as well as sample characteristics, limb treated, and outcomes (percent volume or circumference reduction, if available; where this information was not reported in the publication but able to be calculated from the data provided, it is reported *in italics*). Clinical and statistical significance and adverse events were also extracted for both those investigating IPC alone and IPC in combination with CG.

Results

A total of 2173 studies were identified. After consideration of title, abstract, and full text, 16 studies met inclusion criteria and were accepted for critical appraisal (Fig. 1).

Twelve studies reported the use of IPC alone and four investigated IPC with maintenance CG use between IPC treatments (IPC+CG). Several studies provided results for a device that is not currently commercially available and were excluded unless adequate information regarding dosage allowed replication with a current IPC device. Study characteristics, including population, intervention and comparators, and device and dosage parameters, are provided in Table 1.

Level of evidence

Of 16 studies, Dini et al.[26] (IPC alone) was the only level II (NHMRC) randomized controlled study. Berlin et al.[27] (IPC+CG) included a control group wearing CG only; however, participants were not randomized to group, so rated level III-2. Several studies commented on the ethical dilemma of a control group. Most other studies were either single-case design (before/after) studies or were comparative studies without concurrent controls (level III-3).

Quality of evidence

Comparative studies differed in population with relation to limb, stage, or duration of LE, resulting in nonequivalent comparisons between studies. Study limitations included selection bias (self-selection for group), baseline differences between groups, protocol variation according to participant response, lack of information regarding reliability of outcome measures, lack of evaluation of participant experience, lack of evaluation of between-group differences, and poor reporting and clarity of results (Table 2).

Overall, the quality was poor for IPC-alone studies, with a mean score of 8 out of 15 (12 studies: range 4–11) and fair for

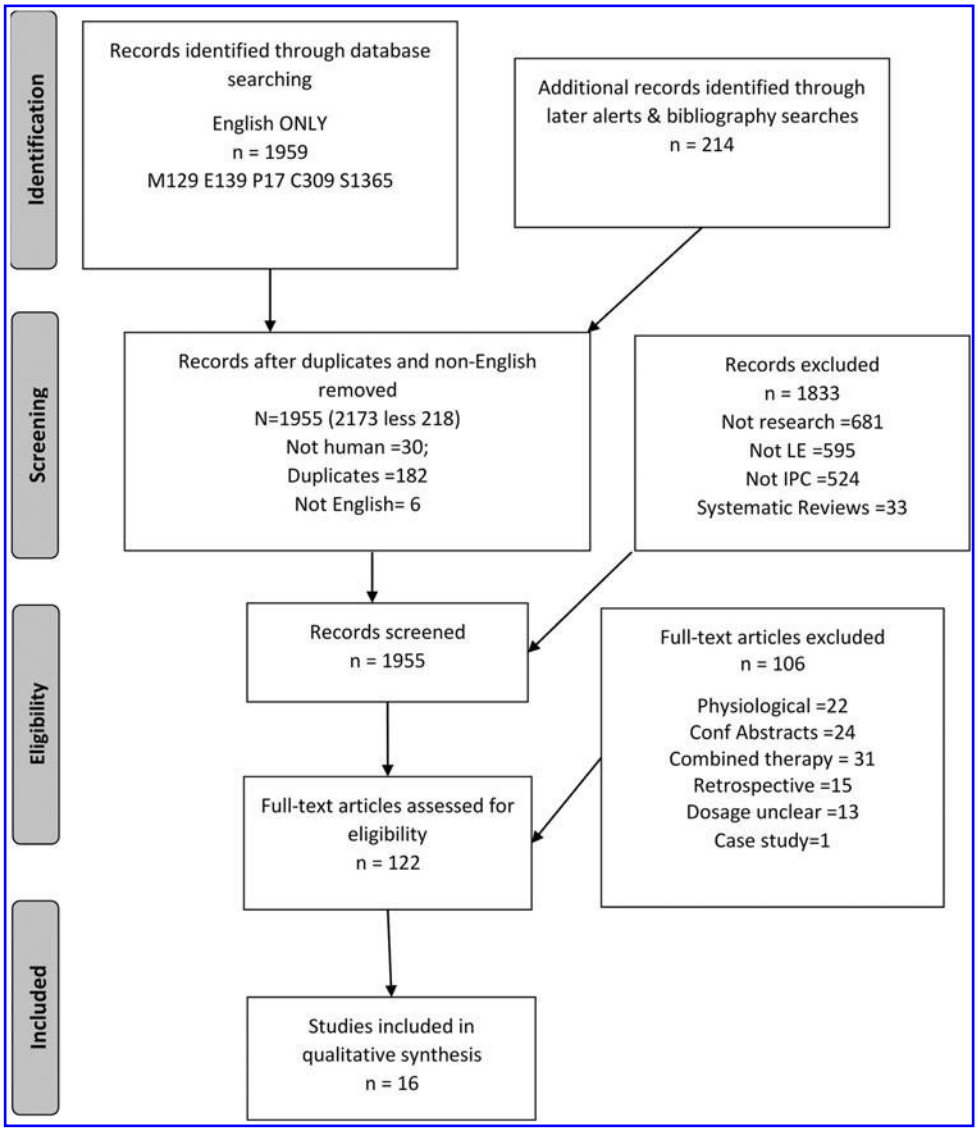

FIG. 1. PRISMA (preferred reporting items for systematic reviews and meta-analyses) diagram[21] of IPC study selection. C, CINAHL; E, Embase; M, Medline; P, PubMed; S, Scopus. IPC, intermittent pneumatic compression.

Table 1. Population, Device Characteristics, and Dosage

Study: First author, year; study rating (NHMRC) Population, mean age (years) (%, range or SD)	*Device/sleeve*		*Cycle duration Seconds unless denoted Minutes*	*Pressure, mmHg*		*Duration of application hours; weeks; months; years*
	Single cell	*Multicell*		*Constant*	*Graduated*	
IPC alone						
Raines[30] 1977 Level III-3 $n=17$ UL 11 LL 6 SLE Age NR	"New device" Single cell		Cycle: 30 InfT 12 DefT 18	60		4 hours (30 minutes off) 16 hours in 24 hours Study period: 1 day
Zelikovski[12] 1980 Level III-3 $n=25$ UL SLE 17/25 (68%) aged 40–50 years		L-P	Cycle: 25 InfT: 20 DefT: 5	100–150 sequential		2–3 hours per day 3 days
Kim-Sing[36] 1987 Level III-3 15 UL SLE median: 67 (35–81) years		WLP	Cycle: 120 InfT: 90 DefT: 30 Distal cell InfT: 90 Middle cell: 70 Proximal cell: 50		Distal cell: midway between systolic and diastolic BP Middle cell: less 20 Proximal cell: less 40	2 hours, 2×4 hours, 2×6 hours, and 2×8 hours=38 hours 1 hour rest between each session. Study period: 48 hours
McLeod[33] 1991 Level III-3 9 LL children F7; M2. Unilateral LO 8 PLE 1 SLE. HF $n=3$ 16.6±1.3 years WLP $n=3$ 10.8±7.1 years L-P $n=3$ 14.7±4.8 years		HF: 3 cells WLP: 3 cells L-P: 9–12 cells	HF: 5 minutes WLP: 120 InfT: 90 DefT + pause: 30 L-P: cycle time 30	HF: 30–120 L-P: mean 122.5	WLP: mean 85/65/4	2 hours on, 30 minutes off when awake AND Night use: 6 hours continuously. Study period: 2 days and nights.
Pohjola[31] 1995 Level III-3 $n=20$ PLE and SLE 10 UL and 14 LL F18 M2; Mean age: 63 years (46–88)	*Jobst Single cell Retro-spective*	Venti-press: model 14 Multicell: number NR	Cycle: 4 InfT: 15 DefT: 25 *Jobst: NT*	60		2 hours per day 5 days *6 hours per day* *5 days (retrospective)*
Bergan[32] 1998 Level III-2 $n=35$ UL: 17 SLE; LL: 11 PLE, 7 SLE Mean age: 26F: 56.9 (36–82) years 9 M: 56.4 (31–83) years	**7000:** Single cell non-gradient	**7500:** 3-cell segmental **2100**: 10-cell graduated		**7000:** 50 **7500**: 50	**2100**: gradient: 80 distal to 30 proximal (80; 74; 69; 63; 68; 52; 47; 41; 36; 30)	2 hours with each device Washout NR
Dini[26] 1998 Level II RCT $n=67$ UL SLE Mean age 62 (±11) years	NR		NR	60		2 hours 20 sessions Study period: 9 weeks
Pilch[35] 2009 Level III-3 UL $n=57$ SLE Mean age/group: 57–58 (39–80) years	FTP Group 1: 1 cell FTF Group 3: 1 cell	FTP Group 2: 3 cells FTF Group 4: 3 cells	FTP (Groups 1 and 2) InfT: 90 DefT: 90 FTF (Groups 3 and 4): InfT: 45 DefT: 15	30–50 Mean (SD): 1: 38.82 (4.16) 2: 39.44 (3.00) 3: 36.36 (5.52) 4: 36.25 (4.25)		1 hours 5 days per week Study period: 5 weeks
Modaghegh[28] 2010 Level III-3 $n=43$ LL 24 PLE 19 SLE Mean age: 37.8 years (14–80) 43 LL		Pn-L: 4 cells	InfT+ hold time: 15	80–120		8 hours per day Study period: 2 days
Ridner[38] 2012 Level III-3 $n=42$ UL SLE Mean age 53.8 years (SD 8.6)		FT 1: Trunk + arm IPC 2: Arm IPC	Hold: "split second"	Mean pressure 9.0±4.2 to 13.7±4.9		1. Trunk + arm IPC: 60 minutes per day 2. Arm IPC: 36 minutes per day Study period: 1 month

(*continued*)

TABLE 1. (CONTINUED)

Study: First author, year; study rating (NHMRC) Population, mean age (years) (%, range or SD)	*Device/sleeve*		*Cycle duration Seconds unless denoted Minutes*	*Pressure, mmHg*		*Duration of application hours; weeks; months; years*
	Single cell	*Multicell*		*Constant*	*Graduated*	
Theys[29] 2015; Level III-3 *n*=9 UL SLE Mean age: 71 years (54–83) Crossover: 1. MLD or 2. IPC		i-Press: 7 cells	Cycle: 6 minutes 15 seconds	40		16 minutes MLD 16 minutes IPC 16 minutes 15 minutes rest before crossover (unclear) Study period: (unclear)
Chmielewska[40] 2016 Level III-3 *n*=21 UL SLE Mean age years (SD): Group A: 60.45 (7.34) Group B: 61.4 (5.44)	Boa device (Metrum CryoFlex). Sequential Number of cells NR		NR	60 A: IPC + exercises B: IPC alone		45 minutes 5 days per week Study period: 4 weeks
IPC+CG studies						
Johansson[37] 1998 Level III-3 *n*=24 UL SLE; Group 1 MLD: Median: 64 years (52.5–69.5) Group 2 IPC: Median: 57.5 years (47.5–69.5)		L-P: 9 cells	NR	40–60		Gp 1: MLD 45 minutes Gp 2: IPC 2 hours 5 days per week Study period: 2 weeks.
Berlin[27] 1999 Level III-2 *n*=55 UL SLE Median age: 70–79 years (range 20 to >80)		CG Flowt L-P	Flowt InfT 2 minutes DefT 2 minutes L-P Cycle 30: InfT 20 hold 6; DefT 4.	CG: 25–50; Flowt: 80 L-P: 90–120		CG: NT Flowt: At least: 20 minutes per day L-P: 20–30 minutes twice per day 5 days per week Study period: 4 weeks
Fife[39] 2012 Level III-3 *n*=36 UL SLE Mean age: 63.9 (±12.2) years		BC FT	BC: Cycle: 112 seconds InfT: 72 Hold: 22 DefT: 18 FT: Cycle 1–3	BC: 30 FT: Mean 9.0±4.2 to 13.7±4.8		1 hours B-C: Cg 23 hours per day + BC 1 hours per day F-T: CG 23 hours per day + FT 1 hours per day Study period: 12 weeks at home
Zaleska[34] 2014 Level III-3 *n*=18 LL Age range: 18–62 years SLE due: Infection 13, Unknown: 4		BC: 8 cells	Total InfT: 400 InfT/cell: 50 DefT 50		At foot: 120 Decrease 20% by groin	45 minutes per day Study period: 24–36 months

BC, bio compression; BP, blood pressure; CG, compression garments; Cycle, InfT+DefT; DefT, deflation time; Flowt, flowtron; FT, flexitouch; FTF, flowtron flowpac; FTP, flowtron plus; HF, hemaflow; InfT, inflation time; IPC, intermittent pneumatic compression; LL, lower limb; LO; L-P, Lympha-Press; MLD, manual lymphatic drainage; NHMRC, National Health and Medical Research Council; NR, not reported; PLE, primary lymphedema; Pn-L, pneumo-lymph; SLE, secondary lymphedema; UL, upper limb; WLP, Wright Linear Pump; SD, standard deviation.

TABLE 2. OUTCOME, SIGNIFICANCE, LIMITATIONS, AND ADVERSE EVENTS

Study: First author, year; study rating (NHMRC) Sample size; population	*Mean edema reduction (SD)% (outcome measure)*	*Statistical significance*	*Clinical conclusions*	*Limitations*	*Adverse events*
IPC alone					
Raines[30] 1977 Level III-3 $n=17$ 11 UL 6 LL SLE	(VWD) UL $n=9$ Hand: 48.5 ± 16.7 Forearm: 19.4 ± 4.9 Arm: 15.4 ± 7.2 LL: $n=6$ Foot: 52 + 15.9 Calf: 41 + 10.9 Thigh: 33 + 6.7	NR	Reduction in calf and forearm related to degree of fibrosis. Minimal response in those with severe/grade 3 fibrosis. Compression therapy contraindicated for fibrosis.	Descriptive statistics only. Concludes and advises contraindications based on small numbers. Unclear if percentage reduction is based on VWD or circ. 2 pts with no response not included in results. Contamination: Bed rest	NR
Zelikovski[12] 1980 Level III-3 $n=25$ UL SLE	(Circ): 51%–70%: in $n=13$ (52%) <35%: $n=3$ (12%) (VWD% reduction): 51%–70%: $n=11$ (44%); <35%: $n=7$ (28%). "Firm consistency": Pretreatment: 88% Post-treatment: 8%	NR	No discussion of results. No conclusion given, except to recommend use of this machine.	Descriptive statistics only. Pressure varied according to tolerance. No measure for fibrosis reported. Contamination: Bed rest	Temporary pain (4 pts) and increased temperature (3 pts) resolved spontaneously. Pressure reduced by 30 mmHg for 2 pts, relieved pain.
Kim-Sing[36] 1987 Level III-3 $n=15$ UL SLE	(circ cm reduction): 0.5–2: $n=8$ 2–4: $n=3$ 4: $n=3$ 5.5: $n=1$ Baseline: difference between limbs of 13.8 cm	NR	No conclusion given small numbers.	Pts self-selected for LO therapy Descriptive statistics only. Contamination: Bed rest	Sleeve slippage overnight, caused some axillary edema. Prolonged bed rest/immobility aggravated arthritis.
McLeod[33] 1991 Level III-3 Children $n=9$ LL 8 PLE; 1 SLE.	HF group: Circ: 8.5 (2.5) VWD: 20 (11) WLP group: Circ: 60 (15) VWD: 38 (26) L-P group: Circ: 33 (25) VWD: 77 (57)	NR	Reduction in all patients related to bed rest. Greatest reduction with L-P.	Descriptive statistics only. Small study groups. Significance of age difference between groups not reported. Contamination: Bed rest with elevation	NR
Pohjola[31] 1995 Level III-3 $n=20$ PLE and SLE 10 UL and 14 LL	(Graphical representation of cross-sectional circumference area) Between group: graph of reduction by area (at 3 points/limb) shows: UL: wrist and forearm reduction $p<0.001$ LL: ankle and calf reduction $p<0.001$	No statistical difference between groups except on basis of time	Marked change in first 2 hours of treatment. Venti-press applied for shorter time with same results.	Retrospective comparator Study conditions not described for retrospective cohort. Unclear presentation of results. Conclusion in conflict with graphical representation of results. Outcome measure not comparable with other studies.	NR

(*continued*)

Table 2. (Continued)

Study: First author, year; study rating (NHMRC) Sample size; population	*Mean edema reduction (SD)% (outcome measure)*	*Statistical significance*	*Clinical conclusions*	*Limitations*	*Adverse events*
Bergan[32] 1998 Level III-2 $n=35$ UL and LL	Mean change (VWD): **By device:** **7000**: +0.4 **7500**: +7.3 **2100**: −32.6. MEDIAN change: **7000**: −2.85 **7500**: −6.9 **2100**: −28.4	Between group: Significant difference ($p<0.001$) Pairwise comparisons: 2100 group greater reduction than 7000 or 7500 group $p<0.05$	Between group: on basis of duration, radiation, gender, history of infection or staging of LO: NS. Clinically: good volume reduction. Between primary and secondary: NS.	Method NR: - recruitment/selection - randomization; - washout; - order of treatment; - effect of order of crossover; Study period unclear: successively with each device. Data for 3 pts not included: did not finish.	NR
Dini[26] 1998 Level II $n=67$ UL SLE	IPC*: 11.8%* (Sum of circ) Absolute mean decrease: IPC: 1.9 + 3.7 cm ($p=0.009$). Control: 0.5 ± 3.3 cm $p=0.33$ NS. Reduction ≥25%: IPC: $n=10$, 25%; (95% CI: 13–41) Control: $n=8$, 20% (95% CI: 9–36) $p=0.59$	Within IPC group $p=0.009$ Between groups ($p=0.084$) NS	Reported clinically NS (required >25% change)	Base-level difference between groups: significance NR. (Results reported adjusted for baseline differences.)	NR
Pilch[35] 2009 Level III-3 UL $n=57$ SLE	(VWD): 1. 90 seconds InfT, 1 cell: 9.6 2. 90 seconds InfT, 3 cells: 8.1 3. 45 seconds InfT, 1 cell: 6.8 4. 45 seconds InfT, 3 cells: 8.3 Greatest absolute reduction: 45 seconds with 3 cells (group 4); Least absolute reduction: 45 seconds with 1 cell (group 3).	Within group, all groups: significant reduction: 6.8%–9.6% ($p<0.05$) Significant difference between 1 and 3 cells in 45 seconds cycle (groups 3 and 4) ($p=0.04$). Significantly less pressure in 45 seconds groups 3 and 4 ($p=0.023$)	All IPC cycles regardless of timing or sleeves produced significant reduction.	Variable pressure applied, based on tissue consistency.	NR
Modaghegh[28] 2010 Level III-3 $n=43$ LL	Unilateral group: (circ, cm) Foot: 94.5 Bilateral group Mean decrease (circ cm): Thigh: 4.22 Foot: 2.25	NR	Greatest % change was seen in the foot in 25 of 32 pts in unilateral group	Descriptive statistics of numbers experiencing reduction Poor reporting of statistical analysis; *p*-values NR Pt satisfaction and skin changes described only at baseline. Contamination: Bed rest	Reports no adverse events occurred

(*continued*)

TABLE 2. (CONTINUED)

Study: First author, year; study rating (NHMRC) Sample size; population	*Mean edema reduction (SD)% (outcome measure)*	*Statistical significance*	*Clinical conclusions*	*Limitations*	*Adverse events*
Ridner[38] 2012 Level III-3 $n=42$ UL SLE	Median change (VTC) [IQR] Arm only: −0.38 [−2.56, +1.34] Arm + trunk: −2.66 [−4.20, −0.55]	Within group: NS in either group. Between groups NS $p=0.481$. Both groups combined: significant reduction $p=0.018$. BIS: Within both groups: significant decrease $p=0.023$ and $p=0.004$	No difference between: trunk + arm IPC and arm-only IPC. Arm-only IPC takes less time thus less burden. Both groups self-reported symptoms and function: significant improvement.	Nil noted	Reports no adverse events occurred
Theys[29] 2015 Level III-3 $n=9$ UL SLE	Vol (plethysmograph) presented as a graph: 1%–6% change. Reduction by both MLD and IPC: Upper arm: 0.03 mL/100mloed/mmHg/minute.	NR	No difference between MLD and IPC.	Study period unclear: successively with each device. Results reporting unclear (graph and language)	Reports no adverse events occurred
Chmielewska[40] 2016 Level III-3 $n=21$ UL SLO	Mean difference (sum of circ): 5.9 cm; 3.6%	Mean significant reduction at all levels of arm; NS in hand	Hand exercise did not affect edema reduction. (NS on comparison with ex + IPC group)	Acknowledged small sample. Usual use of CGs NR. Possibly insensitive tool for hand function assessment (validated in Polish for Carpal Tunnel Syndrome)	No negative effects of exercise; Adverse effects from IPC NR.
IPC+CG studies					
Johansson[37] 1998 Level III-3 $n=24$ UL SLE.	7.1% (VWD) Mean change: MLD: 75 mL $p<0.001$ IPC: 28 mL $p=0.03$	IPC group significant $p=0.03$ % change: NS Between MLD and IPC: NS	No difference between MLD and IPC.	Variation in pressure according to tolerance. Cointervention: Adjunct daily use of CG.	NR
1999 Berlin[27] Level III-2 $n=55$ UL SLE	(VWD) Group 1: CG only $n=28$ Decreased VWD: 15 (54%) No change: 13 Group 2 Flowt: $n=8$ Decreased VWD: 4 (50%) No change: 2; Increased: 2 Group 3: L-P $n=19$ Decreased VWD: 13 (68%); No change: 3 Increase: 3	No statistical difference between groups; p-value NR.	Clinically recommend CGs as simpler treatment: - IPC had greater response, but was not significant	Variation in dosage time between groups; Selection bias: patients self-selected IPC/CGs Baseline between-group difference (IPC groups had larger initial volume); significance NR. Descriptive statistics only. No reporting of statistical analysis: p-value NR Cointervention: Adjunct daily use of CG	Increased swelling: 2 of 8 using Flowt 3 of 19 using L-P

(*continued*)

Table 2. (Continued)

Study: First author, year; study rating (NHMRC) Sample size; population	*Mean edema reduction (SD)% (outcome measure)*	*Statistical significance*	*Clinical conclusions*	*Limitations*	*Adverse events*
Fife[39] 2012 Level III-3 $n=36$ UL SLE	Mean (VTC): FT: −29% ± 44% (−118 mL) BC: +16% ± 63% (+6 mL)	Within group NS Significance between group $p=0.018$	FT: Significant reductions in both TDC and VTC. FT produced better results for home use than BC; difference possibly attributed to device parameters and treatment area. BC: 6/7 adverse events (4 serious) FT: 1/7 adverse events (serious)	Pts self-selected for LE therapy Difference between groups due to exercise: significance NR. Differences between group in control arm, post-intervention not investigated. Cointervention: Adjunct use of CG 23 hours per day.	Seven AE reported: 3 definitely (D) and 3 possibly (P) device-related. - Increased swelling of hand and torso; pain in axilla and back D - pain in forearm and numbness in fingers D - increased hand swelling D - Swelling of lymph nodes, contralateral axilla P - Breast inflammation; increased swelling and pain; infection and fibrosis P - increased arm swelling P - rash on arm: unlikely related
Zaleska[34] 2014 Level III-3 $n=18$ LL	After 1 hour (circ %) $p<0.05$: Lower calf: 2.3 ± 3.6 and thigh 0.9 ± 1.7. After 1 month and 1 year (circ %) $p\leq0.05$: 1 month: Calf: 2.3 ± 3.9 and thigh 2.1 ± 3.8 1 years: Calf 3.6 ± 5.4 and thigh 3.4 ± 3.9 Reduction (circ cm) 1 year: 1.2–2.2; Tonometry: stiffness reduction 1 month Calf: 4% in 80%–90% of pts (p NR).	After 1 month and 1 year: decrease at all levels of leg and thigh: ($p<0.05$).	Decrease or maintenance of circ Increase and maintenance of elasticity of tissues (lower calf and ankle). No relationship between circ decrease and elasticity increase	Variable study period Poor presentation of results and statistical analysis Increased girth in control leg noted in 60% pts over 3 years; not clarified by BMI. Cointervention: Adjunct use of CG	No fibrous thigh ring; No genital edema formed over 3-year study

AE, adverse events; BIS, bioimpedance spectroscopy; Circ, circumference; NS, not significant; Pts, participants; TDC, tissue dielectric constant; Vol, volume; VTC, volume by truncated cone calculation; VWD, volume by water displacement.

Table 3. Critical Appraisal Summary: Evaluation Questions from McMasters Critical Review Form (Quantitative Studies)

	IPC-alone studies												*IPC+CG studies*			
	Raines[30] 1977	Zelikovski[12] 1980	Kim-Sing[36] 1987	McLeod[33] 1991	Pohjola[31] 1995	Bergan[32] 1998	Dini[26] 1998	Pilch[35] 2009	Modaghegh[28] 2010	Ridner[38] 2012	Theys[29] 2015	Chmielewska[40] 2016	Johansson[37] 1998	Berlin[27] 1999	Fife[39] 2012	Zaleska[34] 2014
1. Was the study purpose stated clearly?	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2. Was relevant back ground literature reviewed?	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	0
3. Was the design appropriate for the study question?	1	1	1	0	0	0	1	1	1	1	0	1	1	1	1	0
4. Was the sample described in detail?	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5. Was the sample size justified?	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
6. Were the outcome measures valid?	1	1	1	1	1	1	0	0	1	0	0	1	1	1	1	1
7. Were the outcome measures reliable?	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
8. Was the intervention described in detail?	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1
9. Was contamination avoided?	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
10. Was cointervention avoided?	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
11. Were the results reported in terms of statistical significance?	0	0	0	0	1	1	1	1	0	1	0	1	1	0	1	1
12. Was the analysis method appropriate?	1	1	0	0	1	1	1	1	0	1	1	1	1	0	1	1
13. Were the conclusions appropriate given study methods and results?	1	0	1	0	0	1	0	1	0	1	0	0	1	1	1	1
14. Were the main limitations and biases discussed?	0	0	1	0	0	0	0	0	0	1	0	1	1	0	1	0
15. Was the clinical importance reported?	1	0	1	1	1	1	1	1	1	1	0	0	1	1	1	1
Total	9	7	8	6	8	9	11	9	7	11	4	9	11	8	11	8
Rating	Fair	Poor	Poor	Poor	Poor	Fair	Good	Fair	Poor	Good	Poor	Fair	Good	Poor	Good	Poor
NHMRC level	III-3	III-3	III-3	III-2	III- 3	III-2	II	III-3	III-3	III-3	III-1	III-3	II	III-2	II	III-3

Law et al.[24] Grading Scale[25]: Poor ≤8; Fair 9–10; Good 11–12; Very good 13–14; Excellent 15.

IPC+CG studies: mean 9 out of 15 (4 studies: range 8–11) (Table 3).

Population

Of the total number of people investigated in all studies, those with UL LE (338) were more than double those investigated with LL LE (151). Study sample sizes ranged from 9 to 67. The mean age of participants ranged from 37.8 (14–80) years[28] to 71 (54–83) years,[29] largely reflective of the demography of secondary cancer-related LE. Most studies investigated breast cancer-related UL LE; three included LL LE with a mixed population of secondary and primary LE[30–32] and three investigated LL alone.[28,33,34]

Only one study investigated the use of IPC with children, in a sample of nine with a mean age of 13 years (5.5–17 years).[33]

Study design

One study utilized a control group with skin care only, four IPC alone, two IPC+CG studies investigated the effect of one type of IPC device or dosage or sleeve configuration against another, and five studies investigated a cohort exposed to treatment with a pneumatic device, in a single-case pretest/posttest design. Remaining studies compared retrospective IPC results, manual lymphatic drainage, or IPC combined with exercises with a prospective IPC-only cohort. Study characteristics are described in Table 1.

Devices

The Lympha Press (Orthopaedic Appliances Pty. Ltd., Rowville, Australia, https://www.lympha-press.com/distributors/) (OR: Patriot Medical Distributors, West Chester, PA, www.patriotmedical.com) and Wright Linear Pump (Wright Therapy Products, Inc., Oakdale, PA) were the most commonly used devices (Table 1). Device- and manufacturer-specific factors such as the sleeve configuration, number of cells per sleeve, and the timing cycle of the pressure applied are aspects of dosage not controlled by the clinician in these studies.

Indeed, device variety resulted in differences between studies based on cycle time, sleeve configuration, number of cells per sleeve, and pattern of pressure application, quite apart from dosage (Table 1). Modes of compression described included the following:

a. Single-cell applying intermittent pressure[30,32];
b. multiple cells applying sequential pressure by filling cells one after the other until all were full, then deflating[12,34];
c. pressure in one cell, then the next cell inflated before the previous one deflated, peristaltically moving up the limb before beginning distally again.[28,29]

While many studies compared one IPC with another, one investigated pressure time cycles with either single-cell or three-cell garments with the same device.[35] Having found significant reductions in all groups, it was concluded that all types of timing and sleeve configuration are effective.[35] In contrast, another study[32] reports that a 10-chamber IPC delivered a significantly greater reduction in percentage volume than either single-cell or three-cell devices. However, both studies had limitations that restrain conclusions (Table 2).

Duration of IPC application

Before 1995, IPC was applied for 4–9 hours per day[30,33,36]; then 2 hours per day[26,31,32,37] in the late 1990s and reduced to 1 hour or less over the last 20 years.[29,34,35,38–40] Two studies stand out against the norm of their era for applying IPC for longer (8-hour application in 2010)[28] and shorter (2-hour application in 1980)[12] durations.

Pressure settings

Older studies more than 20 years ago used higher pressures (100–150 mmHg),[12] while nearly all studies from the last 20 years applied pressure between 30 and 60 mmHg. The exceptions were two studies treating LLs that used 80–120 mmHg in 2010[28] and 100–120 mmHg in 2014,[34] and studies using the ''mild'' pressure Flexitouch device (Tactile Medical, Minneapolis, MN).[38,39] The mean standard pressure of the Flexitouch is reported by Mayrovitz[15] to be 13.7 ± 4.9 mmHg for the preparation phase and 9.0 ± 4.2 mmHg for the drainage phase. In 1998, Johansson et al.[37] described application of 40–60 mmHg as being ''standard practice'' (Table 1).

High pressures common to the era (1991) (≥80 mmHg) were also applied to children.[33]

UL versus LL pressures

Studies that included investigation of IPC for UL and LL applied similar pressure irrespective of the limb to be treated.[30–32] Other than those using the low-pressure Flexitouch device, pressure between 30 and 60 mmHg was applied to the UL in five of six investigating UL LE,[26,29,35,37,40] while those investigating LL LE used higher pressure (80–120 mmHg).[28,34]

Variation of pressure within a study

Pressure was varied (Table 1) according to the following:

1. Blood pressure. Maximum pressure was kept below diastolic blood pressure[35] and below the mean of the systolic and diastolic pressure in two.[33,36]
2. Participant tolerance or comfort.[12,37]
3. Skin resistance. Pressure was varied in opposite directions according to tissue condition in two studies, applying either higher[28] or lower pressure[35] in response to increased tissue hardness/fibrosis, with no description of how tissue hardness was determined.

Intervention period

The length of IPC use varied across studies, from one application of 16 minutes[29] to multiple applications over 3 years.[34] Intensity of treatment within the study period also varied from early intensive treatments of 16 hours over a 24-hour period (and hospitalization) to clinic-based studies of 5 weeks (25 applications)[35] or 20 applications over 9 weeks.[26] In contrast, the low-pressure device (Flexitouch) was investigated in home settings with daily applications over 30 days[38] and 84 days.[39]

Outcome

Range of outcome measures. Circumference (10 studies) and volume (by water displacement; 6 studies) were the

most common outcome measures reported (Table 2). Other outcomes were derived from dimensional measures: sum of the difference between limbs[26] or volume calculated from circumferences.[38,39] Other outcome measures included tonometry,[34] tissue dielectric constant,[39] and bioimpedance.[38] Subjective feedback, quality of life (QOL), or symptom improvement were often discussed, but formally reported in only two studies.[37,38] Outcomes of percentage reduction, where available, are reported in Table 2.

Greater response to intensive IPC was found in early than later stage fibrosis using xeroradiography.[30] Tissue softening was observed throughout 3 years of daily IPC using tonometry.[34]

Adverse events

Fife et al.[39] alone reported comprehensively on the range of adverse events, their seriousness, and likelihood of being caused by the IPC, with seven events described as definitely (three), possibly (three), or unlikely (one) to be related to IPC use. Those definitely likely to be related to the IPC treatment included increased swelling of hand and torso; pain in axilla and back; and pain in forearm and numbness in fingers (Table 2). Possibly related events included swelling of lymph nodes in the contralateral axilla; breast inflammation with increased swelling and pain, infection, fibrosis, and increased arm swelling. Assuming 7 events affected 7 participants of a sample of 36, 19.4% is a considerable proportion to be affected. It is unclear how or when these events occurred or were resolved or if participation for those involved was discontinued.

Only three further studies reported adverse events; two related either to transient symptoms or to the issue of pain with high pressure settings where subsequent adjustment to lower pressures relieved pain in most instances.[12,36] In the third, increased swelling was noted in 16%–25% of participants, dependent on group[27] with no further information on site or resolution. Generally, little or no information was provided about resolution of adverse events, particularly of increased swelling.

Discussion

This review of studies using IPC in isolation excluded many recent studies that applied concurrent cointerventions, such as bandaging or wraps,[41–44] CDT,[45–48] CG during IPC treatment,[11] or specific exercises,[49,50] on the basis that the effect of IPC could not be isolated from that of other interventions.

Pressure and timing

Device parameters that are typically adjustable by the clinician or investigator include pressure and duration of application, in contrast with pressure cycle characteristics commonly specific to the device. Assessment of lymphatic function under a range of IPC pressures has used lymphoscintigraphy,[6,51] histology,[52] and more recently NIRFLI[9,19,20] providing evidence of lymph flow at both high and low pressures. However, damage to the lining of lymph vessels following 3–5 minutes of high-pressure manual massage (70–100 mmHg) was reported in 1995 in both dogs and people with LE.[52] These findings may have influenced the IPC dosage choices in subsequent studies.[37,49]

More recent assessment of lymph flow during IPC using NIRFLI has demonstrated optimal flow under pressures up to 80 mmHg[20] and in a comparison of low (45 mmHg) and high (90 mmHg), Kitayama et al.[19] demonstrated optimal flow at the higher pressure. Further studies have also shown fluid movement under high IPC pressure (80–120 mmHg), but with high pressures applied manually, little fluid movement was demonstrated.[10] Others using plethysmography[53] concluded high pressures and long cycle times were needed for fluid flow.

Further investigation of the interaction of IPC with the skin, where uptake of fluid is initiated in the initial lymphatics and where pressure is widely distributed around a limb in comparison with the focused manual application of pressure, may elucidate the optimal mode of application for fluid flow. Furthermore, it has been suggested that NIRFLI to outline fluid pathways before compression therapy might enable individually tailored IPC application.[9]

Many factors influence IPC pressure applied to a limb (1) within the sleeve, (2) at the sleeve/skin interface, and (3) within the tissues, with the result that it is difficult to determine what pressure is translated to the tissues and if that is the key factor influencing lymph flow.

The pressure within an IPC sleeve cell has been reported to be higher than that set at the controls of an IPC,[54] yet the pressure in the tissues has been reported to be far lower than the pressure in the pneumatic sleeve cell.[53] Added to this, some investigators have varied pressure according to tissue resistance, on the basis that lymph flow is affected by this factor, and have applied decreased pressure for hard edema (and increased it for softer tissue)[35]; whereas, conversely, others report much higher pressures were required to move fluid where there was significant tissue resistance caused by fibrosis.[55] Studies investigating tissue pressures during IPC, however, use an *in vivo* needle-wick pressure measurement method[53,55] and cause alteration in the tissues due to the necessarily invasive nature of the method.[56]

The role of tissue resistance in fluid flow under the influence of IPC was supported by *in vitro* simulations, although many assumptions were necessary to this model.[57] Furthermore, an investigation by Theys et al.[58] of pressure at the skin/sleeve interface has reported pressure differences depending on the surface to which it is applied: increasing by 25%–67.5% (dependent on device) with semi-rigid objects but remaining stable for rigid objects; and decreasing by 10%–15% for soft objects. While Pilch et al.[35] give no measurement criteria for rating edema hardness, the decrease of pressure for hard edemas and increase for soft edemas in that study agree with the findings of Theys et al.,[58] at least on the skin surface. Outcomes may then, at least in part, be affected by individual characteristics such as tissue condition, and not dosage alone, in agreement with physiological investigations.[57]

However, when the tonometer was indented to a depth of 10 mm, in a recent investigation of the pressure/flow relationship in different stages of LE, no correlation was reported between tonometry and stage; at least 1000 g/cm^2 force was required for flow (measured using the needle-wick method).[55] Further controlled studies of pressure applied to tissues of differing consistencies may elucidate optimal pressure settings for LE according to stage. Meanwhile, observation by clinicians of the response to IPC proportionate to relative

tissue resistance may assist dosage decisions. (Clinical tools for measurement of tissue resistance are not readily available, a limitation for clinicians.)

Whatever may be demonstrated physiologically or in models, decisions on the optimal pressure and time cycle settings require translation of physiological findings into successful and safe clinical outcomes.

Dosage: evidence from studies with significant outcome

From this review, a limited body of available evidence was found, based on small samples of predominantly UL secondary LE, and equipment that may no longer be available; yet for dosage guidance, studies with significant outcomes and sound methodology, with no adverse events, are sought to provide the basis of evidence for optimal dosage and future research. No studies from this review met these criteria or scored in the upper quartile on critical appraisal (Table 3); those forming the basis for comment in this discussion have the best available rating, yet lack of adverse event reporting and methodological flaws indicate caution in adopting outcomes. Methodological limitations exist for those studies with the best outcomes:

- The greatest change of 32.6% mean volume reduction may be questionable due to lack of information regarding the effect of the crossover design.[32]
- The next best outcome, within an IPC+CG study, was marred by seven adverse effects of varying seriousness and while statistical analysis indicated significant improvement, differences in control limb responses between groups raise questions over conclusions.[39] This study, while allowing usual home self-management to continue, was strengthened by assessment of factors that might have affected outcomes, yet on finding between-group differences, the significance of the difference was not reported.

A reduction in duration of application from 2 hours to 1 or less has occurred over the past 20 years in parallel with the growing need for independent, home-based self-management compared with treatment applied in a clinician-/clinic-centered model. Earlier still (1970–80s), treatments of 8–16 hours were common in a hospital inpatient model of care.[30,36] Self-management models consider the time and burden of any treatment juxtaposed with the potential benefits resulting in the likely adherence to proposed treatment. The impact on the consumer and requirements of managing a chronic condition has been well documented.[59–61]

LE management requires at least once-daily attention, with most strategies (whether self-lymphatic drainage, or bandaging, garment, or IPC application) being particularly time-consuming. Dosage time for IPC application suggested by manufacturers has also decreased, perhaps reflecting both the development of multicell sleeves and sequential pressure applications, as well as being responsive to consumer uptake and needs.

Manufacturers' recommendations for devices that are common and currently available are for treatment duration of 1 hour or less: Medi-Rent's LX9 (30 minutes to 1 hour)[62]; Lympha Press (60 minutes or less, once or twice daily; Lympha Press Protocol; Orthopaedic Appliances Pty. Ltd.). Flexitouch programs vary according to the area, with 45 minutes being recommended for LL only, 30 minutes for UL only, and 60 minutes for LL or UL with adjacent trunk.[63] Device evolution, utilizing different cycle times with sequential pressure, perhaps accounts for improved outcomes with shorter application time.[32,35,39]

Studies investigating LE self-care and IPC in home-based models generally administered IPC for 60 minutes or less[16,38,64] or dependent on the surface area to be treated (greater the area, the longer the treatment time).[42] Given the time commitment required to manage LE, QOL, function, and patient satisfaction in use of IPC were central to studies of home use, as well as objective measures of LE reduction. Adherence to IPC home protocols has varied from less than ideal at 47% and 37% per group[16] to very high (95%–99%).[39] Further research could focus on home dosage programs that combine acceptable time burden for the consumer with satisfactory limb maintenance outcomes. However, even so, most studies report significant positive patient satisfaction and functional outcomes, along with decreases in health care costs, hospitalization, and outpatient care, from IPC use as part of participants' home-based LE management.[16,42,64–66]

Choice of optimal duration and pressure of IPC has been limited by the variation in aims, design, and study period of the above studies, which may have influenced outcomes. The number of times IPC was applied was one source of variation, and sleeve application another, with the root of the limb and upper affected side of the trunk sometimes included.[38,39]

The addition of chest and trunk to IPC treatment of the UL alone resulted in no statistically significant difference in objective (limb circumference) outcomes between groups[38]; flow into adjacent truncal areas (from the LL across the inguinal crease) under IPC has not been demonstrated.[51] While a significant incidence of genital swelling has been identified in a retrospective study of IPC use,[67] reports of increased swelling at the root of the limb have been rare[39] or have not been found in others since,[29,34] despite a reported increase in tissue pressure proximally.[68] Conservatively, clinical guidelines advocate the use of MLD to clear truncal areas and the root of the limb when the trunk is not included in IPC treatment.[69] The findings from NIRFLI of individual variation of drainage pathways and collateral flow[70] highlight the need for individual monitoring of response during treatment.

Despite these variations, small but statistically significant reductions have been demonstrated in studies using 30–60 mmHg, whether the IPC was applied for 2 hours or less than 1 hour[26,32,35,37,39,40] (Table 2).

UL versus LL pressures

Studies in this review investigating both UL and LL applied similar pressure for both. In contrast, upper limits for compression pressures determined using bandages, have been reported to be different for UL and LL: 30 mmHg for UL and 50–60 mmHg for LL.[71,72] While the nature of bandages is quite different to IPC, the latter study demonstrated bandaging pressures above this ceiling having a negative effect on limb volume reduction over a 2-hour period.[72] Differing IPC pressure settings according to limb have been applied in 1985[13] using maximum pressures of 110 mmHg for UL and 150 mmHg for LL. (The latter study[13] was excluded from this systematic review due to variation in dosage.)

The ceiling pressures described by Partsch et al.[72] contrast with studies using NIRFLI[20] and those using plethysmography (and needle-wick measures of pressure),[53] reporting lymph flow under IPC pressures of over 80 mmHg in the LL. Studies from this review investigating IPC use in LL applied between 60 and 120 mmHg in poor-quality, low-level evidence.

Child versus adult dosage

Only one study in 1991 investigated IPC in children: a level III-3 low-quality study with a cohort of nine[33] with inconclusive outcomes of significance. This pediatric study used pressures and device settings in the same range as described for adults in this review, with no comment on the generalizability of dosage from adults to children. Interestingly, pneumatic compression was applied overnight, as in 2 of the 15 adult studies,[30,36] perhaps a reflection of practice common to the era.

Clinical meaning

The one highly rated RCT[26] found a mean 1.9 cm (*11.8%*) decrease in the sum of UL circumferences (compared with 0.5 cm decrease in a control group with only skin care) but deemed it clinically not significant, having set a value of 25% limb reduction to be clinically meaningful. In contrast, later studies have established that to patients, a reduction of 5% limb volume[73] or 8% limb volume[42] can produce positive benefits to QOL, highlighting the importance of including a measure of the outcome from a patient's perspective rather than objective measures alone.

Based on the findings of these latter studies, a reduction of 11.8% would be deemed clinically significant, produced with a dosage of 60 mmHg over 2 hours.[26] As the highest level of evidence available, this dosage is worthy of note. The next greatest reduction of 6.8%–9.6% limb volume was produced with half the IPC duration and a mean pressure of 37.7 mmHg.[35] Both studies applied IPC daily, although device characteristics differ; further investigation of daily versus less frequent application may further elucidate optimal IPC frequency.

A recent device applying lighter pressures (mean 9–13 mmHg) and using short treatment duration (30–60 minutes) has been used daily in conjunction with maintenance CG use over the longer term in home-based studies.[39] Limb reduction and other health-related outcomes, as well as consumer adherence to and satisfaction with maintenance programs, have been significant with the use of this light pressure device.[39,66] However, this device was developed with the aim of supporting self-care by the patient at home, replacing therapist visits for MLD,[74] and has considerably different characteristics from other IPC devices[14,15] currently available: the sleeve through which pressure is applied is of stretchy not inelastic fabric; pressure distribution and cycle characteristics differ from ''standard'' IPCs. Dosage appears to be in preset ''programs'' according to body area,[63] limiting comparative assessments of dosage.

Potential confounders

Bed rest. Five studies applied IPC for 4–8 hours or even longer at a time, often with little break before reapplying,[12,28,30,33,36] requiring participants to be immobilized, often bed-bound for up to 16 hours in 24 hours of 1 day. Results from these studies must be viewed with caution, as even short-term elevation has been shown to reduce edema in ULs[75] and elevation is encouraged as an adjunct to management.[22,76–78]

Body mass index. Body mass index (BMI) is now recognized as a factor in LE[79]; reporting on BMI was absent from one long-term study even where the unaffected limb was noted to have changed in size.[34]

Adverse events

Only one study reported comprehensively on adverse events from IPC, despite using devices with some of the lowest pressures (9–13 and 30 mmHg) and shortest treatment time (1 hour).[39] This perhaps highlights the generally poor reporting of adverse events in the remainder. Of the seven events reported by Fife et al.,[39] five were deemed serious; however, a significantly greater reduction was reported with the advanced programmable device, with only one adverse event, than in those using the standard device program, who did not experience any reduction. This study is one of only two in this review to assess IPC in a home-based model; however, variations in other usual activities (exercise) introduced between-group differences.[39]

Limitations of this systematic review

This systematic review included only studies in English. The number of different outcome measures across studies, as well as the generally low level of evidence of moderate quality, limits the comparisons and conclusions to be drawn.

Conclusion

There is limited low- to moderate-quality evidence for the application of 45–60 minutes of 30–60 mmHg using multicell, sequential IPC programs for the management of UL LE. Whether the addition of the root of the limb and adjacent truncal area to the limb is necessary requires further investigation.[38] Further research on IPC outcomes, utilizing the same application frequency, duration, and pressures, will provide comparative data to build a basis for optimal dosage. The inclusion of outcomes beyond limb volume and dimension, such as tonometry and bioimpedance, will allow control for potential confounding factors such as tissue hardness (stage of LE) and broaden understanding of the impact of IPC on skin condition and fluid flow, relevant to LE management. The inclusion of patient-centered outcomes such as burden of treatment versus symptom management will add optimal IPC use to dosage outcomes.

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Appendix C Reliability of the High Frequency Ultrasound

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ORIGINAL ARTICLE

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Dermal thickness and echogenicity using DermaScan C high frequency ultrasound: Methodology and reliability testing in people with and without primary lymphoedema

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Abstract

Background: DermaScan C high frequency ultrasound was investigated for image capture and analysis of dermal measures in people with and without primary lymphoedema.

Method: Three repeated images were taken at six sites in people without lymphoedema (NLO). Intra-rater reliability was assessed by taking three sets of measures on images from 10 people and inter-session reliability by capturing three images, lifting the probe from the skin in between. Methods were adjusted, and repeated images from four sites were taken in people with primary lymphoedema (PLO) and reliability re-assessed.

Results: Intra-rater reliability in NLO and PLO for echogenicity measures were excellent (NLO $ICC_{(3,1)}$: .989; PLO .997) across all sites and specific to each site (calf: $ICC_{(3,1)}$: .989; and foot: $ICC_{(3,1)}$: .999, respectively). Inter-session reliability was moderate for NLO ($ICC_{(3,1)}$: .727), improving after method modifications for PLO ($ICC_{(3,1)}$: .916). When investigated by site, inter-session reliability was good in the foot ($ICC_{(3,1)}$: .811) and moderate in the calf ($ICC_{(3,1)}$: .616). Mean thickness analysed by site resulted in good inter-session reliability only in the foot ($ICC_{(3,1)}$ .838).

Conclusion: Intra-rater reliability was excellent using the DermaScan C for dermal measures in people with primary lymphoedema. Inter-session reliability required particular attention to method and gain settings.

KEYWORDS

lymphoedema, reproducibility of results, skin, ultrasonography

1 | INTRODUCTION

Ultrasound has been used to measure in vivo skin thickness and fluid content since the 1980s.[1-4] In particular, the superficial focus of high frequency ultrasound (HFU) (15-22 MHz) results in an image of 1-2 mm depth where the dermis and epidermis are clearly demarcated and accessible for measurement. The distance from the entrance echo (on the surface of the epidermis) to the dermal subcutaneous tissue interface measures total skin thickness. This has been used for optimal site selection of dermal injections in diabetes[5] and vaccines in children and adults,[6] and enabled skin and subcutaneous tissue assessment following prednisolone treatment.[7] The validity of HFU to measure skin thickness was demonstrated in early investigations using a 15 MHz ultrasound and A-mode images by charting

the image against an X-ray at the same magnification as the ultrasound.[8,9] As well, HFU (22 MHz) measurement of epidermal thickness has been validated in healthy people (25-40 years) compared with confocal microscopy.[10]

Skin changes are a feature of lymphoedema. The backlog of lymph that characterises lymphoedema accumulates predominantly in the subcutaneous tissues, but is also evident in the dermis.[11] Skin in lymphoedema progresses from soft skin which easily indents or "pits" when pressed in early stages, to hard inflexible non-pitting skin, which may have wart-like papillomatosis or keratosis and skin folds in later stage lymphoedema.[12-15] HFU, along with tissue histology, magnetic resonance imaging and spectroscopy have been used to compare and contrast tissue changes of lymphoedema in the dermis[16,17] as well as the subcutaneous tissue.[18] Skin thickness measured by HFU increases with advancing stages or severity of lymphoedema.[19] Furthermore, skin is the interface for treatment of lymphoedema, whether by manual lymph drainage (a form of massage) or compression applied by elasticised garments or a pneumatic sleeve around the limb. Indeed, 5 days of intensive lymphoedema treatment using manual lymph drainage, pneumatic compression and bandaging has resulted in measurable differences in dermal thickness detected with HFU.[20]

High echogenicity, seen on an HFU image as greater brightness, occurs when tissues reflect more HFU waves. Echogenicity varies with tissue density and content. Tissues with greater water content are hypoechoic.[21-23] HFU studies have described a relatively hypoechoic dermis on the affected side in lymphoedema.[24-26] HFU has been used to document and describe dermal oedema in a range of conditions (noting all chronic oedema may now be regarded as a lymphatic issue.[27]) Significantly less echogenicity was found in the dermis of people with chronic oedema (regardless of whether oedema was due to lymphoedema, lipodermatosclerosis or cardiac insufficiency) compared with healthy skin.[28] HFU has also been used to differentiate between lipoedema and lymphoedema with a blinded assessor correctly diagnosing 100% of lymphoedema images (which were clearly hypoechoic) with no false positives.[29]

While the presence or absence of pitting adds information regarding the condition of the skin, current objective clinical assessment of lymphoedema severity and change relies on volume measures extrapolated from limb circumference measures and whole limb or segment fluid content using bioimpedance.[12] HFU provides the opportunity for non-invasive, direct, valid, and objective measures of dermal thickness and fluid content, yet the equipment is costly and significant training is required. Importantly, no standard protocol for HFU measurement is available. Previous studies have used devices with frequencies varying from 10 to 20 MHz.[18,19,25,26,28] The variation in frequency for image capture has resulted in images of different quality potentially producing non-comparable measures.[30] Other studies have provided little information about the ultrasound settings.[19,20,31]

One important setting which has been variously described is time gain compensation (or gain); this operator-dependent control can make small adjustments or amplifications to account for the loss of amplitude that occurs when echoes travel from deeper tissue (attenuation).[32] These echoes can appear darker than echoes of equal magnitude that are reflected from more superficial structures.[33] The gain compensates for this loss or attenuation of signal and has the effect of increasing both the area and intensity of brightness. Some authors specify keeping the gain setting constant[34,35] while more recent studies have adjusted the gain for some images as needed to improve visualisation of the sub-dermal boundary.[1,29,36-38] In particular, this interface of the dermis with the subcutaneous tissue is not as clear as the epidermal-dermal junction[1,29] and adjusting the gain enables the detection of edges. In contrast, for valid dermal fluid content measures, which specifically measure echogenicity, a "flat" or "horizontal" gain, where no compensation in amplification has been made for attenuation is required (PH Pedersen, R&D Manager, Cortex Technology, personal communication, May 21, 2019). Hence, no one HFU methodology will allow capture of images to assess both fluid content and skin depth which are both of clinical value to understand the status and change in lymphoedema.

A second requisite for image clarity in ultrasonography is water-based gel, which is used as a coupling medium between the skin and transducer. However, gel can alter the distance sound travels depending on its depth, which will alter the echogenicity of images, and then may require gain compensation to produce a clear image. Many specify the standardised application of gel,[4,20,29,39] but this is not uniformly followed by all users where gel is generously applied to the skin.[36,38]

Once HFU images have been captured, the measurements of dermal thickness and fluid content from the images require standardised methodology. Internal software is available with some equipment and varying description[7,23,40-42] limits method reproduction. Other HFU equipment requires exportation of images to MATLAB (a mathematical computing program)[26,38] to perform measures.

High reliability (ICC > .82) of HFU images using a 20 MHz DermaScan C or DermaLab Combo (both Cortex Technology) has been reported for dermal thickness measures in post-burn scars[43-45] and children.[7] There are few reports about the reliability or reproducibility of dermal thickness measurements in lymphoedema. Dylke et al (2018)[37] reported high inter-image, intra-rater and inter-rater reliability (Cronbach's alpha = .995; $ICC_{(3,1)}$ = .962 and .851; and $ICC_{(2,1)}$ = .977) using an 18 MHz device to capture images and measure dermal thickness in 38 women with breast lymphoedema secondary to breast cancer. Further, a change in dermal thickness was detected by HFU simultaneously with the development of clinically detected lymphoedema in the arms of women post-surgery for breast cancer, comparing with an unaffected side.[25] However, there remains no accepted reliable method for HFU to measure dermal thickness or fluid content in the legs of people with primary lymphoedema.

Measurement error must be minimised to reliably determine the outcome of an intervention and understand the impact of the change.[46] In HFU imaging, this requires (a) reliable acquisition of images and (b) reliable analysis of the images. Reliable acquisition

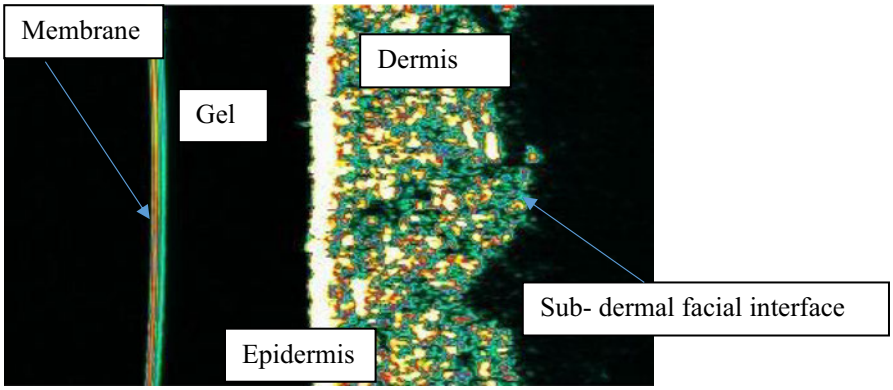

FIGURE 1 DermaScan C image of dermis and epidermis

of images must occur at the same site at different times with the ultrasound probe being lifted on and off the skin between images (inter-session reliability).

The DermaScan C, a particular type of HFU device (20 MHz; Cortex Technology), has been shown to be a valid way of distinguishing changes in water content in the dermis of healthy people (18-65 years) by comparison to MRI.[2] As well, it has been shown to be sensitive, detecting significant difference in skin thickness in the healthy between young (2-13 years) and old (25-40 years)[47] and between different body sites.[34,42] In a study of healthy skin thickness and echogenicity to assess ageing at different body sites, Gniadecka and Jemec (1998)[23] reported a Spearman correlation coefficient of .88 (95% CI: 0.72-1.0) between skin thickness and echogenicity.

The aim of this study was to develop and test a standardised HFU image capture method using the DermaScan C. The method was piloted in people without lymphoedema, refined and then tested with people who had primary lower limb lymphoedema. The intra-rater reliability of image measurement for skin thickness and dermal fluid content was investigated, and HFU images captured at different times were investigated for inter-session reliability.

2 | METHODS

2.1 | Ethics

Ethics approval for a study recruiting people with primary lymphoedema across three states was granted by Royal Children's Hospital Melbourne Australia (HREC/16/RCHM/136). Lymphoedema participants gave written informed consent and provided images for assessment in this reliability study.

2.2 | Population

Initially, people with no lymphoedema (NLO) were recruited from friends and colleagues of the primary researcher to pilot the proposed methodology. After the initial pilot, children and adults aged 3-40 years with primary lymphoedema (PLO) diagnosed by Mercy Health Lymphoedema Services assessment clinic or the Royal Children's Hospital Melbourne were recruited. Exclusion criteria included pregnancy, any skin condition in the assessable area such as dermatitis or eczema; uncontrolled cardiac, embolic or thrombotic conditions; and connective tissue conditions such as Marfan's disease, inflammatory conditions such as rheumatoid arthritis and infective conditions especially history of cellulitis within the past 2 months.

2.3 | Positioning

Participants lay supine on a plinth with one pillow under the head and another under the limb being measured. For posterior limb image capture, they lay prone.

2.4 | High frequency ultrasound: equipment, image capture and measurement

The DermaScan C (Cortex Technology) provides 20 MHz B-scanning at 60 × 150-micron resolution, with 13 mm penetration.[48]

The head of the transducer (probe) was held perpendicular to the skin[21,41] at a standardised distance from the skin,[4,49] producing images where the epidermis is parallel with the membrane within the transducer (Figure 1). Water-based gel (DANE-GEL R1, Rohdé Produits, Gl. Holte, Denmark) was applied within a "spacer," a slot on the probe head which provides a uniform distance between the HFU transducer and the skin surface. Air bubbles within the gel required removal or re-application of the gel.

An area the size of the transducer head was marked on each image capture site using a body pencil. This ensured repeat placement of the transducer on the same site for multiple image captures. Image capture was performed in a climate-controlled room with the participant always in the same position to avoid discrepancies due to temperature or body position.

One gain setting was consistently used to provide images for fluid content measures (mode one, gain profile 13 in the DermaScan C). To determine the best gain for skin thickness image clarity, three different gain settings were tested: mode one, gain

profile 19 and mode two gain profiles 16 and 19, chosen from initial NLO pilot testing and with reference to the manufacturer's manual on image capture.[49] For repeat image capture, the head of the probe was removed from the skin, gel re-applied and the head of the probe replaced on the same site (dorsum of the foot or calf) for three successive sets of four images. This provided images for the inter-session reliability analysis.

Ten images were chosen randomly across sites and people. Measures were taken on all images before being repeated twice more, separated in time by approximately two hours, ensuring no recall of individual images between measurement sessions.

2.4.1 | Intra-rater reliability: Dermal thickness measures

Images with the same gain setting were used for repeated dermal thickness measures at the same site. Lines were established to include both the epidermis and the dermis, along the entrance echo on the surface of the epidermis and the underside of the dermis (along the interface with the subcutaneous tissue), using automated edge detection software from the DermaScan C (DScan version 3 application software for Windows, advanced configuration), with the threshold set at 20.[49] The line produced by the edge detection function, determined "automatically" by default in the DermaScan software, may also be manipulated manually. In some images, a small gap in echogenicity allowed the measurement line to follow the threshold (of echogenicity it was following) within the dermis, which created a loop that deviated in and out of the dermis at the same point and affected the minimum measure (Figure 2). The small gap in echogenicity was "bridged" manually, to avoid an artificial minimum.

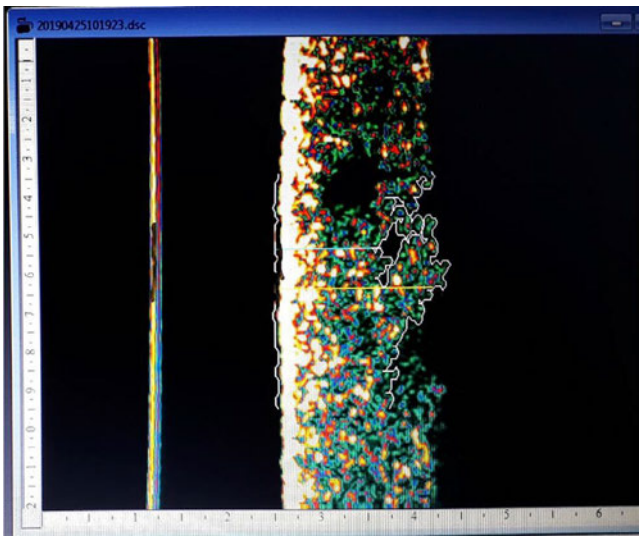

FIGURE 2 DermaScan C image showing edge detection line following area of low echogenicity and resultant "false" minimum skin thickness

2.4.2 | Intra-rater reliability: Dermal fluid content measures

Dermal fluid content measures were determined by using the "region of interest" (ROI) function in the DermaScan software. This is a standardised shape and size, which may be placed within the dermis to establish the area for assessment. Shape 1, with a standard rectangular area of 6.894712 mm^2, was set with the long boundary along the underside of the epidermis, completely within the dermis extending the length of the field of view (12.1 mm). The threshold for detection was set to 30[2,4,22,47] and on requesting "segmentation," the area (mm^2) and intensity (pixels and percentage) are produced of both the total ROI and the proportion (segmented area) of the ROI which was represented by 0-30, or fluid.

Measures were exported in a.csv (comma separated values) file, and the image showing the ROI, segmented area and dermal thickness measurements was saved as an image in the DermaScan software.

2.4.3 | Inter-session reliability

Images captured successively at approximately 5 minute intervals were analysed for dermal thickness and fluid content as described above.

2.5 | NLO pilot reliability study

Ten people with no lymphoedema (NLO), eight females and two males, aged 17-54 years, provided sites on one upper and one lower limb each for ultrasound image capture. Six sites were imaged: dorsum of the foot, posterior mid-calf, posterior mid-thigh and dorsum of the hand, medial anterior forearm, a quarter of the way from wrist to medial epicondyle, and anterior upper arm, a quarter of the way from the medial epicondyle to the anterior edge of the acromion in the anatomical position. Ten images from both upper and lower limbs were randomly chosen for image analysis and consisted of ten images for fluid analysis and ten for thickness measure analysis.

2.6 | Statistical analysis

The intraclass correlation coefficient (ICC), used to assess intra-rater or test-retest reliability, combines both correlation and agreement.[50,51] SPSS version 25 (IBM SPSS Statistics for Windows 2017) was used to calculate ICC, denoted $ICC_{(3,1),}$ using a two-way model (mixed effects), single score and absolute agreement.[52]

Both the ICC and the confidence intervals were considered in interpretation of reliability scores. ICC values over .90 indicate excellent reliability (repeatability), while .75 to .90 indicate good reliability and .50 to .75 moderate reliability.[50,51] Where the confidence interval extended below 0.75, even with a higher value ICC, the reliability

was rated as a range indicating the lower level (for example, an ICC of .92 with a lower level CI of 0.70 would be rated as good to excellent, not excellent).

Intraclass correlation coefficients (ICC) calculated for intra-rater reliability were good to excellent (CI: 0.836-0.998) (Table 1A) while inter-session reliability was lower, being generally moderate to good (Table 2).

2.7 | Pilot methodology modifications for PLO measurements

2.7.1 | Image capture

To enable more reproducible image capture, vertical lines were drawn on the screen of the monitor to assist visual vertical alignment of the epidermis for each image (checking that the probe is held perpendicular to the skin). A visual check of the screen marks against the image also highlighted discrepancies in the thickness of the gel.[1,4] Site position reproduction accuracy is important as is standardisation of coupling gel thickness.[2,22,41,53,54] Attention was paid to scraping excess gel from the probe surface as small variations in gel were seen to increase the gap between probe membrane and epidermal surface. Secondly, to ensure that the placement of the probe was consistent on the same site, instead of using pen markings on the skin, a small adhesive template was used, just big enough for the head of the probe.

Based on the NLO pilot, mode two gain profile level 16 (2/16) produced most images enabling edge detection of the sub-dermal boundary. However, as it was unclear whether the same would be true in participants with PLO, three gains were captured to enable the clearest image to be used for skin thickness measures. Images using the same gains were used for reliability analysis.

A set of four images using four different gain settings (comprising one to enable fluid measures (mode one, gain profile 13) and three for thickness measures: mode one, gain profile 19 and mode two gain profiles 16 and 19,) were taken three times at the same site, lifting the probe and re-applying gel between image capture; this provided data for the inter-session reliability analysis.

2.7.2 | Intra-rater reliability: Skin thickness measures

Repeated measures of skin thickness (comprising both epidermis and dermis) were taken on ten randomised images from ten lymphoedema (PLO) participants, (as per NLO participants), but with an amended measurement procedure. The central six millimetres of the full image length (12.1 mm) was used for edge detection (whereas the full length of the image had been used for those with NLO); this enabled a more consistent edge detection process than when including the top and bottom of the image. Edge detection lines for both the outer surface of the epidermis and the sub-dermal boundary began on the scale line mark at 3.5 cm and finished at 9.5 cm; the minimum, maximum and mean distance between the two boundary lines were used for analysis.

2.7.3 | Intra-rater reliability: Dermal fluid content measures

Consistent with skin thickness analysis, the centre of the image was used for LO images to assess fluid content: a small standardised rectangle set by the DermaScan C software (Shape 3:2.287931 mm^2; previously Shape 1 with 6.894712 mm^2 was used in NLO) was chosen as the Region of Interest (ROI). This ROI was

TABLE 1 Lymphoedema Reliability Study: Intra-rater reliability comparing outcomes from amended methodology of primary lymphoedema (PLO) with initial methodology in non-lymphedema (NLO) population

A. NLO reliability pilot						B. PLO reliability pilot				
			Confidence interval					Confidence interval		
Measure	N	ICC	Lower	Upper	Result	N	ICC	Lower	Upper	Result
Minimum distance	10	.951	0.868	0.986	Good-excellent	10	.997	0.992	0.999	Excellent
Maximum distance	10	.940	0.836	0.983	Good-excellent	10	.999	0.998	1.000	Excellent
Average distance	10	.962	0.898	0.990	Good-excellent	10	1.000	0.999	1.000	Excellent
Segmented area (mm)	10	.991	0.943	0.998	Excellent	10	.999	0.998	1.000	Excellent
Segmented area (pixels)	10	.991	0.943	0.998	Excellent	10	.999	0.998	1.000	Excellent
Total intensity	10	.993	0.966	0.998	Excellent	10	1.000	0.999	1.000	Excellent
Total intensity within range %	10	.989	0.925	0.998	Excellent	10	.997	0.991	0.999	Excellent

Note: Reliability rating based on ICC < .5 = Poor; Moderate: .5-.75; Good .75-.90 and Excellent > .90.
Abbreviation: ICC, Intraclass Correlation Coefficient.

set around the centre of the vertical scale (at 6.5) and was aligned with the underside of the epidermis, with the edge of the ROI just brushing the line of brighter intensity of the epidermis, rather than a rectangle that stretched the whole length of the image (as used for those with NLO).

2.7.4 | Inter-session reliability

The reliability of image capture at intervals of approximately 5 minutes with the probe removed in between image capture was assessed as for NLO. The procedure for dermal thickness and fluid content measurement from images was the same used in intra-rater reliability.

2.8 | Data cleaning

To check data entry, a random 15% of all data entered for NLO were double-checked with no errors found. For the PLO data, having twice the amount of data entered (measurement of both legs), double data entry into Microsoft Excel (Microsoft, Washington, US) was used to check for errors.

3 | RESULTS

Ten people with primary lymphoedema of the lower limb provided ultrasound images. Images using mode one gain profile 13 and mode two gain profile 16 were used for fluid content and thickness measure analysis, respectively.

3.1 | Intra-rater reliability

Images from five people (two females aged three and thirteen; three males aged eight, eleven and thirty-four) provided measures using images from the foot and calf in affected and unaffected lower limbs. Intraclass correlation coefficients ($ICC_{(3,1)}$) calculated for intra-rater reliability were good to excellent (95% confidence interval [95% CI]: 0.991-1.000) (Table 1B).

3.2 | Inter-session reliability

Images were provided by four participants (a male aged 16 and three females, one aged 31 and two aged 40) from the foot and calf in affected lower limbs. Inter-session reliability improved compared with NLO results, with good to excellent ICCs for mean thickness measures (95% CI: 0.809-0.980) and fluid measures (total intensity within range) (95% CI: 0.783-0.976) (Table 3).

Further analysis was undertaken with the data divided by site. While the analysis of intra-rater reliability in the PLO population shows reliability increased with technique improvements compared with the NLO population (Table 1A), the separation of PLO data into specific sites for fluid analysis (Table 4) resulted in slightly lower reliability, although all higher than ICCs than in the NLO reliability study, and still all excellent.

However, inter-session reliability was not as high. In the NLO population, both fluid and thickness measures generally had good reliability (Table 2), although minimum thickness was moderate (ICC .667; CI 95% 0.543-0.772), as was the measure of the range representing fluid within a specified area of the image. Technique improvements implemented in the PLO population increased the ICC generally from good to excellent (Table 3). However, when refining the data by site (Table 5), reliability became variable at specific sites in those with lymphoedema. Measures for fluid were generally good (ICC > .765) although again, the measure representing fluid within a specified area ("total intensity within range" represented by 0-30 from the intensity range 0-255) was lower, particularly in the calf (ICC: .616, CI: 0.332-0.828). Measures from the foot however were good (ICC .811; CI: 0.623-0.922). The foot also had higher reliability for minimum and mean thickness measures than the calf.

4 | DISCUSSION

Future research and clinical use of high frequency ultrasound (HFU) would benefit from using a standardised method to allow comparison of outcomes from intervention studies and consistent description of the tissues of people with and without lymphoedema. The method described here was developed and piloted to reliably measure dermal thickness and fluid content in people with (PLO) and without primary lymphoedema (NLO).

4.1 | Device

Those using the DermaScan C are advised to develop their own skill by practice, there being "no formal training or education in dermatological echography" Ref. 4, p.478 A training pathway with supporting manuals would assist in the reliable clinical and research use of this device. Key factors for good imaging are the situation and angle of the probe, the gain setting and the gel layer.[4] Attention to these factors, particularly the use of a fixed gain setting, the addition of marks on the screen to monitor the verticality of the probe and the thickness of the gel, improved the reliability of the capture and analysis of HFU images in this study, in assessing and comparing different body sites and different populations.

Images vary in brightness and in clarity of image at depth due to tissue properties (eg density), device properties (both fixed properties, eg frequency, and variable settings, eg time gain compensation) and procedural differences (eg thickness of gel, angle of probe on the skin).[4] The time gain compensation may be adjusted to allow for signals from those deep tissues to be intensified, making up for the attenuation of echoes originating from deeper tissue

TABLE 2 Non-lymphoedema Pilot: Inter-session Reliability

Measure	N	ICC	Confidence interval		Result	*F* Test with True Value 0			
			Lower	Upper		Value	*df*1	*df*2	Sig
Minimum distance	59	.667	0.543	0.772	Moderate	7.034	58	116	0.000
Maximum distance	59	.784	0.692	0.857	Good	11.9	58	116	0.000
Average distance	59	.813	0.731	0.877	Good	13.897	58	116	0.000
Segmented area (mm)	57	.867	0.804	0.914	Good	20.631	56	112	0.000
Segmented area (pixels)	57	.867	0.804	0.914	Good	20.631	56	112	0.000
Total intensity	57	.890	0.836	0.930	Good	25.47	56	112	0.000
Total intensity within range %	57	.727	0.614	0.817	Moderate	8.889	56	112	0.000

Note: Reliability rating based on ICC < .5 = Poor; Moderate: .5-.75; Good .75-.90 and Excellent > .90.

Abbreviation: ICC, Intraclass Correlation Coefficient.

TABLE 3 Primary Lymphoedema (PLO) Reliability Study: Inter-session reliability

Measure	N	ICC	Confidence interval		Result	*F* Test with True Value 0				Mean of 3 means	Mean of 3 variances
			Lower	Upper		Value	*df*1	*df*2	Sig		
Minimum distance	10	.881	0.705	0.966	Moderate to good	22.915	9	18	0.000	0.894	0.209
Maximum distance	10	.908	0.765	0.974	Good-excellent	29.866	9	18	0.000	1.469	0.210
Average distance	10	.929	0.809	0.980	Good-excellent	36.816	9	18	0.000	1.177	0.214
Segmented area (mm)	10	.917	0.786	0.977	Good-excellent	35.953	9	18	0.000	1.701	0.168
Segmented area (pixels)	10	.917	0.786	0.977	Good-excellent	35.953	9	18	0.000	2255.633	294 843
Total intensity	10	.892	0.693	0.970	Moderate to good	35.564	9	18	0.000	9.302	11.266
Total intensity within range %	10	.916	0.783	0.976	Good-excellent	36.164	9	18	0.000	6.466	1.818

Note: Images from two lymphoedema participants utilising two sites (dorsum foot and calf). Reliability rating based on ICC < .5 = Poor; Moderate: .5-.75; Good .75-.90 and Excellent > .90.

Abbreviation: ICC, Intraclass Correlation Coefficient.

TABLE 4 Primary Lymphoedema (PLO) Reliability Study: Intra-rater Reliability (Specific to site)

Measure	Site	N	ICC	Confidence interval		Result
				Lower	Upper	
Segmented area (mm)	Calf	10	.992	0.977	0.998	Excellent
	Foot	10	.999	0.998	1.000	Excellent
Segmented area (pixels)	Calf	10	.992	0.977	0.998	Excellent
	Foot	10	.999	0.998	1.000	Excellent
Total intensity	Calf	10	.997	0.992	0.999	Excellent
	Foot	10	1.000	1.000	1.000	Excellent
Total intensity within range %	Calf	10	.989	0.968	0.997	Excellent
	Foot	10	.999	0.996	1.000	Excellent

Note: Reliability rating based on ICC < .5 = Poor; Moderate: .5-.75; Good .75-.90 and Excellent > .90. Image Analysis by site.

Abbreviation: ICC, Intraclass Correlation Coefficient.

TABLE 5 Primary lymphoedema (PLO): Inter-session reliability by site

Measure	Site	N	ICC	Confidence Interval		ICC rating	*F* Test with True Value 0				Cronbach's alpha
				Lower	Upper		Value	*df*1	*df*2	Sig	
Minimum distance	Calf	16	.302	0.014	0.622	Poor	2.380	15	30	0.021	.580
	Foot	16	.834	0.667	0.932	Good	15.606	15	30	0.000	.936
Maximum distance	Calf	16	.864	0.722	0.945	Good	19.814	15	30	0.000	.950
	Foot	16	.705	0.460	0.872	Moderate	7.940	15	30	0.000	.874
Average distance	Calf	16	.321	0.032	0.637	Poor	2.522	15	30	0.015	.603
	Foot	16	.838	0.673	0.934	Good	15.748	15	30	0.000	.936
Segmented area (mm)	Calf	16	.767	0.551	0.903	Good	12.289	15	30	0.000	.919
	Foot	16	.887	0.765	0.955	Good	24.175	15	30	0.000	.959
Segmented area (pixels)	Calf	16	.767	0.551	0.903	Good	12.289	15	30	0.000	.919
	Foot	16	.887	0.765	0.955	Good	24.175	15	30	0.000	.959
Total intensity	Calf	16	.765	0.540	0.902	Good	12.709	15	30	0.000	.921
	Foot	16	.872	0.737	0.949	Good	21.375	15	30	0.000	.953
Total intensity within range %	Calf	16	.616	0.332	0.828	Moderate	5.544	15	30	0.000	.820
	Foot	16	.811	0.623	0.922	Good	13.071	15	30	0.000	.923

Note: Primary lymphoedema (PLO): Inter-session reliability by site. Reliability rating based on ICC < .5 = Poor; Moderate: .5-.75; Good .75-.90 and Excellent > .90. Abbreviation: ICC, Intraclass Correlation Coefficient.

that occurs with high frequency. Attenuation can make echoes of the same echogenicity appear darker, if originating from deeper tissue.[39,55] Given the time gain compensation may be altered to make images brighter, it is important to standardise this setting when making comparisons across anatomical sites, participants and repeated measures.[39] Images used for assessment of dermal thickness require enough clarity for an edge to be seen, which software can detect, so that measurements can be made. When using HFU diagnostically, the ability to adjust settings (gain) in real time has allowed for visualisation of structures not otherwise clearly identifiable. A relatively high gain (mode four gain profile 13, using the DermaScan C) was used for image capture to measure thickness in post-burn scars, which produce hypoechoic images.[56] However, when assessing change in tissue over time, or differences between populations, many factors may affect repeated scanning, with accurate repositioning and the gain settings being key factors. Previous studies utilising HFU vary, with the gain setting being adjusted if thickness measures are being assessed [42,43,53] or standardised if echogenicity is assessed.[41] The importance of using the same specific gain settings for each site in each person in follow-up images was stressed by Schou et al [7] in an investigation of changes in skin thickness in children over weeks of prednisolone use. Gel depth can also alter echogenicity of images by altering the distance sound travels; the DermaScan C has addressed this by adding a spacer to the head of the probe. However, where gel is applied non-uniformly and the gain requires adjustment for image clarity as a result, there is room for variation and technical error. Thickness measures rely on echogenicity thresholds for edge detection in the DermaScan C software; therefore any device property or method that affects echogenicity and attenuation including gain and gel depth would ideally be kept constant to reduce any potential source of error. Images used for fluid assessment rely on the echogenic properties of the tissue so the gain setting of the HFU needs to be constant if tissue properties are to be compared with others.[4] Determining what differences are due to tissue change or true difference between populations, is central to a study of this nature.

4.2 | Population differences

Standardised settings become problematic where there is marked lower echogenicity due to tissue type, as in lymphoedematous skin.[28] Ideally, the same settings should be used for comparison of dermal thickness between lymphoedematous skin and normal skin, but the low echogenicity of the dermis in lymphoedema means that the gain setting that produces acceptable images in normal dermis are too low to produce images in lymphoedematous dermis that provide clear depiction of the lower boundary of the dermis (and allow thickness measurements to be made). On the other hand, if settings are used that produce acceptable images in lymphoedema, (time gain compensation "turned up"), the resultant dermal image in healthy normal skin may be too bright (hyperechoic). Consequently, the lowest gain setting that produced acceptable images in both was sought.

4.3 | Body site differences

Further, when comparing sites around the body, higher echogenicity has been observed in limb skin than truncal skin in previous

studies, prompting the gain to be altered to obtain images depicting clear boundaries.[23] Different echogenicity was evident according to site during preparations for this current study, with proximal limb segments (upper arm and thigh) generally higher in echogenicity than distal segments. Investigation, according to site however, exposed the variability of HFU images in some areas of the body. The reliability of measures from the posterior calf specifically rated far lower when analysed individually than when it was included with measures from all sites. A reason for this may be the variable underside of the dermis (deep boundary), where "shadows" frequently appear in the calf image possibly representing veins (Figure 3). Generally, foot images were higher in intensity, except in lymphoedematous feet (Figure 4) where images were found to require a higher gain for accurate thickness measures to be taken; the presence of extra fluid in the dermis has been noted to disrupt collagen fibres,[41] resulting in less density, lower resolution and clarity in the image.[57]

In this study, reliability investigation of the capture and analysis of HFU images, undertaken in people with no lymphoedema, led to improvements in technique which resulted in greater reliability in a dermal study in people with lymphoedema. *Intra-rater reliability* outcomes in the non-lymphoedema population were excellent, but thickness measures had lower confidence limits below 0.9. Ideally, excellent scores above 0.90 were sought for clinical measures. Methods amended for both image capture and image analysis following the NLO pilot outcomes, resulted in subsequent images taken from ten PLO participants showing improved intra-rater reliability, achieving "excellent" for all measures.

However, *Inter-session reliability* investigated by site showed relatively low ICC for images taken over the calf in the leg, raising questions as to the variability of the tissue itself, or if technical error occurred. Inter-session reliability analysis by site in the NLO population may reveal whether this low reliability extends to both populations or if it was specific to the PLO calf for repeated measures. Thickness measures had lower reliability than fluid measures; possible causes of this lower reliability may include the tissue responding differently to prolonged ultrasound transmission[32] or the lower uniformity of the underside of the calf dermis, resulting in greater variability in echogenicity and the measurement line detecting that border curving inwards frequently.

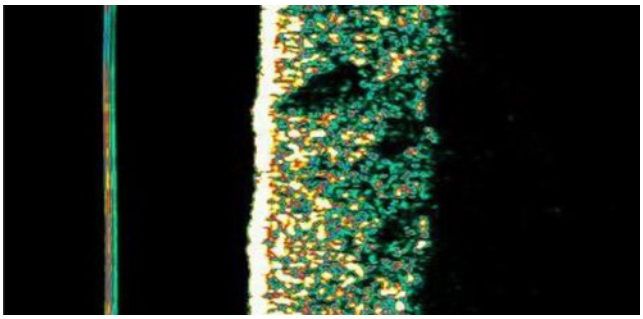

FIGURE 3 DermaScan C image from the leg over the calf muscle, showing dermis with variable sub-dermal border

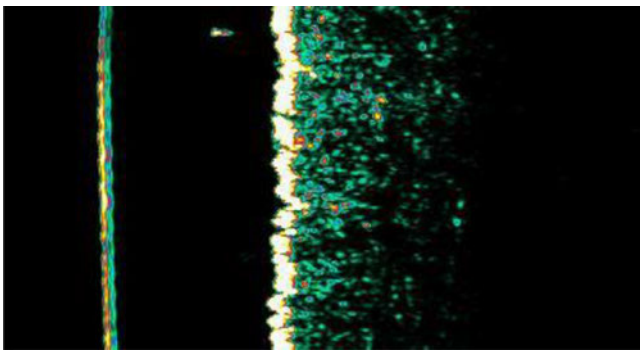

FIGURE 4 DermaScan C image from the foot showing oedematous dermis with low echogenicity

4.4 | Limitations of this study

The reliability outcomes of this study are specific to the populations assessed, to one operator and to the DermaScan C; outcomes may not apply to other operators and other high frequency ultrasound devices. The number of images captured using different gains varied between participants, in order to investigate optimal gain settings. Repeated images for each type of reliability (inter-session in particular) were restricted to a subset of participants (as described in the Section 3).

5 | CONCLUSION

Known procedural factors in high frequency ultrasound image capture such as gain setting and operator technique such as gel thickness and probe angle affect dermal measures produced using the DermaScan C. Based on the pilot study in people with no lymphoedema, amended methods improved reliability in a subsequent study in the primary lymphoedema population. Reliability outcomes determining the repeatability of HFU measures in both these populations suggest that the time gain compensation and measurement method for thickness and echogenicity be specified by anatomical site in the method of ultrasound studies and particularly in the use of the DermaScan C. Further analysis of lymphoedematous images showed good to excellent intra-rater reliability in measurement of images and good inter-session reliability for fluid measures.

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CONFLICT OF INTEREST

None to declare. Cortex Technology had no part in this study, including the conception, design or analysis of results.

AUTHOR CONTRIBUTIONS

Sue Gordon contributed to the concept, design and writing review; Karen Reynolds contributed to the design, method and writing review; and Jane Phillips contributed to the concept, design, method, data collection, data analysis and writing.

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Certificate of Calibration

Certificate number: 20082019-MDC1110

Date of calibration: 20 Aug 2019

Due: Oct 2021

Instrument type: MoistureMeterD Compact (1st gen)

Serial number: MDC1110

Customer: Flinders University, Murdoch Children's Research Institute
Royal Children's Hospital,
Flemington Rd, Parkville VIC 3052

Instrument condition on return

Meets all specifications

Calibration procedure

Standard calibration according to Internal Working Instructions
Externally Audited Quality Handbook, (ISO 13485:2016)

We certify that the above equipment meets or exceeds published specifications and has been duly inspected and calibrated using standards and instruments whose accuracies are traceable to International Standards, standard measuring equipment and methods for the realization of physical units of measuring according to the International Systems of Units (SI).

Calibration performed by

Richard Walmsley

Calibration approved by

Juha Pärnänen

The calibration has been performed at Delfin's manufacturing plant:
Delfin Technologies Ltd, Microkatu 1, 70210 Kuopio, Finland

Delfin Technologies Ltd
Microkatu 1, Kuopio
70210 Finland
www.delfintech.com

Calibration results MoistureMeterD Compact (1st gen) s/n MDC1110

Parameter: Tissue Dielectric Constant (TDC)

Measurement source	Reference value	Allowed tolerance	Observed difference
Ethanol	21.5	±5 %	1 %
Distilled Water - Ethanol Mixture	35.5	±5 %	1 %
Distilled Water	79.0	±5 %	-1 %

Pre-calibration values

Measurement source	Reference value	Observed difference
Ethanol	21.5	12 %
Distilled Water - Ethanol Mixture	35.5	-4 %
Distilled Water	79.0	-1 %

APPENDIX E ETHICS

Ethics approved study documents

The following documents were approved for use in the SkiPL study, copies of which are below

1. Initial and final approval letters from RCH and Mercy Health and confidentiality agreement with Mt Wilga Private Rehabilitation Hospital (**Appendix E**)
2. Protocol final version (8) dated 9th October 2019, outlining amendments. (**Appendix F**). Approved extra study sites for data collection in Melbourne are in the appendix to the Study Protocol (**Appendix F.1**)
3. Recruitment letters (**Appendix G**)
    a. RCH/MCRI Victoria
    b. Recruitment Letter Mercy Health Victoria
    c. Letter to therapists advising of the study
    d. Recruitment posters
    e. Recruitment letters to organisations such as the Australasian Lymphology Association and Lymphoedema Association of Victoria (consumer support group) with contact details form.
    f. Tracing letter (RCH and Mercy Health versions)
    g. Initial contact screening questionnaire (for both Primary Lymphoedema (PLO) and those without lymphoedema (NLO). Those with no lymphoedema did not complete full questionnaire.)
4. Participant information and consent forms (PICFs): RCH, Mercy Health and Sydney versions for each
    a. Master adult PICF – Primary Lymphoedema
    b. Master adult PICF – Non-Primary Lymphoedema
    c. Master parent/guardian PICF – Primary Lymphoedema
    d. Master parent/guardian PICF – non-Primary Lymphoedema
5. Data collection documents
    a. Attendance questionnaire LEG – Primary Lymphoedema

Appendix E.1 Ethics and Governance Approval: Royal Children's Hospital, Melbourne Victoria

ETHICS APPROVAL

19 December 2016

Ms Jane Phillips
General Medicine
The Royal Children's Hospital

Dear Ms Phillips,

Project Title: Dermal composition and related measures: response to pneumatic compression in people with and without primary lymphoedema

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HREC Reference Number: HREC/16/RCHM/136
RCH HREC Reference Number: 36273A

I am pleased to advise that the above project has received ethical approval from The Royal Children's Hospital Melbourne Human Research Ethics Committee (HREC).

The HREC confirms that your proposal meets the requirements of the National Statement on Ethical Conduct in Human Research (2007). This HREC is organised and operates in accordance with the National Health and Medical Research Council's (NHRMC) National Statement on Ethical Conduct in Human Research (2007), and all subsequent updates, and in accordance with the Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95), the Health Privacy Principles described in the Health Records Act 2001 (Vic) and Section 95A of the Privacy Act 1988 (and subsequent Guidelines).

HREC Approval Date: 16 December 2016*
Please note the HREC are no longer issuing pre-determined approval periods. Ethical approval is now ongoing, subject to the submission of an annual report on the anniversary of approval.

Participating Sites:
Ethical approval for this project applies at the following sites:

Site Name
• The Melbourne Children's Campus • Lymphoedema Research Unit, Flinders Medical Centre • Mt Wilga Private Hospital • Westmead Children's Hospital • Sydney Children's Hospital • Private Physiotherapy practice, Lymphoedema and Laser Therapy, 140 Payneham Rd, Stepney, South Australia 5069.

Approved Documents:
The following documents have been reviewed and approved:

Document	Version	Date
Master adult PICF – Primary Lymphoedema	1	8 December 2016
Master adult PICF - non-Primary Lymphoedema	1	8 December 2016
Master parent/guardian PICF – Primary Lymphoedema	1	8 December 2016
Master parent/guardian PICF – non-Primary Lymphoedema	1	8 December 2016
Protocol	3	14 December 2016
Participant Measures Form - Primary Lymphoedema	1	14 November 2016
Master recruitment letter - Primary Lymphoedema	1	16 November 2016
Letter to therapists – Primary Lymphoedema	2	18 November 2016
Letter to organisations	2	18 November 2016

Letter to Australasian Lymphology Association	1	18 November 2016
Initial Screening Questionnaire	2	16 November 2016
Attendance Questionnaire LEG – Primary Lymphoedema	2	16 November 2016
Attendance Questionnaire ARM Primary Lymphoedema	2	16 November 2016
Poster advertising study	2	15 November 2016

Site Specific Assessment:
Site-specific governance authorisation must be obtained by each participating site before the study can commence at that site.

You are required to provide a copy of this HREC approval letter to the principal investigator at each site covered by this ethics approval to assist each site PI with obtaining governance approval to commence the project at that site.

Conditions of Ethics Approval:

- You are required to submit to the HREC:
  - An Annual Progress Report (that covers all sites listed on approval) for the duration of the project. This report is due on the anniversary of HREC approval. Continuation of ethics approval is contingent on submission of an annual report, due within one month of the approval anniversary. Failure to comply with this requirement may result in suspension of the project by the HREC.
  - A comprehensive Final Report upon completion of the project.
- Submit to the reviewing HREC for approval any proposed amendments to the project including any proposed changes to the Protocol, Participant Information and Consent Form/s and the Investigator Brochure.
- Notify the reviewing HREC of any adverse events that have a material impact on the conduct of the research in accordance with the NHMRC Position Statement: *Monitoring and reporting of safety for clinical trials involving therapeutic products May 2009*.
- Notify the reviewing HREC of your inability to continue as Coordinating Principal Investigator.
- Notify the reviewing HREC of the failure to commence the study within 12 months of the HREC approval date or if a decision is taken to end the study at any of the sites prior to the expected date of completion.
- Notify the reviewing HREC of any matters which may impact the conduct of the project.
- If your project involves radiation, you are legally obliged to conduct your research in accordance with the Australian Radiation Protection and Nuclear Safety Agency Code of Practice 'Exposure of Humans to Ionizing Radiation for Research Purposes' Radiation Protection series Publication No.8 (May 2005)(ARPANSA Code).
- The HREC, authorising institution and/or their delegate/s may conduct an audit of the project at any time.

Yours sincerely

Dr Monique Baldwin
Research Ethics Manager
Research Ethics and Governance
The Royal Children's Hospital Melbourne
Phone : (03) 9345 5044
Email : rch.ethics@rch.org.au
Web : www.rch.org.au

GOVERNANCE AUTHORISATION

18 May 2017

Ms Jane Phillips
General Medicine
The Royal Children's Hospital

Dear Ms Phillips,

Project Title: Dermal composition and related measures: response to pneumatic compression in people with and without primary lymphoedema

---

HREC Reference Number: HREC/16/RCHM/136
SSA Reference Number: SSA/16/RCHM/142
RCH HREC Reference Number: 36273A

I am pleased to advise that the above project has received governance authorisation at the Melbourne Children's Campus (incorporating The Royal Children's Hospital, Murdoch Childrens Research Institute and the University of Melbourne Department of Paediatrics).

Governance Authorisation Date: 18 May 2017*
Please note that governance authorisation is ongoing, subject to the submission of an annual report on the anniversary of approval.

Authorised Documents:
As per the documents listed on the HREC approval letter, the following documents have been authorised for use at the Melbourne Children's Campus:

Document	Version	Date
MCRI recruitment letter Primary LO	1	23 November 2016
MCRI PICF - ParentGuardian - lymphoedema	1	8 December 2016
MCRI PICF – ParentGuardian - Non-LO PICF	1	8 December 2016
MCRI Adult PICF - lymphoedema	1	8 December 2016
MCRI Adult PICF - Non-LO	1	8 December 2016
Protocol	3	14 December 2016
Participant Measures Form - Primary Lymphoedema	1	14 November 2016
Letter to therapists – Primary Lymphoedema	2	18 November 2016
Letter to organisations	2	18 November 2016
Letter to Australasian Lymphology Association	1	18 November 2016
Initial Screening Questionnaire	2	16 November 2016
Attendance Questionnaire LEG – Primary Lymphoedema	2	16 November 2016
Attendance Questionnaire ARM Primary Lymphoedema	2	16 November 2016
Poster advertising study	2	15 November 2016

Conditions of Governance Authorisation

As Principal Investigator, you are required to:

1. Comply with the Investigator's responsibilities as outlined in the *Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95).*
2. Submit a copy of this letter to the person responsible for radiation safety at this site. **This condition only applies if** the project involves exposure to ionising radiation that exceeds dose constraints, and the Medical Physicist's report has advised that the project needs to be added to the site's *Licence for Research Involving Human Volunteers* issued by the Department of Health Radiation Safety Section (for more information, visit http://www.health.vic.gov.au/radiation/).

Note: If the Medical Physicist's report has advised that the project needs to be added to the site's licence, the project cannot commence at site until you have confirmed that the project has been added to the site's licence.

3. Notify the RGO of:
    - The actual start date of the project.
    - Any amendments to the project after these have been approved by the reviewing HREC.
    - Any adverse events involving patients at this site, in accordance with the NHMRC Position Statement: *Monitoring and reporting of safety for clinical trials involving therapeutic products May 2009.*
    - Any changes to the indemnity, insurance arrangements or Clinical Trial Research Agreement for this project. This includes changes to the project budget or other changes which may have financial or other resource implications at this site.
    - Your inability to continue as Principal Investigator or any other change in research personnel involved in this project.
    - Failure to commence the study within 12 months of the Reviewing HREC approval date or if a decision is taken to end the study at this site.
    - Any other unforeseen events.
    - Any other matters which may impact the conduct of the project at this site.
4. Ensure that HREC approval remains current for the entire duration of the project. Investigators undertaking projects without current Reviewing HREC approval risk their indemnity, funding and publication rights.
5. Submit an annual progress report every 12 months for the duration of the project. This report is due on the anniversary of HREC approval. Continued Governance Authorisation is contingent on receipt of an annual report by the RGO. In addition, a comprehensive final report should be submitted to the RGO upon completion of the project.

You must also abide by the following requirements:

1. Where applicable, ensure that the CTN has been electronically lodged to the TGA by the sponsor.
2. For clinical trials where the site is the Sponsor, you are required to contact MCTC to organise submission of the electronic Clinical Trial Notification (e-CTN) to the TGA. <u>This must be completed before commencement of your project.</u>
3. It is the Principal Investigator's responsibility to ensure that copies of the complete submitted e-CTN and TGA issued acknowledgement are included in the study Site File for the project at this site.
4. Ensure that the Clinical Trial Research Agreement (CTRA) and Indemnities (or other research agreements as applicable) are fully executed, i.e. signed by all parties; and an original version (or copy) placed in the study file.

The RGO may conduct an audit of the project at any time.

If you have any matters that arise regarding conduct of the research at this site, please ensure you contact the Research Governance Manager on 03 9345 5044.

I wish you and your colleagues every success in your research.

Yours sincerely

Dr Monique Baldwin
Research Ethics Manager
Research Ethics and Governance
The Royal Children's Hospital Melbourne
Phone : (03) 9345 5044
Email : rch.ethics@rch.org.au
Web : www.rch.org.au

ETHICS APPROVAL

11 October 2019

Ms J Phillips
School of Health Sciences
Flinders University

Dear Ms Phillips,

Project Title: **Dermal composition and related measures: response to pneumatic compression in people with and without primary lymphoedema (Skin in Primary Lymphoedema)**

HREC Reference Number: **HREC/16/RCHM/136**
RCH HREC Reference Number: **36273**

I am pleased to advise that the below amendment has received ethical approval from The Royal Children's Hospital Melbourne Human Research Ethics Committee (HREC).

The HREC confirms that your proposal meets the requirements of the National Statement on Ethical Conduct in Human Research (2007). This HREC is organised and operates in accordance with the National Health and Medical Research Council's (NHRMC) National Statement on Ethical Conduct in Human Research (2007), and all subsequent updates, and in accordance with the Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95), the Health Privacy Principles described in the Health Records Act 2001 (Vic) and Section 95A of the Privacy Act 1988 (and subsequent Guidelines).

HREC Amendment Approval Date: 11 October 2019
Please note the HREC are no longer issuing pre-determined approval periods. Ethical approval is now ongoing, subject to the submission of an annual report on the anniversary of approval.

Participating Sites:
Ethical approval for this project applies at the following sites:

Site Name
• Melbourne Children's Campus (incorporating The Royal Children's Hospital, Murdoch Children's Research Institute and the University of Melbourne Department of Paediatrics)
• Flinders Medical Centre (Lymphoedema Research Unit)
• Westmead Children's Hospital
• Sydney Children's Hospital

Approved Documents:
The following documents have been reviewed and approved:

Document	Version	Date
Protocol	8.0	9 October 2019
Appendix A	-	10 October 2019

Site Specific Assessment:
Site-specific governance authorisation must be obtained by each participating site before the study can commence at that site.

You are required to provide a copy of this HREC approval letter to the principal investigator at each site covered by this ethics approval to assist each site PI with obtaining governance approval to commence the project at that site.

Conditions of Ethics Approval:

- You are required to submit to the HREC:
  - An Annual Progress Report (that covers all sites listed on approval) for the duration of the project. This report is due on the anniversary of HREC approval. Continuation of ethics approval is contingent on submission of an annual report, due within one month of the approval anniversary. Failure to comply with this requirement may result in suspension of the project by the HREC.
  - A comprehensive Final Report upon completion of the project.
- Submit to the reviewing HREC for approval any proposed amendments to the project including any proposed changes to the Protocol, Participant Information and Consent Form/s and the Investigator Brochure.
- Notify the reviewing HREC of any adverse events that have a material impact on the conduct of the research in accordance with the NHMRC Position Statement: *Safety monitoring and reporting in clinical trials involving therapeutic goods November 2016*.
- Notify the reviewing HREC of your inability to continue as Coordinating Principal Investigator.
- Notify the reviewing HREC of the failure to commence the study within 12 months of the HREC approval date or if a decision is taken to end the study at any of the sites prior to the expected date of completion.
- Notify the reviewing HREC of any matters which may impact the conduct of the project.
- If your project involves radiation, you are legally obliged to conduct your research in accordance with the Australian Radiation Protection and Nuclear Safety Agency Code of Practice 'Exposure of Humans to Ionizing Radiation for Research Purposes' Radiation Protection series Publication No.8 (May 2005)(ARPANSA Code).
- The HREC, authorising institution and/or their delegate/s may conduct an audit of the project at any time.

Yours sincerely

Deeptika Chauhan
Research Ethics and Governance Officer
Research Ethics and Governance
The Royal Children's Hospital Melbourne
Phone: (03) 9345 5044
Email: rch.ethics@rch.org.au
Web: www.rch.org.au

GOVERNANCE AUTHORISATION

11 October 2019

Ms J Phillips
School of Health Sciences
Flinders University

Dear Ms Phillips,

Project Title: **Dermal composition and related measures: response to pneumatic compression in people with and without primary lymphoedema (Skin in Primary Lymphoedema)**

HREC Reference Number: **HREC/16/RCHM/136**
SSA Reference Number: **SSA/16/RCHM/142**
RCH HREC Reference Number: **36273**

I am pleased to advise that the below amendment has received governance authorisation at the Melbourne Children's Campus (incorporating The Royal Children's Hospital, Murdoch Children's Research Institute and the University of Melbourne Department of Paediatrics).

HREC Approval Date: 18 May 2017
HREC Amendment Approval Date: 11 October 2019
Governance Amendment Authorisation Date: 11 October 2019*
**Please note that governance authorisation is ongoing, subject to the submission of an annual report on the anniversary of HREC approval.*

Authorised Documents:
The following documents have been authorised for use at the Melbourne Children's Campus:

Document	Version	Date
Participant ICF (Non-LO)	3.1	8 October 2019
Parent/Guardian ICF (Non-LO)	2.1	8 October 2019

Conditions of Governance Authorisation

As Principal Investigator, you are required to:

1. Comply with the Investigator's responsibilities as outlined in the *Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95).*
2. Submit a copy of this letter to the person responsible for radiation safety at this site. **This condition only applies if** the project involves exposure to ionising radiation that exceeds dose constraints, and the Medical Physicist's report has advised that the project needs to be added to the site's *Licence for Research Involving Human Volunteers* issued by the Department of Health Radiation Safety Section (for more information, visit http://www.health.vic.gov.au/radiation/). *Note: If the Medical Physicist's report has advised that the project needs to be added to the site's licence, the project cannot commence at site until you have confirmed that the project has been added to the site's licence.*
3. Notify the RGO of:
    - The actual start date of the project.
    - Any amendments to the project after these have been approved by the reviewing HREC.

- Any adverse events involving patients at this site, in accordance with the NHMRC Position Statement: *Safety monitoring and reporting in clinical trials involving therapeutic goods November 2016*.
- Any changes to the indemnity, insurance arrangements or Clinical Trial Research Agreement for this project. This includes changes to the project budget or other changes which may have financial or other resource implications at this site.
- Your inability to continue as Principal Investigator or any other change in research personnel involved in this project.
- Failure to commence the study within 12 months of the Reviewing HREC approval date or if a decision is taken to end the study at this site.
- Any other unforeseen events.
- Any other matters which may impact the conduct of the project at this site.

4. Ensure that HREC approval remains current for the entire duration of the project. Investigators undertaking projects without current Reviewing HREC approval risk their indemnity, funding and publication rights.
5. Submit an annual progress report every 12 months for the duration of the project. This report is due on the anniversary of HREC approval. Continued Governance Authorisation is contingent on receipt of an annual report by the RGO. In addition, a comprehensive final report should be submitted to the RGO upon completion of the project.

You must also abide by the following requirements:

1. Where applicable, ensure that the CTN has been electronically lodged to the TGA by the sponsor.
2. For clinical trials where the site is the Sponsor, you are required to contact MCTC to organise submission of the electronic Clinical Trial Notification (e-CTN) to the TGA. This must be completed before commencement of your project.
3. It is the Principal Investigator's responsibility to ensure that copies of the complete submitted e-CTN and TGA issued acknowledgement are included in the study Site File for the project at this site.
4. Ensure that the Clinical Trial Research Agreement (CTRA) and Indemnities (or other research agreements as applicable) are fully executed, i.e. signed by all parties; and an original version (or copy) placed in the study file.

The RGO may conduct an audit of the project at any time.

If you have any matters that arise regarding conduct of the research at this site, please ensure you contact the Research Governance Manager on 03 9345 5044.

I wish you and your colleagues every success in your research.

Yours sincerely

Deeptika Chauhan
Research Ethics and Governance Officer
Research Ethics and Governance
The Royal Children's Hospital Melbourne
Phone: (03) 9345 5044
Email: rch.ethics@rch.org.au
Web: www.rch.org.au

Mercy Health
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Fax: +61 3 8416 7888
mercyhealth.com.au

21 February 2017

Ms Jane Phillips
Flinders University PhD Candidate
[redacted]

Dear Ms Phillips

Re: R16/67: Dermal Composition and Related Measures: responses to pneumatic compression in people with and without Primary Lymphoedema. (Skin in Primary Lymphoedema).

I am pleased to advise following discussion on 14 February 2017, Mercy Health Human Research Ethics Committee agreed that this research can be ***approved*** as ***low risk research***.
Specifically, the following documentation is approved:

Mercy Application	Dated 5 February 2017
Victorian Specific Module	Dated 23 January 2017
NEAF Multi-site Application	Dated 13 September 2016
Protocol	Version 3, Dated 14 December 2016
Participant Information & Consent Form (Non-Lymphoedema Group) Mercy Hospital for Women- Parent /Guardian	Version 1, Dated 6 January 2017
Participant Information & Consent Form (Lymphoedema Group) Mercy Hospital for Women – Parent/ Guardian	Version 1, Dated 6 January 2017
Participant Information & Consent Form (Adult Lymphoedema) Mercy Hospital for Women	Version 1, Dated 6 January 2017
Participant Information & Consent Form (Adult Non- Lymphoedema) Mercy Hospital for Women	Version 1, Dated 6 January 2017
Mercy Health Recruitment Letter	Version 1, Dated 23 November 2016
Mercy Poster - Lymphoedema	Version 2, Dated 15 November 2016
Mercy Poster – Non-Lymphoedema	Version 1, Dated 15 November 2016
Organisation Letter	Version 2, Dated 29 December 2016
ALA Letter	Version 1, Dated 18 November 2016
Letter to Therapists	Version 2, Dated 29 December 2016
Initial Contact Questionnaire	Version 2, Dated 16 November 2016
Attendance Questionnaire- Adult ARM	Version 2, Dated 16 November 2016
Attendance Questionnaire – Adult LEG	Version 2, Dated 16 November 2016
Master Participant Measures Form ARM	Version 1, Dated 9 November 2016
Master Participant Measures Form LEG	Version 1, Dated 9 November 2016

The Human Research Ethics Committee is constituted and functions in accordance with the National Health and Medical Research Council National Statement on Ethical Conduct in Human Research (2007- updated May 2015).

This approval by the Mercy Health Human Research Ethics Committee is valid from 21 February 2017 to 20 February 2019. That is, the project should be completed by the approval expiry date, of **20 February 2019**.

Should you require an extension of ethics approval, the HREC office must be contacted before the ethics approval runs out and your request must be accompanied with a current progress report. (Blank copy emailed with this letter)

Please note that the research project should have commenced within 6 months from the date of this letter.

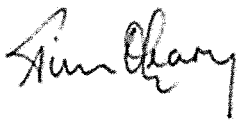
1. Immediate notification to the Administrative Officer, Human Research Ethics Committee and sponsor, of any serious adverse effects on participants;
2. Immediate notification of any unforeseen events that may affect the continuing ethical acceptability of the project;
3. Notification and reasons for ceasing the project prior to its expected date of completion;
4. The completion of a progress report annually for the duration of the project;
5. Human Research Ethics Committee approval of any proposed modifications to the project;
6. The submission of a final report and papers published on completion of the project.

Please also note:

7. Consent Forms must be available for audit by the Human Research Ethics Committee and retained for the period required by law;
8. The Principal Investigator upon leaving the Institution must inform the Human Research Ethics Committee as to the nominated person to replace him/her.

If you have any queries, please do not hesitate to contact Ms Carole Branch, Administrative Officer, Mercy Health Human Research Ethics Committee on (03) 8458 4808.

Yours sincerely



Mr Tim O'Leary
Chair, Mercy Health Human Research Ethics Committee

Mercy Health
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mercyhealth.com.au

January 4 2018

Ms Jane Phillips
Flinders University PhD Candidate
[redacted]

Dear Ms Phillips

Re: R16/67: Dermal composition and related measures: Response to pneumatic compression in people with and without primary lymphoedema.

I am pleased to advise that at the Mercy Health Human Research Ethics Committee meeting 12th December 2017, your request for updated documents related to this approved research were ***approved.***

Updated Equipment details – 1. Victorian – Specific Module	Dated 3 October 2017
Protocol - Skin in Primary Lymphoedema	Version 5, Dated 2 November 2017
Initial Contact Questionnaire	Version 3, Dated 3 October 2017
Participant Information and Consent Form – Adult Lymphoedema	Version 2, Dated 10 October 2017
Participant Information and Consent Form – Adult Non-Lymphoedema	Version 2, Dated 10 October 2017
Participant Information and Consent Form – Child/Parent Lymphoedema	Version 2, Dated 10 October 2017
Participant Information and Consent Form – Child/Parent Non-Lymphoedema	Version 2, Dated 10 October 2017
Attendance Questionnaire – LEG	Version 3, Dated 2 November 2017
Attendance Questionnaire – ARM	Version 3, Dated 2 November 2017
Participant Measures Form – ARM	Version 2, Dated 3 October 2017
Participant Measures Form – LEG	Version 2, Dated 3 October 2017

The Human Research Ethics Committee is constituted and functions in accordance with the National Health and Medical Research Council's National Statement on Ethical Conduct in Human Research (2007). In accordance with the NHMRC Guidelines, approval is subject to:

1. Immediate notification to the Administrative Officer, Human Research Ethics Committee and sponsor, of any serious adverse effects on participants;
2. Immediate notification of any unforeseen events that may affect the continuing ethical acceptability of the project;
3. Notification and reasons for ceasing the project prior to its expected date of completion;

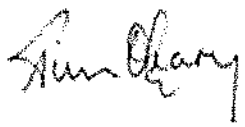
4. The completion of a progress report annually for the duration of the project;
5. Human Research Ethics Committee approval of any proposed modifications to the project;
6. The submission of a final report and papers published on completion of the project.

Please also note:

7. Consent Forms must be available for audit by the Human Research Ethics Committee and retained for the period required by law;
8. The Principal Investigator upon leaving the Institution must inform the Human Research Ethics Committee as to the nominated person to replace him/her.

If you have any queries, please do not hesitate to contact Ms Carole Branch, Administrative Officer, Mercy Health Human Research Ethics Committee on (03) 8458 4808.

Yours sincerely



Mr Tim O'Leary
Chair, Mercy Health Human Research Ethics Committee

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Richmond Vic 3121
Phone: +61 3 8416 7777
Fax: +61 3 8416 7888
mercyhealth.com.au

16 April 2019

Ms Jane Phillips
PhD candidate
Flinders University

Dear Ms Phillips,

Re: R16-67: Dermal composition and related measures: a comparison of people with and without primary lymphoedema.

I am pleased to advise that at the Mercy Health Human Research Ethics Committee meeting held on 09 April 2019 your amendment request to this approved research was **approved and endorsed** by the committee.

In particular, the following is approved:

Document	Version	Date
Mercy Health Amendment Request Form	1	15 February 2019
Mercy Health Tracing letter	2	15 February 2019
RCH clinical trial protocol	7	02 July 2018

In accordance with the NHMRC National Statement on Ethical Conduct in Human Research (2007), approval is subject to:

- Immediate notification to the Administrative Officer, Human Research Ethics Committee and sponsor, of any serious adverse effects on participants;
- Immediate notification of any unforeseen events that may affect the continuing ethical acceptability of the project;
- Notification and reasons for ceasing the project prior to its expected date of completion;
- The completion of a progress report annually for the duration of the project;
- Human Research Ethics Committee approval of any proposed modifications to the project;
- The submission of a final report and papers published on completion of the project.

Please also note:

- Consent Forms must be available for audit by the Human Research Ethics Committee and retained for the period required by law;
- The Principal Investigator upon leaving the Institution must inform the Human Research Ethics Committee as to the nominated person to replace him/her.

If you have any queries, please do not hesitate to contact Ms Natasha Rooney, Administrative Officer, Mercy Health Human Research Ethics Committee on 8458 4808 or email ethics@mercy.com.au.

Yours sincerely,

Mr Tim O'Leary

Chair, Mercy Health Human Research Ethics Committee

Mt Wilga
Private Hospital
Part of Ramsay Health Care

Mt Wilga Private Hospital
ABN 54 003 222 080
66 Rosamond Street
Hornsby NSW 2077
Telephone: 02 9847 5000
Facsimile: 02 9477 1253
mtwilgaprivate.com.au

ANNEXURE A

Dear Jane

Confidentiality & Privacy Agreement

You have been listed as a Clinical Research Representative as part of *Dermal composition and related measures: response to pneumatic compression in people with and without primary lymphoedema trial* which is being run out of Facilities owned and operated by Mt Wilga Pty Limited trading as Mt Wilga Private Hospital ('the Hospital'), a member of the Ramsay Health Care Group ('Ramsay').

As part of the Clinical Trial Research Agreement (CTRA) between Flinders University and the Hospital, Representatives of the CTRA have agreed to comply with certain obligations of confidentiality, which extend to ensuring that you comply with these obligations of confidentiality, public liability and professional indemnity cover.

The purpose of this letter is to ensure that you understand the expectations of Ramsay and the Hospital regarding its Confidential Information during your attendance at the hospital.

Confidential Information means confidential information regarding or belonging to any person, including information regarding Ramsay, the Hospital, practitioners and patients, whether verbal, visual, written, electronic or in some other form relating to:

(a) knowledge or information regarding the business transactions, affairs, property, policies, procedures or activities of Ramsay or the Hospital;

(b) any intellectual property of the Hospital or Ramsay;

(c) any document which is marked confidential or which you are advised or should reasonably be aware is confidential;

(d) medical records or health information of any patient; and

(e) personal information of any person.

You acknowledge and agree that:

1. You will not disclose to any third party, retain for your own records or use in any way, any Confidential Information of which you become aware of or which may come into your possession during your attendance at the Hospital for participation in the CTRA. This obligation does not extend to information which:
   (a) is, or becomes public knowledge that is not of your doing; or
   (b) is, or becomes available to you from a source other than Ramsay or other than through your attendance at the Hospital for participation in the Event.
   (c) is, or specifically related to the materials and information required to conduct the CTRA.

2. You will not, without the prior written consent of Ramsay, provide to any other person any Confidential Information, a copy of this document or disclose the contents of this document to any other person unless necessary to conform to all applicable laws and regulations.

3. You agree to comply with Ramsay's privacy policy and all applicable laws which apply to patient health information or medical records, including laws of confidentiality and privacy.

4. You will supply copies of Certificates of Currency and right to practice as a registered Physiotherapist to fulfil the requirements listed in the CRP agreement.

Please indicate that you have read and accept these terms by signing and dating the attached copy of this letter along with evidence as stated in item 4 and returning these to the undersigned.

Yours sincerely

Lorrie Mohsen
Chief Executive Officer
Mt Wilga Private Hospital

APPENDIX F STUDY PROTOCOL

PROTOCOL

Skin in Primary Lymphoedema

Dermal composition and related measures: response to pneumatic compression in people with and without primary lymphoedema.

Protocol Version 8 9th October 2019

Revision Chronology:

Date of change	Summary of changes
12 September 2016	Version 1
16 November 2016	Version 2
14th December 2016	Version 3
3rd October 2017	Version 4 Change to equipment
2nd November 2017	Version 5 Addition of PedsQL
14th June 2018	Version 6 Equipment change
2nd July 2018	Version 7 Recruitment amended
9th October 2019	Version 8 Recruitment amended

Statement of Compliance

This document is a protocol for a research project. This study will be conducted in compliance with all stipulation of this protocol, the conditions of the ethics committee approval, the NHMRC National Statement on ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

CONTENTS

PROTOCOL SYNOPSIS

TITLE	**Dermal composition and related measures: response to pneumatic compression in people with and without primary lymphoedema.**
OBJECTIVES	**Primary Objective**: To understand the effect of pneumatic compression on dermal depth and composition, and soft tissue properties in people with and without primary LO. **Secondary Objectives:** 1. To compare the response of the dermis and soft tissues to intermittent pneumatic compression of: a. Children, young adults and adults with primary lymphoedema b. People with different stages or duration of primary lymphoedema c. Age, gender and limb dominance matched people with and without lymphoedema

	2. To determine the degree of equivalence of clinical assessment measures with dermal depth and composition as identified by high frequency ultrasound.
DESIGN	Age and gender matched control pretest, post-test design.
OUTCOMES	Outcome measures: 1. Dermal thickness and composition – high frequency ultrasound 2. Dermal water content – high frequency ultrasound Related measures: 3. Tissue dielectric constant – MoistureMeterD 4. Soft tissue compressibility - Indurometer 5. Soft tissue elasticity – DermaLab 6. Volume – calculated from tape circumferences 6. Relative measure of fluid content in a limb segment - Bioimpedance spectroscopy
STUDY DURATION	24 months
INTERVENTIONS	Intermittent Pneumatic Compression at a dosage specific to adults and children
NUMBER OF PARTICIPANTS	Pilot study sample size of 10 participants. An interim analysis will be provided to RCH HREC, reviewing and providing feedback regarding the protocol (particularly with relation to children from 3-5 years old and 5-10 years old). One hundred and twenty total participants: up to 60 people with and 60 without primary lymphoedema across three states (including pilot participants).
POPULATION	Participants with primary lymphoedema aged between 3 and 40 years old will be sought through children's hospitals, lymphoedema treatment services and lymphoedema support networks in Adelaide, Melbourne and Sydney. People without lymphoedema will be recruited by requesting participants to invite a friend to the study, matched for age and gender; other non-lymphoedema participants will be sought through word of mouth, community groups, and from friends and colleagues at each site.

GLOSSARY OF ABBREVIATIONS

ABBREVIATION	*TERM*
ANOVA	*Analysis of Variance*
BIS	*Bioimpedance Spectroscopy*
DMC	*Data Monitoring Committee*

FU	*Flinders University*
HFU	*High Frequency Ultrasound*
HREC	*Human Research Ethics Committee*
IPC	*Intermittent Pneumatic Compression*
LO	*Lymphoedema*
MCRI	*Murdoch Children's Research Institute*
MHLS	*Mercy Health Lymphoedema Services*
MM	*Moisture Meter*
NHMRC	*National Health and Medical Research Council*
Pitting	*The presence of an indentation in the tissues following pressure applied by a finger or device to the skin of a lymphoedematous body part.*
RCH	*Royal Children's Hospital*
VA	*Vascular Anomaly*

1. ADMINISTRATIVE INFORMATION

1.1. Trial registration

1.1.1. Registry

This is not a commercial trial; all devices and interventions are being used within the range of usual therapeutic care.

This study has been registered under the ANZCTR with registration number: ACTRN12618000162213

1.2. Sponsor

Study Sponsor	Flinders University
Contact name	Professor Susan Gordon – Primary Supervisor of Principal Investigator
Address	17 Rockville Avenue Daw Park, South Australia 5041

This is a PhD project with supervision of all aspects of the study from design to data collection and management, analysis and interpretation. Decisions to submit report for publication will be made jointly and with approval of Flinders University PhD supervisors.

1.3. Expected duration of study

This is a multi-centre study which includes a before and after intervention assessment of matched participants. Participants will be sourced from lymphoedema treatment centres in major cities around Australia, and be asked to attend for one visit only. Recruitment will take place over 12 months using snowballing recruitment in a sample of convenience; once enough participants have been identified at a site, several days of assessment will be offered, where a participant needs attend on one day only, for approximately 3 hours duration. There is no follow-up period, as the study consists of one visit, with assessment before and after the intervention.

1.4. Contributorship

Name	Summary of contribution
Jane Phillips - PhD candidate, Flinders University - Lymphoedema Physiotherapist, Children's Private Medical Group	Study concept and design Recruitment Data collection, analysis and interpretation Write up
Professor Susan Gordon - PhD Supervisor, School of Health Sciences, Flinders University	Supervision, study design and oversight of all aspects of study, including review of protocol
Professor Neil Piller, School of Medicine – PhD Supervisor, Flinders University, SA	Review of protocol, equipment support, site liaison at Flinders Medical Centre and assistance with recruitment in South Australia
Professor Tony Penington, Murdoch Children's Research Institute (MCRI), Vic - PhD Supervisor MCRI, Adjunct supervisor Flinders University;	Supervision and project oversight; paediatric perspective
Professor Karen Reynolds, School of Computer Science, Engineering and Mathematics Flinders University SA - PhD Supervisor, Flinders University SA	Oversight of use and interpretation of equipment
Dr Helen Mackie Rehabilitation Specialist, Mt Wilga Private Hospital NSW	Assistance with recruitment and site liaison in NSW
Dr Zerina Tomkins Head of Vascular Biology Laboratory, Murdoch Children's Research Institute	Assistance with recruitment and site liaison at MCRI, Melbourne
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2. INTRODUCTION AND BACKGROUND

2.1. Background and rationale

Clinical assessment of treatment effectiveness and disease progression in lymphoedema (LO) commonly uses volume measurement calculated from circumferential measures or the pitting test, a highly subjective test where a finger is used to compress the soft tissues and assess the response. There is however no current understanding of the relationship between these measurements and the composition of the dermis - which is a target tissue of LO therapy. Therapy for LO involves the skin through management strategies that include a type of massage known as Manual Lymphatic Drainage (MLD), compression, and skin care to prevent infection. Arguably, movement is also affected by the stiffness of the skin in a limb, resulting in difficulty performing daily functional activities. Early stages of LO are characterized by fluctuant swelling with the presence of pitting, while in later stages the tissues become harder and pitting may be absent (Framework, 2006). Significant negative impacts on quality of life have been attributed to the tissue changes associated with LO (Auriol et al., 1994; S. G. Rockson,

2006) and it is suggested the hardness or increased tissue resistance impedes s the success of management strategies (Bagheri, Ohlin, Olsson, & Brorson, 2005).

New tissue measurement tools including the Indurometer, DermaLab and MoistureMeter have been developed to measure tissue compressibility, tissue elasticity and moisture 'at a point' in an attempt to improve objectivity of LO assessment. However again there is no current understanding of how these measures correlate with the composition of the underlying tissues. High Frequency Ultrasound (HFU) provides the opportunity to assess the changes in the dermis. HFU has been used to investigate the dermis and subcutaneous changes in secondary LO on comparison with MRI (Tassenoy et al., 2011; Tassenoy et al., 2009) and to assess skin thickness in secondary LO (Mellor et al., 2004) being found an accurate method for measurement of skin thickness since the early 1990s (Fornage, 1995; Serup, Keiding, Fullerton, Gniadecka, & Gniadecki, 2006). Having a clinical tool that provides an objective measurement of dermal change that also correlates to the changes seen on HFU will form the basis for targeted treatment plans and evaluation. The addition of children to this study will provide information for evidence based treatment where currently there is none: guidelines for treatment of children with LO are based broadly on those relating to adults, with little evidence of effectiveness (Damstra & Mortimer, 2008b; Phillips & Gordon, 2014).

Compression has long been a key management strategy for LO (Szuba & Rockson, 1998) and is also used extensively in the healthy population during travel to prevent deep vein thrombosis (Clarke, Hopewell, Juszczak, Eisinga, & Kjeldstrøm, 2006) and for prevention and treatment of muscle soreness after exercise (Valle et al., 2013). While dermal changes related to use of compression have been investigated with HFU post DVT (Volikova, Edwards, Stacey, & Wallace, 2009) and skin depth in lymphoedema (Hacard et al., 2014), age related differences in dermal composition in lymphoedema have not. Investigations into the skin of children without LO have shown that dermal thickness changes with age (Seidenari, Giusti, Bertoni, Magnoni, & Pellacani, 2000) and it is known that the maturation and thickness of the skin is related to collagen content (Waller & Maibach, 2005). The structure of elastic and collagen fibres differ at birth to that of adults (Shachner & Hansen, 2003); clinically this is reflected in the 'feel' of the skin, as presence of oedema is assessed by a pitting test. (Oedematous skin will leave an indentation or "pit" when pressed upon by a finger, a test known as "pitting".) The impact of the relative immaturity of the skin in young children compared with adults on treatment for lymphoedema such as compression, is unknown. The inclusion in this study of the healthy, age and gender matched population and the understudied childhood lymphoedema population will add to the understanding of the effect of compression on the dermis in all groups and form the basis for targeted treatment guidelines. Most lymphoedema research has investigated secondary LO, despite there being strong growing evidence of underlying genetic pre-disposition or covert primary LO in those who do develop secondary LO (Stanley G Rockson, 2010). The findings of this study will provide a basis for understanding the progression of primary LO and inform future primary and secondary LO research.
This project will use High Frequency Ultrasound (HFU) to:
1. Provide clinicians and researchers with information about differences in the composition of the dermis in those with and without lymphoedema (LO).

2. Provide clinicians and researchers with information about differences in the dermis in people with LO at different ages.
3. Determine the relationship between new tissue measures (Indurometry, elasticity and moisture meter) and current common measurement (circumferential measurement), and dermal composition in those with and without LO.
4. Determine the dermal and soft tissue response to a standardised dose of pneumatic compression in those with and without LO.

The findings of this research will inform the adoption of appropriate objective assessment tools and development of conservative treatment regimens based on a scientific understanding of dermal composition and the dermal response to compression in people with and without LO. This will inform the development of age appropriate treatment guidelines based on dermal variations. Further it will allow clinicians to target their treatment according to the specific alterations in dermal composition when LO is present as evidenced by the objective tissue measurements.

HFU is very expensive and not accessible to most clinicians; hence the need to correlate the dermal characteristics identified using HFU with other objective soft tissue assessment tools. If a measure of equivalence is identified this will provide a proxy measure of a dermal characteristic relevant to clinical practice. As all the tools are portable and affordable for clinical practice this will improve objective measurement of LO and change in LO.

The outcomes (description and change in dermal composition) for the group who do not have LO will be of interest to clinicians who apply compression for other conditions, such as prevention and treatment of deep vein thromboses (DVTs), and treatment of delayed onset muscle soreness from sport.

2.2. Aims

The aim of this study is to measure the depth and composition of the dermis using HFU, investigate correlation with other physical measures and to investigate dermal and soft tissue change following treatment with intermittent pneumatic compression (IPC) in children and young adults with and without primary lymphoedema.

3 STUDY OBJECTIVES

3.1 Primary objective

To understand the effect of pneumatic compression on dermal depth and composition, and soft tissue properties in people with and without primary lymphoedema.

3.2 Secondary objectives

1. To compare the effect on the dermis and soft tissues of intermittent pneumatic compression in:
   a. Children, young adults and adults with primary lymphoedema
   b. People with different duration of primary lymphoedema
   c. Age, gender and limb dominance matched people with and without lymphoedema
2. To determine the degree of equivalence of clinical assessment measures with dermal depth and composition as identified by high frequency ultrasound.

4 STUDY DESIGN

4.1 Type of Study

This is a multicentre pretest/post-test study, with a non-equivalent multi-group design (primary lymphoedema and an age, gender and limb dominance matched control /without lymphoedema group). Both groups will be assessed with high frequency ultrasound and other physical measures before and after a single application of intermittent pneumatic compression (IPC), a common lymphoedema treatment. Clinically used physical assessment tools will be investigated for equivalence with dermal thickness and composition as seen on high frequency ultrasound. It is expected that a participant will be asked to attend once for a period of approximately 3 hours. The first ten matched pairs will provide data for establishing intra-rater reliability. Repeated measures will be taken with both the DermaLab elasticity probe and HFU. A blinded research assistant will undertake independent assessment of HFU images for dermal thickness and water content to establish reliability. The reliability of the principal investigator has previously been established for Indurometry and circumferential measures in a primary lymphoedema population. The MoistureMeter (Mayrovitz, Davey, & Shapiro, 2009) and Bioimpedance (S. Rockson, 2007) have also been shown reliable for use in people with lymphoedema.

4.2 Study Setting

Murdoch Children's Research Institute, Victoria.

Mercy Health Lymphoedema Services, Victoria.

Mt Wilga Private Hospital – Sydney NSW

Lymphoedema Clinical Research Unit, Flinders Centre for Innovation in Cancer, Flinders Medical Centre, Adelaide, South Australia.

Sydney Hospital for Children – not confirmed

Hospital for Children at Westmead – not confirmed.

5 PARTICIPANTS AND RECRUITMENT

5.1 Number of Participants

Sixty people with and 60 people without primary lymphoedema will be sought across all sites.

ELIGIBILITY CRITERIA

Participants will be included in the study if they meet all of the inclusion criteria and none of the exclusion criteria.

5.1.1 Inclusion criteria

Participants must meet all of the following criteria to be enrolled in the study group:

- Have a diagnosis of primary lymphoedema, as assessed by screening questionnaire.
- Be aged between three and 40 years old

Participants must meet the following criteria to be enrolled in the control/comparison group of this study:

- Have no primary lymphoedema
- Be aged between three and 40 years old

- Be age, gender and limb dominance matched to a primary lymphoedema participant.

5.1.2 Exclusion criteria

Participants meeting any of the following criteria will be excluded from this study:

- Have had any skin infection (cellulitis) within previous two months
- Have a diagnosed connective tissue disorder e.g. lipodermatosclerosis or local occurrence of a skin condition such as psoriasis;
- Have a congenital condition which affects the skin such as Ehlers-Danlos Syndrome or Marfan's Disease

Have any contraindications for bioimpedance spectroscopy: Participants meeting any of the following criteria will be excluded from bioimpedance spectroscopy:

- a. Are pregnant
- b. Have a pacemaker or other implanted electronic device
- c. Have a metal implant such as pins or plates in bones

Have any contraindication for Intermittent Pneumatic Compression (IPC): Participants meeting any of the following criteria will be excluded from the study:

- a. Active metastatic disease
- b. Known or suspected deep venous thrombosis
- c. Pulmonary embolism
- d. Thrombophlebitis
- e. Uncontrolled cardiac failure
- f. Current cellulitis (infection of skin)
- g. Pulmonary oedema
- h. Ischaemic vascular disease
- i. Severe peripheral neuropathy
- j. Chronic regional pain syndrome

5.2 Recruitment and identification of potential participants

A convenience sample of adults and children with primary lymphoedema will be recruited through snowball sampling by poster through lymphoedema health professionals, and lymphoedema support groups, as well as community groups across three states.

Snowball recruitment will be undertaken via participants, as well as by providing information about the study to lymphoedema therapists, lymphoedema support groups and to the Australasian Lymphology Association for dissemination to people with primary lymphoedema; and to paediatricians, plastic surgeons and dermatologists at children's hospital clinics. Community groups such as Rotary, Apex, Lions Clubs and Probus will be approached with information regarding the project, to request information to be spread among their members, by newsletter advertisement, presentation or further information as requested. (Rotary Health has an interest in the project, having provided funding for a scholarship to investigate primary lymphoedema, which supports the lead investigator during this project.) Both lymphoedema and non-lymphoedema participants will be sought from among community groups.

The associate investigator for the site (person with appropriate authority) at children's hospital networks (Melbourne Children's Network: Royal Children's Hospital and Murdoch Children's Research

Institute, Melbourne; The Sydney Children's Hospitals Network and Flinders Medical Centre, Adelaide) will be approached to send a letter of invitation to those identified from the site's database with a diagnosis of primary lymphoedema over the past 20 years. Where the date of last contact with a person is more than two years prior, a tracing letter will be sent to check contact details prior to sending the letter of invitation. Prior to sending any correspondence, the deceased status within the medical record will be checked, as well as the diagnostic status of lymphoedema (confirmed, primary or secondary). In some cases where the diagnosis may not be possible to confirm for certain without speaking to the person, the screening questionnaire on initial contact will resolve this. Those who subsequently are found NOT to have primary lymphoedema, for whom the study is inappropriate, will be thanked for their time, with apologies for contacting them unnecessarily (if that is the case). A letter of invitation will also be provided to the Australasian Lymphology Association (ALA) for distribution to those who are registered on the ALA Lymphoedema Registry who have given consent to be contacted for research purposes.

It is also intended to advertise the study in the community; large churches may be approached to request a poster advertising the project be circulated via a newsletter or the noticeboard throughout the church network.

Those responding to the study advertisement will be asked to invite a friend of the same age and gender to participate on the same day (as an age and gender matched control), particularly for the younger participants. Further non-lymphoedema participants will be sought by word of mouth through friends and colleagues, and community groups by poster invitation to contact the lead investigator; Melbourne-based control participants recruited under MCRI/RCH may attend for data collection at an appropriate site closer to their location, for ease of attendance, if possible. Those non-lymphoedema participants who are not invited by a participant as a matched control, will be matched for age, gender and limb dominance, where possible, to those already recruited with primary lymphoedema.

Section 15.1. Posters advertising study;
Following Section 15.2:
Letter to organisations Primary LO;
Letter to therapists Primary LO
Letter to ALA
Master recruitment letter (to people with primary lymphoedema)

5.3 Consent

Recruitment will occur either by potential participants responding to a poster invitation, or by response to a letter of invitation. If responding to the poster invitation, potential participants are asked to contact the investigator by phone or email. Verbal consent will be sought to ask general health screening questions, to ensure suitability and safety of participation for that person. If eligible, an information sheet, consent form and proposed date and time will be sent to participants. (Attendance at an appropriate venue closer to their location will be arranged if possible, for matched control participants

attending in Melbourne. If attending at an appropriate venue of convenience to the participant, a colleague will be alerted prior to and following attendance at the site (as in a buddy system) and local emergency procedures followed as needed, in accordance with off-site field-work procedures.) Contact a week prior to the appointment will be made by phone or email to confirm stable health, suitability and the appointment time. If ineligible, the questionnaire will cease at the point that ineligibility becomes clear and the name, date and reason for ineligibility will be recorded. On arrival for their appointment, they will be asked for a signed consent form before any further questionnaire or other study specific procedure. If potential participants are responding to a letter of invitation, they may return the consent form in which case they will be contacted by phone, to answer any questions they may have, to verify they are eligible and to make an appointment time. If they have not responded after two weeks, they will be contacted by phone, at which stage they may ask any questions or be taken off the list if they indicate they wish not to participate.

For participants below the legal age, a parent, legal guardian, or person with power of attorney, must also sign a consent form. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. The investigator will conduct the informed consent discussion and will check that the participant and their legally acceptable representative comprehend the information provided and answer any questions about the study. The principal investigator may be known to some participants in Melbourne, from prior employment at Mercy Health and a small number of children may currently be in the clinical care of the PI. It will be made clear that participation in the study is entirely the choice of the potential participant and will not in any way affect their relationship with the researcher, or the institution where they may be accessing treatment if they decide not to participate or if they change their mind and wish to withdraw after initial consent. Where possible, other members of the research team not involved in the clinical care of the potential participant will conduct the consent process, to ensure that consent is voluntary and free from coercion. The investigator who conducted the consent discussion will also sign the informed consent form. A copy of the consent form will be given to the participant or their legally acceptable representative and the fact that the participant has been consented to the study will be documented in the participant's record.

5.4.1 Consent forms and screening tools to be used may be found following Section 15.2, and include:

- Information sheet and consent form – Adult
- Information sheet and consent form – Parent/ Guardian
- Information sheet and consent form – Adult Non-lymphoedema
- Information sheet and consent form – Parent/Guardian Non-lymphoedema
- Initial Screening Questionnaire

5.4.2 Documentation of consent will be recorded in a password protected file, along with a record of those who were invited but were ineligible, and the reason for their ineligibility. Details of name, date of birth, contact details, geographic locality/research site, type and site of lymphoedema, and age at diagnosis will be recorded, along with their study identifier. This information will be kept separate to the data collected in the study and will be accessed only by the principal investigator.

6 INTERVENTION

6.1 Treatment arms

Participants in both study groups (lymphoedema and no lymphoedema) will undergo the same procedure of measurement with High Frequency Ultrasound (HFU), Indurometer, tape measure, MoistureMeterD, DermaLab elasticity probe and Bioimpedance (SFB7). Once this data is collected, all will undergo treatment with a sequential intermittent pneumatic compression device, (Lymphapress or LX9) and be remeasured with all assessment tools.

6.2 Intervention

Upon completion of the measurement process, each person will be positioned supine with a pillow under the affected limb to undergo a standardised intermittent pneumatic compression session. The standard lymphatic drainage techniques preparatory for intermittent pneumatic compression (IPC) will be applied (deep breathing, standardised application of nodal massage in armpit, groin, and trunk massage) prior to the sleeve being applied to the limb. (*Queensland Health lymphoedema clinical practice guideline 2014*). The IPC unit used for the study is the LX9 supplied by Medi-Rent or the Lympha-Press supplied by Orthopaedic Appliances Pty Ltd (OAPL). An inflatable sleeve will be applied to either the arm or the leg of the participant depending on the site of the lymphoedema. A participant-specific liner will be applied to the limb prior to application of the sleeve, for hygiene purposes.

6.2.1 Dosage of intervention

Background

No standard treatment dosage has been agreed upon for IPC (Feldman et al., 2012; Maclellan, 2015; Zaleska et al., 2013) while debate continues around the length of treatment, the dosage of pressure applied and the cycle of time pressure is applied compared with the time of deflation, it is commonly agreed that IPC is a useful adjunct to treatment (Framework, 2006) (*Queensland Health lymphoedema clinical practice guideline 2014*) (Shao, Qi, Zhou, & Zhong, 2014). Its use in this study provides a method to apply a standardised "dose" of compression that is reproducible. Factors that affect the outcome of IPC (increased fluid movement expressed in softened tissues and reduced limb volume) include device dosage of the pressure applied, the time it is applied for and recipient factors including the resistance of the tissues. Extremes of tissue fibrosis or increased skin rigidity, which both increase tissue resistance and are features of later stage lymphoedema, are not expected in the population for this study. Further, given transmitted pressure is greater on a surface of smaller diameter, compared to surfaces of larger diameters, pressures at the lower end of the range will be selected for arms and pressures in the higher range for legs. In the absence of specific guidelines on pressure range for arms compared to legs, it can be seen from studies investigating upper limb lymphoedema, pressures in the range of 30-60 mmHg have more frequently been used and higher pressures for lower limb lymphoedema (40 – 120mmHg)(Feldman et al., 2012; Zaleska et al., 2013).

Dosage

Based on the literature, a consistent dosage of low to moderate pressure will be applied. Low to moderate pressure is described as 30-60mmHg (Feldman et al., 2012; Framework, 2006) which is clinically acceptable for adults and young adults (over the age of 9 for girls and 10 years for boys, based on developmental age) (Schook et al., 2011).
In general, lower pressures and shorter pressure cycle times have been used for young children with small limbs, but studies have had small sample sizes with a wide range of ages included (Hassall, Graveline, & Hilliard, 2001; McLeod et al., 1991). Hence, the lower end of the pressure range will be applied.
All participants will be monitored for comfort during IPC application and pressures applied will be appropriate to age and size of limb in those under 10 (pre-puberty). No adverse events have been reported in reviews of recent studies in adults and in children. (Feldman et al., 2012; Shao et al., 2014)

The device will be set for a 50 minute period of treatment. This will consist of preclearance of the proximal segment of the limb of 10 minutes, before a compression treatment program of 40 minutes in young adults and adults and 30 minutes in prepubescent children will be applied to the whole length of the limb. (Framework, 2006; *Queensland Health lymphoedema clinical practice guideline 2014*). Dosage has been adapted for prepubescent children following a review of the literature by shortening application time and lowering the pressure applied in adults and in concordance with the literature where the use of pneumatic compression in children is reported. This is in response to the relatively shorter limb of children of this age, and in accordance with lower pressures of compression garments used clinically for children of this age.

Dosage for adults:
Preclearance for 10 minutes at 30mmHg, then:
- 40 mmHg pressure over 40 minutes for upper limbs, and
- 60 mmHg pressure over 40 minutes for lower limbs.

Dosage for young adults:
Young adults of 10- 18 years of age (male) and 9-18 years of age (female), preclearance at 30mmHg, then:
- 40 mmHg over 40 minutes for upper limbs, and
- 50 mmHg over 40 minutes for lower limbs.

Dosage for children:
For children of 5- 9 (male) and 5-8 (female) years of age:
- 5 minutes preclearance at 20 mmHg and then 30 mmHg over 30 minutes for upper limbs and
- 5 minutes preclearance at 20 mmHg and then 40 mmHg over 30 minutes for lower limbs;

For children of 3-4 years of age:
- 5 minutes preclearance at 20 mmHg and then 20 mmHg over 30 minutes for upper limbs and
- 5 minutes preclearance at 20 mmHg and then 30 mmHg over 30 minutes for lower limbs;

The principal investigator will apply the IPC and monitor the participant during IPC use. The amount of clinical space for data collection has not been confirmed. It is most likely that only one space will

be used, and the principal investigator would remain in the room to monitor the participant. In the event that a child participant becomes distressed during the treatment period, this will be managed in conjunction with the parent, first checking there is no physical cause for distress, and providing alternative distraction or entertainment. If the child continues to be restless or distressed, treatment will be interrupted or aborted, as appropriate. If this occurs at any stage, but particularly during the pilot sample, the protocol may be amended if procedural changes are required.

Use of a second room to measure a second participant whilst the first has IPC set up may be a possibility and would depend on the ability of the principal investigator to monitor participants. Participants will be provided with a bell in case the attention of the principal investigator is required. It is anticipated that once a participant is undergoing treatment with pneumatic compression and is comfortable, the principal investigator may begin the process of measurement with a second participant. During this time, the bell would be utilised if there is need for the principal investigator's attention. The bell is provided for reassurance of the participant that they will be able to call for assistance if required, and is standard clinical practice within physiotherapy departments if for any reason the therapist needs to leave the room. On completion of the IPC treatment time, the PI would carry out the post-intervention measures, (HFU, Bioimpedance, circumferences, Moisture Meter, Indurometry and elasticity measure) whilst the IPC is on the second participant. No more than two participants would be asked to attend at overlapping periods. This would facilitate the measures of an age and gender matched control to attend with younger participants. However, a parent or guardian would be required to attend with each child under the age of 16. Participants will be invited to bring in some entertainment such as an iPod to listen to music or an iPad to read or watch a movie.

During application of the IPC, participants would remain stationary, lying on a treatment bed. The majority of participants are expected to have lower limb lymphoedema (In a study of 138 children with primary lymphoedema, 91.7% were reported to be lower limb lymphoedema (Schook et al., 2011)) and will thus be able to use their hands to hold a book or tablet for entertainment. A small bookstand to support a book or tablet will be supplied for the use of those with upper limb lymphoedema who are not able to use one arm while IPC is applied.

7 STUDY VISITS AND PROCEDURES

7.1 Screening

Potential participants will contact the principal investigator directly by phone or email. To verify eligibility using inclusion and exclusion criteria for the study, verbal consent will be sought to ask a number of screening questions. (Initial screening questionnaire accompanies this document.)

7.2 Baseline measures

On arrival participants will be asked to visit the bathroom before measures of height, weight, blood pressure and skin temperature are taken. In a private measurement area, they will then be asked to undress the body part involved, and will be draped with a towel or appropriate cover, as per usual

lymphoedema therapy treatment. If wearing a compression garment they will be asked to remove this, and rest supine (to counteract the effect of gravity) with a pillow under their head for a period of 20 minutes prior to measurements being recorded. During this time they will be asked questions relating to general matters such as skin care and, if they have lymphoedema, how they manage the condition on a day to day basis. The questionnaire will include a Quality of Life (QOL) tool.
Attendance Questionnaires.

- Attendance Questionnaire ARM
- Attendance Questionnaire LEG
- Attendance Questionnaire ARM Child (to be included once child QOL questions available)
- Attendance Questionnaire LEG Child (to be included once child QOL questions available)

Outcome measures including HFU, Indurometer, circumferences, MoistureMeterD, elasticity and Bioimpedance will be collected before (baseline) and after the application of compression using IPC. Participants need attend only once. Each measure will only require a few minutes to record, although some time is also needed for setting up to measure. During the set up time, participants will be able to move; it is only during the short period for recording of each measure that they will be required to stay still.

7.2.1 Procedure for Assessment

Sites will be measured in the arm if upper limb lymphoedema is present, and the leg if lower limb lymphoedema is present. Matched controls with no lymphoedema will have the limb measured that corresponds with the limb of interest in their matched participant. Measurement will be undertaken at three sites on the affected limb (which may be arm or leg) standardised by anatomical site and marked with a removable skin pencil in order to be repeated at the same site after the intervention. Each site of six cm^2 will be outlined with a removable body pencil, (using a template), so that pencil marks are not within the site of interest.

The point measurement sites to be outlined are described below (7.2.1.1) and will be used for measures taken with HFU, MoistureMeterD, Indurometer and elasticity. Segments will be delineated for segmental measurement of bioimpedance, with circumferential measures taken at the boundaries marking the segments, as well as circumferences at the point measurement sites. The segmental measurements are described in section 7. The mark-up procedure prior to the measurements being taken is described below.

7.2.1.1 Sites for HFU, MoistureMeter, Indurometer and elasticity

Point measurement sites are required for HFU, MoistureMeter, Indurometer and elasticity.

7.2.1.1.1 Arm point measurement sites

Point A. Dorsum of hand: Between the second and third metacarpals, just distal to the carpometacarpal joint.

Point B. Medial forearm: Along the anteromedial border of the ulna in the forearm, at the most proximal point lateral to the flexor carpi ulnaris tendon. If unable to be palpated, this point will equate to ¼ of the distance from the superior border of the ulnar styloid process to the olecranon, along the anteromedial border of the ulna.

Point C. Medial arm: Superior to the medial humeral epicondyle at the lower edge of the brachialis or biceps muscle bulk. If unable to be palpated, this point will equate to a point ¼ of the distance from the medial humeral epicondyle to the anterior border of the acromion, along the medial border of the brachialis or of the biceps, with the arm in the anatomical position.

7.2.1.1.2 Leg Point Measurement Sites

Point D. Dorsum of the foot: Between the second and third metatarsals, just distal to the tarsometatarsal joint.

Point E. Lower leg: With participant lying prone, with foot over the end of the plinth, foot in plantar grade; at a point half-way from the midpoint of the knee crease to the lower edge of the heel.

Point F. Posterior thigh: With participant lying prone, with feet over the end of the plinth, at a point half-way from the midpoint of the knee crease to the midpoint of the gluteal fold.

7.2.1.2 Segment boundaries for Bioimpedance and levels for Circumferential Measures

Body segments for measurement of bioimpedance will be marked by limb circumferential measures taken at the boundaries of the segments. Limb circumferences will also be recorded at the point measurement sites. Limb circumference measurement levels to be marked medially and laterally with a body pencil, to ensure accurate placement of later measurements.

7.2.1.2.1 Arm segment measurement levels

Segment A: Hand	
1. Metacarpophalangeal joint (MCP)	MCP joint line
2. Arm point measurement A	Dorsum of hand: distal to Carpometacarpal joint (CMC) joint line, between the second and third metacarpals.
Segment B: Forearm	
3. Ulnar Styloid	Ulnar styloid (least girth)
4. Arm point measurement B	Medial Forearm
5. Upper forearm	Distal to head of radius (maximum girth of forearm)
Segment C: Arm	
6. Proximal Epicondyle	Proximal to medial humeral epicondyle, inferior to biceps muscle bulk
7. Arm point measurement C	Lower third of upper arm.
8. Upper arm	Deltoid insertion (maximum girth of upper arm)

7.2.1.2.2 Leg segment measurement levels

Segment D: Foot	
1. Metatarsophalangeal (MTP) heads	MTP joint line
2. Leg point measurement D.	Dorsum of foot: distal to Tarsometatarsal (TMT) joint line
Segment E: Leg	
3. Minimum Ankle	Minimum Ankle above medial malleolus
4. Leg point measurement E	Lower leg
5. Upper Calf	Distal to head of fibula (maximum girth of calf)
Segment C	
6. Patella	Proximal pole of patella, in anatomical position
7. Leg point measurement F	Posterior thigh
8. Upper thigh	Distal to greater trochanter (maximum girth of thigh).

7.2.1.2.3 Length measurements

Length measurements of the appropriate limb segments (pertaining to either arm or leg) will be recorded to enable volume calculations to be made.

Arm	
i. Length of hand	Length of hand from MCP to ulnar styloid.
ii. Length of forearm	Length from Ulnar Styloid to Upper Forearm
iii. Length of arm	Length from Proximal Epicondyle to Upper Arm
Leg	
iv. Length of foot	Length from MTP to back of heel
Length of calf	Length from Minimum Ankle to Upper Calf
Length of thigh	Length from Patellar Pole to Upper Thigh

7.2.1.3 Measurement Protocol

The order in which measures are taken is planned so that later measures are not affected by the recording of earlier measures.

1. HFU images.

2. MoistureMeterD (tissue dielectric constant)

3. Indurometer (tissue compressibility).

4. Circumferential and length measures

5. Bioimpedance measures of the limb segments (thigh, leg and foot; OR arm, forearm and hand, depending on site of lymphoedema). In children the proximity of the hand to the forearm may require the hand and forearm to be measured as one segment; similarly for the foot and leg)

6. Elasticity (skin extensibility).

The measures of compressibility and extensibility (Indurometer and elasticity) may affect subsequent measures; for this reason, measures of bioimpedance and circumference will be taken after Indurometer and before elasticity measures to allow time for tissue recovery. Likewise, post-intervention measures will happen after an hour has elapsed during which the intervention (IPC) and other post-intervention measurement recording will take place, which will be sufficient time for tissue recovery following elasticity measures (Barel, Courage, & Clarys, 2006).

Each measurement will take only a few minutes or less, to record; the participant will be free to move in between measures.

7.2.1.3.1 High Frequency Ultrasound (HFU)

Recording of images with high frequency ultrasound requires the use of a water based gel as interface between the ultrasound head and the skin. This is consistent with diagnostic ultrasound use. Images will be recorded at point measurement sites A, B and C on both arms, or and point measurement sites D, E and F on both legs.

7.2.1.3.2 MoistureMeter (Tissue Dielectric Constant)

The MoistureMeter is a small hand held instrument with a head of approximately 2 cm which will be held against the skin. An electronic reading is produced after a few seconds. Readings will be taken at point measurement sites A, B and C on both arms, or and point measurement sites D, E and F on both legs.

7.2.1.3.3 Indurometer (Tissue compressibility)

This is a small hand held instrument (Model BME 1563G) consisting of a 6 cm disc which rests on the skin. A plunger protrudes through the disc on application of downward pressure by the researcher, until a force of 200g is reached. A beep is heard and a reading is produced at this point. This gives a measure of the resistance of the tissues to compression. Readings will be taken at point measurement sites A, B and C on both arms, or and point measurement sites D, E and F on both legs.

7.2.1.3.4 Circumferential and length measures (Limb volume calculation)

A tape measure will be used to record circumferential and length measures at each of the levels described in 7.2.1.2.

7.2.1.3.5 Bioimpedance (Relative measure of fluid)

Bioimpedance is a measure of resistance to flow of a small electrical current (powered by a battery) where resistance to flow is inversely proportional to the amount of extracellular fluid present. Limb segments of foot, leg and thigh in those with leg lymphoedema and hand, forearm and arm in those with arm lymphoedema will be measured with boundaries as described in 7.2.1.2.1 and 7.2.1.2.2. Four electrodes are required for each measure, with one being at each end of the segment to be measured, a third one is distal to the segment, and the fourth one more proximal (at the root of the limb or on the trunk). Electrodes consist of a small tabs with a gel-like substance that is applied to the skin; leads are attached to these electrodes. Measures take only a few seconds and are painless.

7.2.1.3.6 Elasticity Probe (Tissue Extensibility)

This measure will be undertaken using the elasticity probe. The probe is applied to the skin with double sided tape, to prevent skin "creeping"; this seals a section of the skin under a small cuplike device, which exerts a negative pressure sucking the skin upwards to a point when a reading of the skin extensibility is given. This is a pain free process.

7.3 Participant Withdrawal

7.3.1 Reasons for withdrawal

Participants may request to withdraw from the study at any time by signing the "Withdrawal from study" form and sending it to the principal investigator. If their measurements have already been taken, they may request these not be included in the study results. If the participant has not yet attended for measures to be taken, they will not be included in the study. The "Withdrawal from Study" form lists reasons for withdrawal.

The investigator may withdraw a patient from the study treatment if the patient:

- Is in violation of the protocol;
- Experiences a serious or intolerable adverse event
- Develops, during the course of the study, symptoms or conditions listed in the exclusion criteria
- Requires a medication that is prohibited by the protocol
- Requires early discontinuation for any reason

The investigator will also withdraw all participants from the study if the study is terminated. Patients are free to withdraw from the study at any time upon their request or the request of their legally acceptable representative. Withdrawing from the study will not affect their access to standard treatment or their relationship with the hospital and affiliated health care professionals.

7.3.2 Handling of withdrawals and losses to follow-up

When a participant withdraws from the study, the reason for withdrawal will be recorded by the investigator on the Data collection form (if during attendance) and the withdrawal from study form. If

measurement and/or treatment is suspended on the day, the reason shall be recorded, and an alternative time offered for measurement if appropriate.

7.3.3 Replacements

Further recruitment may be required to maintain the required sample size.

7.4 Trial Closure

If the study has not reached the number of participants for the required sample size, the study may be extended to further areas of Australia, or repeat visits made to existing research sites to gather further participants at a later stage. However, as this is a PhD project, it is time-limited. This decision will be made with study supervisors of the PhD project, in particular Professor Susan Gordon and Professor Neil Piller.

7.5 Continuation of therapy

No further treatment will be arranged for the participant following the study except as part of their existing treatment plan with their lymphoedema therapist. Details will be provided for access to pneumatic compression if requested.

Details to be made available to participants will include

- contact details of the company that provided the study project IPC
- other companies in Australia who provide appropriate IPCs (at least two other companies are known)
- the website address for the National Lymphoedema Practitioners Register of therapists who provide lymphoedema management.

The principal investigator and all the researchers have no conflict of interest or any connection with the companies providing compression units. The company providing the IPC has no control over study protocol, results, publications or any other aspect of the study except for provision of the equipment.

If, during the course of the assessment, an associated clinical problem is found, the participant will be directed to their treating doctor; if the researcher has any concerns, these will be discussed with a doctor on the team, Prof. Tony Penington and/or the lymphoedema paediatrician, Dr Julian Kelly. (For example, should signs suggesting tinea be evident – which has been associated with cellulitis in people with lymphoedema - this may be documented photographically with the participant's consent, for the purpose of discussion with the doctor, and appropriate clinical tests arranged, with results to go to their treating doctor.)

8 OUTCOMES

8.1 Primary outcome

The primary outcome measures will reflect the expected outcomes of compression: to reduce volume of extracellular fluid in dermis and soft tissues, and promote the movement of fluid away from the compressed tissue. Measures of outcomes will include all measures of dermal thickness and composition, and soft tissue properties before and after pneumatic compression.

Measures of improvement will include:

1. Reduction of dermal thickness and water content (determined from HFU images).
2. Reduction in tissue dielectric constant reading from MoistureMeterD.
3. Increased tissue compressibility read with the Indurometer.
4. Increased tissue extensibility read with the elasticity probe.
5. Decreased limb segment volume.
6. Increased bioimpedance reading (where amount of fluid is inversely proportional to the impedance).

8.2 Secondary outcome

Secondary outcome measures will include

A. Description of the dermis and soft tissues to determine difference between LO and nonLO groups and include the following measures:

1. Dermal depth and water content determined from HFU images and calculated using software provided with the Dermascan HFU.
2. Tissue dielectric constant as measured with the MoistureMeterD;
3. Tissue compressibility as measured with the Indurometer;
4. Tissue extensibility as measured with the elasticity probe;
5. Limb segment volumes (calculated from circumferential measures, using the truncated cone formula) (Taylor, Jayasinghe, Koelmeyer, Ung, & Boyages, 2006) or extra-cellular fluid volumes using information from the SFB7, and
6. Ratio measure of extracellular fluid in limb segment measured with Bioimpedance Spectroscopy (SFB7).

B. A comparison of all dermal and soft tissue measures within the LO group for difference with respect to:

- Age
- Stage and duration of lymphoedema

C. The measure of equivalence of each clinical assessment tool will be assessed with respect to the dermal depth and water content determined from HFU images.

9 ADVERSE EVENTS AND RISKS

9.1 Definitions

Unanticipated Problems

Unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- Is unexpected in terms of nature or severity given (a) the research procedures that are described in the protocol-related documents, and (b) the characteristics of the participant population being studied;
- related or possibly related to participation in the research;
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognised.

Unexpected Adverse Reaction:

- This is a reaction that may be related or possibly related to the use of IPC and is unexpected (i.e. not consistent with applicable product information).

Adverse Event (AE): Any untoward medical occurrence in a patient enrolled into this study regardless of its causal relationship to the use of IPC.

Serious Adverse Event (SAE)

Adverse events are classified as serious or non-serious.

An SAE is defined as any AE that:

- results in death; or
- is immediately life threatening; or
- requires inpatient hospitalisation; or
- requires prolongation of existing hospitalisation; or
- results in persistent or significant disability/incapacity; or
- is a congenital anomaly/birth defect.

Important medical events will be considered an SAE when, based upon appropriate medical judgement, they may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Suspected Unexpected Serious Adverse Reaction (SUSAR):

A SUSAR is any SAE that is both suspected to be related to the study treatment and is unexpected (i.e. not consistent with applicable product information)."

9.2 Assessment and documentation of adverse events

The investigator will record any adverse events (AE) and unanticipated problems during the study, with the following exceptions:

- Conditions that are present at screening and do not deteriorate will not be considered adverse events.

Any adverse event or unanticipated problems will be recorded on the Case Report Form (CRF) for the participant, and include:

- A description of the adverse event
- The time of onset of the event
- Nature of reaction/event
- Severity of reaction (mild, moderate or severe)
- Any action taken
- The outcome (resolved, continuing action, follow-up planned)
- The likelihood of the relationship of the AE to the use of IPC (Unrelated, Possible, Probable, Definite)
- If related to use of the IPC, application details including timing of event within treatment time, IPC dosage and application of sleeve

Adverse events or unanticipated problems characterised as intermittent will be documented and followed to resolution, where possible. The severity and relationship of an AE will be assessed as per

Appendix 15.2. The seriousness of an AE will be assessed by an investigator according to the definition in section 9.1, with the following exception:

- Hospitalisation due to progression of disease will not be considered an SAE for the purposes of this study.

In the unlikely event that it is an SAE requiring urgent care, appropriate medical attention will be sought as per the procedure within the health facility or if in a community setting, by accessing emergency services.

9.3 Eliciting adverse event information

Adverse event information will be collected by monitoring of the participant during application of IPC. The decision to discontinue treatment or participation in the study will be made by the principal researcher on assessment of the AE.

9.4 Serious adverse event reporting

Any serious adverse event will be reported via the reporting requirements of the State or Regulatory Agency responsible for SAEs in the State in which the SAE occurred.

9.4.1 SAES

Any SAE occurring in a study participant will be reported to the HREC within 24-72 hours of occurrence, in accordance with the safety reporting policy of the HREC. The HREC safety reporting form will be completed, signed and submitted by the principal investigator.

10 DATA MANAGEMENT

10.1 Data Collection

The investigator will be responsible for ensuring the accuracy, completeness, legibility and timeliness of the data reported. All source documents will be completed in a neat, legible manner to ensure accurate interpretation of the data. The investigator will maintain case information of study participants (including the identification code for participants, kept separate to the study data) and initial screening questionnaires.

10.1.1 Source Data

Source documents will include initial screening questionnaire and communications regarding appointment and contact details. Information collected in the questionnaire will be used
- To ensure eligibility;
- To subgroup participants
- To age- and gender-match a comparison group

These details will be kept separately from the data report form (CRF), which will be given a code to identify it. The code to identify participants will be kept separately to the CRF and will be in the care of the principal investigator, stored separately.

- Data Report Form V1 Primary LO (Follows Section 15.2)

CRFs will record data collected, which includes the attendance questionnaire, the measures recorded using the HFU and all other measurement tools, as well as details of treatment with IPC. The attendance questionnaire will record current management strategies (PL group) as well as general information such as limb dominance, relevant to the analysis of data. Images and measurement calculations from the HFU will be stored separately on a password protected electronic file.

Research data will only be accessed by the research team.

All outcome measures of interest will be recorded on the CRF:
1. Ultrasound images (from an arm or a leg) - to be assessed for dermal depth and composition
2. Measures of skin properties (all taken at the same sites on arm or leg).
a. Tissue compressibility: Indurometer measures
b. Skin elasticity – elasticity measures
c. Fluid component - Bioimpedance measures and Moisture Meter measures
3. Volume of the limb - calculated from circumferences measures, using a tape measure.

10.1.2 Data Capture Methods

Images and measurements from all instruments will be recorded electronically and stored under password protected conditions on a dedicated research computer.
- The HFU images and measurements will be recorded electronically as part of the data capture for the study.
- Measurements from the Indurometer, elasticity, Bioimpedance, Moisture meter and circumference measures will be recorded on an assessment sheet and transferred to password protected electronic storage on computer.
The principal investigator will take all measurements and be responsible for transfer to electronic data storage. Data quality checks will be carried out by regular review process.

10.2 Data Storage

Data storage will be on password protected electronic file and backup on a secure password protected online file accessed only by the research team linked with this project.
Privacy will be protected by participants being given a code; data will be gathered and held in non-identifiable form during collection, storage to analysis and reporting stages of the project. The key to the code will be held separately to the data. The key to the code will be safeguarded by Principal Investigator.

10.3 Record Retention

Data will be kept for 15 years after the completion of the study or until the 25th birthday of the youngest participant, whichever is the later, in accordance with the requirements of the Health Privacy Principles. In the instance that the ethical requirements for South Australia or NSW specify a longer retention period, the data will be kept for the longer period. Study related documents will be stored in secure password protected format within the School of Health Sciences, Flinders University

with the principal supervisor or head of school having responsibility for the documents. Disposal of documents at the end of the period required will be in accordance with the ethical requirements of the university and the National Health and Medical Research Council's Australian Code for the Responsible Conduct of Research

11 STUDY OVERSIGHT

11.1 Governance structure

This study is part of a PhD project and as such is subject to oversight by PhD supervisors from Flinders University and Murdoch Children's Research Institute. As a before and after study of one attendance only, there are no long term or follow-up visits required of participants; there will be no Trial Steering Committee, Safety Review Committee or Endpoint Review Committee.

11.2 Independent Data Monitoring Committee

The principal investigator will be responsible for all data collection and ensuring the quality of processes, the safety and storage of the data and all documentation associated with clinical research activities. Oversight responsibility rests with the Flinders University principal supervisor (Professor Susan Gordon, email address: sue.gordon@flinders.edu.au; Phone: (08) 7221-8745

11.3 Quality Control and Quality Assurance

Procedural documents outlining the process for data collection and measures to be taken will be followed at each data collection site. As applicable, calibration exercises will be conducted prior to and during the study to ensure equipment is reliable and accurate and to maintain acceptable intra-examiner agreement.

12 STATISTICAL METHODS

12.1 Sample Size Estimation

Primary lymphoedema is a rare condition. Based on the data available, including a study on Indurometry in primary lymphoedema where 13 people comprised the sample (Phillips and Gordon, unpublished, presented at the Asia Pacific Lymphology Conference, 2016), numbers are likely to be limited.

Sample size will be re-evaluated after measurement of 10 people in the pilot; at this stage sample size estimate is 100 participants, with 50 per group. Sample size will be determined by numbers of people with primary lymphoedema within the age and inclusion criteria available to participate, within the time frame of a PhD project.

Internationally, there are relatively low numbers of people with primary lymphoedema with 1 in 6000 being reported at a London, UK, clinic in 1985 (Dale, 1985) and 1.15 per 100,000 of those under 20 years in a town in the USA in 1985 (Smeltzer, Stickler, & Schirger, 1985). Little is reported in Australia on the prevalence, but based on experience with a previous study on Indurometry in primary lymphoedema (Phillips and Gordon, unpublished), the number of children recruited may be few. In this instance, children will be reported on as a case series.

In order to achieve an optimum sample size, recruitment will be conducted across three states of Australia, one of which houses a support group specifically for children with lymphoedema (NSW). In Victoria, RCH Health Information Services report that fifty people are recorded over the past twenty years as having primary lymphoedema. While not all of these people will still be children, a similar number have been identified by Mercy Health Lymphoedema Services, also within Victoria. Thirdly, collaboration with colleagues in the UK, who report having a data base of 200 children with primary lymphoedema, has raised the possibility of recruiting children with lymphoedema from a larger population.

The impact of the relative immaturity of the skin in young children compared with adults on treatment for lymphoedema such as compression, is unknown. Children under the age of five have been included, as changes in collagen and elastic properties of the skin occur during early childhood, just when treatment for lymphoedema begins, including the application of compression. While the numbers of children under five years recruited to this study are expected to be small, inclusion of children under five years old will provide important preliminary information regarding the response of the dermis to the standard treatment strategy of compression in this age group.

During later childhood or in adolescence, changes in the limb - both in size and in skin properties – occur such that greater compression is required to contain lymphoedema to a minimum. The inclusion of pre-pubertal children (aged from 5-10 years) will provide information regarding the properties of skin and its response to compression for this age group which could form foundations towards the development of clinical guidelines.

While low numbers of children in the study reduces the strength of data analyses involving age, the inclusion of children is a core element of this study, providing a base for further investigation, given the paucity of evidence regarding paediatric therapeutic management (Damstra & Mortimer, 2008a; Phillips & Gordon, 2014).

12.2 Statistical Analysis Plan

The primary objective of this study is to investigate the effect of pneumatic compression on dermal depth and composition and soft tissue properties in children and adults with primary lymphoedema (PLO) compared with children and adults, matched for age, gender and limb dominance, without primary lymphoedema (NLO).

The statistical analysis plan will include:

1. High frequency ultrasound (HFU) images of the dermis from all data (both pilot and main study) will be used to investigate the differences in dermal depth and composition in the limbs of those with and without primary lymphoedema. Comparative data, including mean, median, standard deviation and range will be described for dermal depth and composition as well as for all measures of physical properties of soft tissues.
2. Analysis of comparative data of dermal composition and depth as identified with HFU will be investigated before and after pneumatic compression is applied. Baseline and post treatment measures of HFU dermal properties and clinical assessment tools (Indurometer, elasticity,

Bioimpedance, volume and MoistureMeter) will be described with mean, median, standard deviation and range. Post treatment measures will be investigated for difference using a mixed ANOVA analysis, within factor for treatment and between factor of PLO/NLO groups and between factor for age.
3. The analysis of the response to compression will highlight the differences within each group with respect to age and comparison made with PLO and NLO groups.

Secondary objectives:
1. To compare the response of the dermis and soft tissues to intermittent pneumatic compression of:
a. Children, young adults and adults with primary lymphoedema
b. People with different stages or severity of primary lymphoedema
c. Age, gender and limb dominance matched people with and without lymphoedema
2. To determine the degree of equivalence of clinical assessment measures with dermal depth and composition as identified by high frequency ultrasound.

Statistical Analysis Plan:
1. Comparative data, including mean, median, standard deviation and range will be described for all measures of dermal and soft tissue properties and, for within group analysis will be sub-grouped for age and gender, as NLO and PLO are matched across groups. As numbers at each age may be small, the effect of age will also be investigated by groups of ages: e.g. children from 3-4 years and from 5-9 years, adolescents from 10-18 years and adults 19-40 years. Evaluation of HFU images by the PI will be investigated for inter-rater reliability with that of an independent research assistant, blinded as to group.
2. Comparative measures will be investigated for significant difference within those with lymphoedema (within group PLO), stratified by age and duration of lymphoedema. Stage of lymphoedema (defined by skin changes) could be expected to have an effect on the outcome measures; however given the age of the sample population, it is expected that the majority would be of a similar stage. For this reason, duration of lymphoedema will be investigated, as an exploratory analysis.
3. Comparative measures will be investigated for significant difference between study groups matched by age, gender and limb dominance and stratified for ethnicity. Matching of the PLO group with a NLO control group by age, gender and limb dominance will offset the relatively low numbers in each age and control for effect of limb dominance in volume measures.

4. The convergence validity of clinical assessment tools:
- Circumference measures (at point of interest and used to calculate volume using the truncated cone formula (Taylor et al., 2006),
- Bioimpedance,
- MoistureMeter,
- Indurometer and
- Elasticity will be investigated, in measures of agreement with dermal changes as identified with HFU, utilising SPSS.

12.2.1 Population to be analysed

The population to be analysed will consist of two groups, with (PLO) and without primary lymphoedema (NLO). Participants attend for one session, consisting of pre-test assessment, intervention and post-test assessment.

Two points about the study population are of note:

1. The inclusion of children: The majority of the sample recruited are expected to be adults. While children and adolescents are sought, and included, it is recognised that the number recruited in these age groups may be small. As many as possible will be sought in a convenience sample and through snowball recruitment, but where numbers are small, analysis of children in these age groups will be undertaken as a case series, separate to adult results. Due to the paucity of evidence around management of children with lymphoedema, analysis of outcomes in children is deemed worthy of inclusion. See notes in section 12.1 Sample Size
2. Primary lymphoedema to the exclusion of secondary: Primary lymphoedema occurs due to anomalies of formation in the lymphatic system. Some anomalies may affect the development of the initial lymphatic vessels in the dermis, and consequently lymph drainage is affected at the very periphery. In contrast, secondary lymphoedema more commonly results from an interruption to the lymph drainage at a more central point: e.g. at the lymph nodes or due to radiation at the root of the limb (armpit or groin). While it is not clear if this would have any impact on the outcomes, to ensure there is no confounding effect within the dermis due to different causation of lymphoedema, those with secondary lymphoedema have been excluded.

12.2.2 Handling of missing data

Screening for allergy to tape may result in some participants providing data for all of the other assessment tools except those that require tape for accurate placement (tape allergy). In this instance, data provided will be included in the relevant analyses.

12.2.3 Methods of analysis

Comparative data including the mean, median, standard deviation and range will describe the thickness and water content of the images from HFU across groups and the outcome measures for all assessment tools. Differences between groups will be investigated, as well as the difference within group for those with lymphoedema. Analysis within the lymphoedema group will be stratified according to the duration of lymphoedema (related to age, and stage of LO). The stratification test used will depend on whether there is normal distribution of data.

Pretest/post-test comparisons will involve an analysis of covariance with a between factor of age and presence of lymphoedema or not. Possible covariates of stage and duration of lymphoedema will be investigated where possible. Appropriate analyses of sensitivity will be used to investigate violations of assumptions of normality and alternative tests used where necessary to correct for these violations. Convergence validity of the clinical tools (elasticity, Bioimpedance, Indurometer, MoistureMeter and circumferences) will be assessed against the standard of the depth and composition of the dermis determined from HFU images.

13 ETHICS AND DISSEMINATION

13.1 Research Ethics Approval

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the human research ethics committee (HREC) via the National Ethics Application Form (NEAF). National Mutual Acceptance sought across Victoria, South Australia and New South Wales and site specific adaptation. A letter of protocol approval by the site specific HREC will be obtained prior to the commencement of the study at that site, as well as approval for other study documents participant to HREC review.

13.2 Modifications to the protocol

This study will be conducted in compliance with the current version of the protocol. Any change to the protocol document or Informed Consent Form that affects the scientific intent, study design, patient safety, or may affect a participants willingness to continue participation in the study is considered an amendment, and therefore will be written and filed as an amendment to this protocol and/or informed consent form. All such amendments will be submitted to the HREC, for approval prior to becoming effective.

13.3 Protocol Deviations

All protocol deviations will be recorded in the patient record (source document) and on the CRF. Protocol deviations will be assessed for significance by the Principal Investigator. Those deviations deemed to have a potential impact on the integrity of the study results, patient safety or the ethical acceptability of the trial will be reported to the HREC in a timely manner. Where deviations to the protocol identify issues for protocol review, the protocol will be amended as per section 11.3.

13.4 Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, research staff, and the sponsoring institution and their agents. The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorised third party, without prior approval of the sponsoring institution. Authorised representatives of the sponsoring institution may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records. All evaluation forms, reports and other records that leave the site will be identified only by the Participant Identification Number (SID) to maintain participant confidentiality. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by HREC or regulatory agencies.

13.5 Participant Reimbursement

There will be no participant payment made. A discount parking voucher will be provided for those attending at The Royal Children's Hospital Melbourne (Murdoch Children's Research Institute) and car parking costs may be covered for those attending Flinders Medical Centre. Following completion of all

measures, participants will be given a copy of their individual measures (Participant Measures Form) and a Hush CD as a thank you gift. Individual measures taken with each clinical tool may be useful to participants in self- management of their lymphoedema, or with their treating therapist, and as a record for comparison at a later date, particularly for children. Measures will be explained verbally if participants are unfamiliar with any of them.

- Participant Measures Form (Follows Section 15.2)

13.6 Financial Disclosure and Conflicts of Interest

There are no financial disclosures or conflicts of interest. The intermittent pneumatic compression device for the study was supplied by Medi-Rent Pty Ltd and Orthopaedic Appliances Pty Ltd Australia. The elasticity probe was supplied by Cortex Technology, Denmark.

13.7 Dissemination and translation plan

Results of this study will be disseminated to healthcare professionals, participants and the public via publication of articles in scientific publications, and by presentation at conferences, in particular the Australasian Lymphology Conference. Presentations will be made to Rotary Health and the Rotary Club who support this PhD project with a scholarship. A summary of results of the group findings will be made available to participants upon request (indicated on consent form).

The principal investigator holds the primary responsibility for publication of results with Flinders University supervisors of this PhD project.

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14.1 Causality and Assessment of Severity – Adverse Events

The severity of an Adverse Event or Adverse Reaction will be assessed as follows:

- **Mild**: Events that require minimal or no treatment and do not interfere with the patient's daily activities.
- **Moderate**: Events that cause sufficient discomfort to interfere with daily activity and/or require some therapeutic intervention (e.g. manual lymphatic drainage).
- **Severe**: Events that prevent usual daily activity or require complex treatment.

The relationship of the event to the use of IPC will be assessed as follows:

- **Unrelated**: There is no association between the application of IPC and the reported event. AEs in this category do not have a reasonable temporal relationship to exposure to the intervention device, or can be explained by a commonly occurring alternative aetiology.

- **Possible**: The event could have caused or contributed to the AE. AEs in this category follow a reasonable temporal sequence from the time of treatment with the device intervention and/or follow a known response pattern to the intervention, but could also have been produced by other factors.
- **Probable**: The association of the event with the study treatment seems likely. AEs in this category follow a reasonable temporal sequence from the time of exposure to the intervention and are consistent with the known action of the device, known or previously reported adverse reactions to the device, or judgement based on the investigators clinical experience.
- **Definite**: The AE is a consequence of administration of the intervention. AEs in this category cannot be explained by concurrent illness, progression of disease state or concurrent medication reaction. Such events may be widely documented as having an association with the intervention.

End of Document

Appendix A. Skin in Primary Lymphoedema
Offsite Study Visits in Melbourne

Study visits in Melbourne may be held at locations more convenient to study participants than attending MCRI.

1. Offsite locations will consist of clinical spaces and supply:
    a. A private room/space for data collection.
    b. A plinth of appropriate height.
    c. Accessible power points (3).
    d. Toilet facilities.

2. The PI is a private consulting physiotherapist and the sites proposed for offsite data collection are clinical rooms of colleagues; as such, the PI will be aware of the local emergency response. There is limited scope for adverse events given all procedures undertaken for the study protocol fall within usual clinical practice.

3. Acknowledgement from the Head of each site for use of the site for this study was obtained prior to use.

    a. Sites:
        i. Victorian Lymphoedema Practice, 5 Warrigal Rd Surrey Hills.
        ii. Surrey Hills Medical Practice 174 Union Rd, Surrey Hills.
        iii. Vermont Health and Lifestyle, 133 Heatherdale Rd, Vermont.

4. Flinders University provides Insurance for the PI as a PhD student to cover work at an external site.

APPENDIX G RECRUITMENT DOCUMENTS

Appendix G.1 Recruitment Letter RCH

Number/Street Name

Suburb

State/Postcode

Date

Dear Mr/Ms Surname

Research Project Title: Skin in Primary Lymphoedema

I am writing to let you know about a new research project that is taking place at [site] which you/ your child may be interested in participating.

Why are you being asked?

I am sending you this information because our records indicate you/your child may have primary lymphoedema and may be suitable to take part.

What is the research about?

Enclosed is an information statement and consent form describing the project. The form explains the research in detail, including:

- What the aims of the research are
- What is involved if you take part
- What the risks and benefits of participation are
- What happens to information collected and how confidentiality of information is protected

What do you need to do?

Please take some time to read the information statement and consider taking part.

Please call or email the research team to let them know if you are interested in taking part or you can sign the consent form and return it, along with the contact details form.

If the research team does not hear from you in the next 2 weeks, they will contact you to check you received this letter and tell you more about the project.

Kind regards

Dr Julian Kelly MBBS FRACP
Consultant Paediatrician
Department of General Medicine
The Royal Children's Hospital
Melbourne
3 West Clinical Offices, 50 Flemington Road, Parkville 3052
The Children's Private Medical Group
Suite 3.3 48 Flemington Rd
Parkville, 3052
Ph: 9345 6688

Jane Phillips

Lymphoedema Physiotherapist
PhD Candidate
School of Health Sciences
Flinders University
The Children's Private Medical Group
Suite 3.3 Level 3, 48 Flemington Road
Parkville 3052
Jane.phillips@flinders.edu.au
Ph: 9345 6688

Appendix G.2 Recruitment Letter Mercy Health

Number/Street Name
Suburb
State/Postcode

Date

Dear Mr/Ms Surname

Research Project Title: Skin in Primary Lymphoedema

I am writing to let you know about a new research project that is taking place at Murdoch Children's Research Institute/ site which you/ your child may be interested in participating.

Why are you being asked?
I am sending you this information because you/your child may have primary lymphoedema and may be suitable to take part.

What is the research about?
Enclosed is an information statement and consent form describing the project. The form explains the research in detail, including:

- What the aims of the research are
- What is involved if you take part
- What the risks and benefits of participation are
- What happens to information collected and how confidentiality of information is protected

What do you need to do?
Please take some time to read the information statement and consider taking part.

Please call or email the research team to let them know if you are interested in taking part or you can sign the consent form and return it.

If the research team does not hear from you in the next 2 weeks, they will contact you to check you received this letter and tell you more about the project.

Kind regards

Tanya Darrer
Manager
Mercy Health Lymphoedema Services
Mercy Hospital for Women
163 Studley Rd Heidelberg Vic 3084
Ph: 03 8458 4156

Jane Phillips
Lymphoedema Physiotherapist
PhD Candidate
School of Health Sciences
Flinders University
at Murdoch Children's Research Institute
The Children's Private Medical Group
Suite 3.3 Level 3, 48 Flemington Road
Parkville 3052
Jane.phillips@flinders.edu.au

Appendix G.3 Recruitment Letter to Therapists

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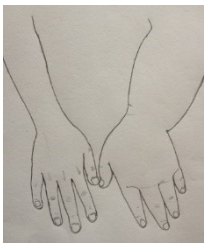
inspiring achievement

[Date]

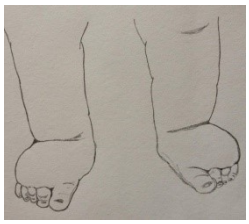
Primary lymphoedema and the skin



My name is Jane Phillips; I'm a physiotherapist and lymphoedema therapist investigating the skin in primary lymphoedema –before and after compression. Lymphoedema clinical assessment tools will be investigated for correlation to the changes measured.

Participants required

I will be recruiting participants with primary lymphoedema from age 3 to 40 years old. As primary lymphoedema is not common, participants will be sought in three states in Australia, with measuring sites planned for Melbourne, Adelaide and Sydney. I will also need age and gender matched participants without lymphoedema, so plenty of scope for others to be involved! The study will start in Melbourne, with Sydney and Adelaide to follow.



Why am I telling you this? What do I need?

National ethical approval has been granted with HREC/16/RCHM/136. The information sheet and poster advertising the study are available to prospective participants. If you work in a clinic which sees people with primary lymphoedema, it would be appreciated if you could display a poster or provide the information sheet regarding the study to people with primary lymphoedema who may be eligible or interested. Participants will need attend only once, for about three hours, for measures to be taken, before and after a session with pneumatic compression.

If you know people with primary lymphoedema and may be able to help spread the information regarding this study, please provide copies of the poster and participant information sheet and consent form, (attached) or for further information, please contact me:

Jane Phillips

jane.phillips@flinders.edu.au

With thanks,

Jane Phillips

PhD Candidate Flinders University

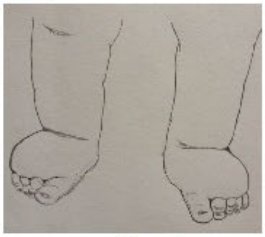
Lymphoedema Physiotherapist

Appendix G.4 Recruitment Posters

Flinders UNIVERSITY inspiring achievement

People with Lymphoedema

murdoch children's research institute



- ✓ Are you between 3 and 40 years old?
- ✓ Have you had swelling in your arm or leg since you were born, or were a child?
- ✓ Do you have primary lymphoedema?
- ✓ Do you want to understand what happens in the skin with compression?

Could you spare two or three hours of your time to have some limb measures taken before and after a treatment for lymphoedema?

If you are interested in taking part in this research, please call or email

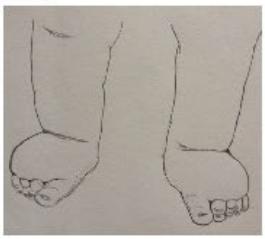
Jane [redacted] jane.phillips@flinders.edu.au

Seeking information does not commit you to taking part in the research, and your involvement or non-involvement in this research will not affect your relationship with any health professional or researcher at Royal Children's Hospital or Murdoch Children's Research Institute

Flinders UNIVERSITY inspiring achievement

Research Study on Lymphoedema
-- Seeking people with NO lymphoedema --

murdoch children's research institute



- ✓ Are you between 3 and 40 years old?
- ✓ Do you want to understand what happens in the skin with compression?
- ✓ Could you spare two or three hours of your time to have some limb measures taken before and after a treatment for lymphoedema?

We are seeking **people without lymphoedema** to take part in a research study as a comparison group. This research is to see what happens in the skin with compression (compression is often used in sports recovery or for travel).

If you are interested in taking part in this research, please call or email:

Jane [redacted] jane.phillips@flinders.edu.au

Seeking information does not commit you to taking part in the research, and your involvement or non-involvement in this research will not affect your relationship with any health professional or researcher at Royal Children's Hospital or Murdoch Children's Research Institute

Appendix G.5 Recruitment Letters to Organisations

Flinders UNIVERSITY
Inspiring achievement

President
Australasian Lymphology Association
<address>

<Date >

Dear [ ],

My name is Jane Phillips and I am a PhD candidate at Flinders University. My research project is investigating the differences in the skin before and after compression, in children and adults with and without primary lymphoedema. Information from this study will contribute to the development of treatment guidelines for children with lymphoedema and as well, contribute to our understanding of changes with compression for people with lymphoedema.

I seek the support and assistance of the ALA to disseminate information regarding this study to members of the ALA and registrants on the Australia New Zealand Lymphoedema Registry.

Ethical review and approval under National Mutual Acceptance through the National Ethics Application Form (NEAF) has been provided by the Human Research Ethics Committee at Royal Children's Hospital Melbourne and is attached for your consideration.

I have also provided:

1. Participant information sheet and consent form
2. Invitation to participate letter and
3. Poster advertising the study.

Your assistance in providing this information to potential participants would be most appreciated.

Thank you for your consideration.

Kind regards

Jane Phillips

PhD Candidate
School of Health Sciences
Flinders University
Jane.phillips@flinders.edu.au
[redacted]

President < Date >
Lymphoedema Support Groups NSW / SA/ Lymphoedema Association of Victoria/Rotary Health

Dear [ ],

My name is Jane Phillips and I am a PhD candidate at Flinders University. My research project is investigating the differences in the skin before and after compression, in children and adults with and without primary lymphoedema. Information from this study will contribute to the development of treatment guidelines for children with lymphoedema and as well, contribute to our understanding of changes with compression for people with lymphoedema.

< **Remove for organisations familiar with lymphoedema**: Primary lymphoedema occurs from birth or in childhood from an inherited or congenital predisposition, and is not as common as lymphoedema from secondary causes such as cancer. (However it is now postulated that the occurrence of secondary lymphoedema in some people and not others may be due to a primary disposition. This research will have useful application for both primary and secondary lymphoedema.)>
Ethical review and approval under National Mutual Acceptance through the National Ethics Application Form (NEAF) has been provided by the Human Research Ethics Committee at Royal Children's Hospital Melbourne (HREC/16/RCHM/136).

I am seeking people with primary lymphoedema between the ages of 3 and 40 years old who may be able to spare time for one visit involving about three hours to participate in this study. The visit would involve having the skin measured, before and after having some compression applied.

As people with primary lymphoedema are not easy to find, I ask your help in disseminating information regarding this study to people with primary lymphoedema. You may know or have access to people with primary lymphoedema, through a regular newsletter or other publication. I have attached the Poster advertising the study; your assistance in providing this information to potential participants would be most appreciated.

For further information, please contact me:
Jane Phillips jane.phillips@flinders.edu.au

Thank you for your consideration,

Kind regards

Jane

Jane Phillips

PhD Candidate
School of Health Sciences
Flinders University
Jane.phillips@flinders.edu.au

Contact Details (please complete and return with signed consent form)

Name :

Address:

Home phone:

Mobile :

Email:

Preferred time for contact:

APPENDIX H
PARTICIPANT INFORMATION AND CONSENT FORMS

Appendix H.1 Master Adult Primary Lymphoedema

Add institution logo

HREC Project Number:	HREC/16/RCHM/136		
Research Project Title:	Skin in Primary Lymphoedema		
Principal Researcher:	Ms Jane Phillips		
Version Number:	3	**Version Date:**	10/10/2017

Thank you for taking the time to read this **Participant Information Statement and Consent Form (Lymphoedema Group)**. We would like to invite you to participate in a research project that is explained below.

This document is 5 pages long. Please make sure you have all the pages.

What is an Information Statement?
These pages tell you about the research project. It explains to you clearly and openly all the steps and procedures of the project. The information is to help you to decide whether or not you would like to take part in the research. Please read this Information Statement carefully.

Before you decide to take part or not, you can ask us any questions you have about the project. You may want to talk about the project with your family, friends or health care worker.

Important things you need to know

- It is your choice whether or not you take part in the research. You do not have to agree if you do not want to
- If you decide you do not want to take part, it will not affect the treatment and care you get at <site >

If you would like to take part in the research project, please sign the consent form at the end of this information statement. By signing the consent form you are telling us that you:

- understand what you have read
- had a chance to ask questions and received satisfactory answers
- consent to taking part in the project

We will give you a copy of this information and consent form to keep.

1. What is the research project about?

Primary Lymphoedema is a rare condition which can occur in some children and adults. It is characterized by swelling of the limbs. We do not know how often it occurs in Australia. Currently, the standard treatment is to manage the risk of skin infection (that can happen with swelling) and reduce swelling with exercise, massage and compression. We do not know how the skin responds to compression or the best way to measure it.

This project aims to compare the skin of children, young people and adults aged between 3-40 years old, with and without lymphoedema, before and after a compression treatment is applied. This will provide information to help provide better management of lymphoedema and to help development of lymphoedema treatment guidelines for children.

It is hoped a total of 100 people will take part in this research.

2. Who is funding this research project?

The research is being conducted by Jane Phillips, as part of her PhD thesis, supported by Flinders University, South Australia. The project will take place at hospitals in Melbourne, Adelaide and Sydney.

3. Why am I being asked to be in this research project?

We are asking you because you have primary lymphoedema and are aged between 3 and 40 years of age. We are also looking for people without lymphoedema to take part in this study as well. This is because it is important to know what is normal. People without lymphoedema are not usually tested in this way, especially young people and children.
We are asking you if you could “bring a buddy”: invite an unaffected friend to take part too. If you have a friend of the same age, please tell them about the study and ask them to contact us for more information. (Principal Researcher: Jane Phillips)
If this is not possible, we would still very much like you to take part. However, it is important for the study to have people with no lymphoedema as well.

4. What do I need to do in this research project?

We would like you to attend an appointment at <insert site name>. This appointment will take up to three hours to complete and will be organised at a time that suits you best. The appointment involves some assessment procedures and a compression treatment.

Assessment Procedures

We will complete the following procedures before you are given the compression treatment:

- Ask you to visit the bathroom to empty your bladder before we complete any procedures
- Measure your height, weight and skin temperature
- Ask you to lie on a bed for 20 minutes to allow your body to adjust before taking some ultrasound pictures. You will need to remove any compression garment you are wearing. We will cover your affected arm or leg with a towel except while measuring
- Ask you some questions during this 20 minute adjustment time, including what is your preferred hand or leg, and how you manage the lymphoedema on a day-to-day basis
- Take ultrasound pictures of three different places on your arms or legs
- Following the ultrasound we will measure:
    1. Around your arms or legs using a tape measure
    2. How much the skin pushes back using a small machine called an Indurometer. This rests on your arm or leg and presses on the skin
    3. How stretchy your skin is by using an elasticity measure, which has a tiny cup that sucks on the skin
    4. How much moisture or water there is in the skin using a moisture meter
    5. The amount of water in the arm or leg by doing a Bioimpedance test. This involves placing some sticky patches in different places on your arms and legs.

Compression Treatment

We will massage (called manual lymphatic drainage) your armpits, the top of your legs and your trunk for approximately five minutes.

We will use an Intermittent Pneumatic Compression (IPC) device on your affected arm or leg. This treatment will take about 50 minutes to complete. This is a standard treatment for lymphoedema and is sometimes used to treat swelling related to sports injuries. An air-filled sleeve will apply waves of compression, in the same pattern as lymphatic massage is given. This will feel like gentle pressing on the leg or arm, starting at the top (thigh or arm) and gradually will include the hand or foot. The pressing is usually on for about 30 seconds, and then off for about 10 seconds. This will continue for about 40 minutes.

Following the compression treatment, we will repeat all the assessment procedures, as described above.

Other important information

Before the appointment, please ensure you:

- Do not exercise for two hours
- Do not drink any caffeine (coffee or tea, sports drinks such as Red Bull or cola) for two hours

- Do not drink alcohol (if applicable) for 12 hours
- Do not apply moisturizer to your skin on the day of the appointment
- Wear light, loose-fitting clothing (such as t-shirt and tracksuit pants)
- Brings something to do while lying down, such as:
  - a book
  - an iPod with music to listen to
  - an iPad with a movie to watch.

Bring a buddy

We are also looking for people without lymphoedema to take part in this study as well. We are asking you if you could "bring a buddy": invite a friend to take part in this study too. If you have a friend of the same age, please tell them about the study and ask them to contact us for more information. (Principal Researcher contact details are at the end of this section).

5. Can I withdraw from the project?

If you give your consent and change your mind, you can withdraw from the project. You do not need to tell us the reason why you want to stop being in the project. If you leave the project, we will use any information already collected unless you tell us not to.

6. What are the possible benefits for me and other people in the future?

This project may give you some benefits. We will give you a copy of your results with your individual measures before and after compression treatment. This information may help you to manage your lymphoedema and could be useful if you wish to share the information with other health professionals in the future.

If you have not had compression treatment before, it may help your condition. If you would like more information on how to access this treatment after you have completed the research project, please ask us.

We hope the information we get will benefit others in the future, by giving therapists more information for managing lymphoedema in both children and adults as well as helping to form treatment guidelines.

7. What are the possible risks, side-effects, discomforts and/or inconveniences?

None of the measurements should hurt or cause discomfort. If you are uncomfortable, please tell us. You will have a towel to cover you and we will only uncover the areas near your ankle and knee or your arm where measurements are taken. You will be monitored during the compression treatment and a bell will be available for you to use if our attention is needed.

Measurement and treatment time will take 2 ½ -3 hours, during which time you will need to lie still for a few minutes at a time while measures are recorded. In between, while we set up each new measure, you will be able to wriggle. During the compression treatment, lying still is preferable, for approximately 40 minutes, which is when a game, book or other entertainment device could be useful. A bookstand which

may assist in holding devices or books will be available. (If you bring an electronic game, please consider if it can be played lying down, or, if you will need both hands available to play it with, as you might not have the use of both arms all the time during measures and treatment. You can check this with us before you agree to participate.)

Taking part in this study may cause some inconvenience as it needs approximately 2 ½ to 3 hours of your time. Appointments will be made for a time that suits you best, and out-of- business hours and weekends will be available wherever possible. We will pay for parking costs at the <insert site name>. You will be given a Hush Foundation CD as a thank you for taking part in the research project.

8. What will be done to make sure my information is confidential?

Any information we collect that can identify you will be treated as confidential. It will be used only in this project, unless otherwise specified. We can disclose the information only with your permission, except as required by law.

All information will be stored securely in the School of Health Sciences at Flinders University. The results will be kept until the youngest participant is 25 years old. The research information may be destroyed or kept indefinitely in secure storage after this time. The only people who can access this information are the research team involved with this project and members of the Human Research Ethics Committee.

The stored information will be re-identifiable. This means that we will remove identifying information such as your name and give the information a special code number. Only the research team can match your name to your code number, if it is necessary to do so.

In accordance with relevant Australian and/or <insert applicable state name> privacy and other relevant laws, you have the right to access and correct the information we collect and store about you. Please contact us if you would like to access this information.

At the end of the study, results may be presented at conferences or published in medical journals. This will be done in such a way that you cannot be identified. The results of this research will be used by Jane Phillips as part of her PhD thesis requirements.

9. Will we be informed of the results when the research project is finished?

We will send you a summary of group results at the end of the study. The summary will be of the whole group of research study participants, not individual results.

10. Who should I contact for more information?

If you would like more information about the project or if you need to speak to a member of the research team in an emergency please contact:

Name: Jane Phillips

Email: jane.phillips@flinders.edu.au

If you have any concerns and/or complaints about the project, the way it is being conducted or your child's rights as a research participant, and would like to speak to someone independent of the project, please contact: Director, Research Ethics & Governance, The Royal Children's Hospital Melbourne on telephone: (03) 9345 5044.

CONSENT FORM

HREC Project Number: HREC/16/RCHM/136

Research Project Title: Skin in Primary Lymphoedema

Version Number: 3 **Version Date:** 10/10/2017

- I have read, or had read to me in my first language, the information statement version listed above and I understand its contents.
- I believe I understand the purpose, extent and possible risks of my involvement in this project.
- I voluntarily consent to take part in this research project.
- I have had an opportunity to ask questions and I am satisfied with the answers I have received.
- I understand that this project has been approved by The Royal Children's Hospital Melbourne Human Research Ethics Committee and will be carried out in line with the National Statement on Ethical Conduct in Human Research (2007) - including all updates.
- I understand I will receive a copy of this Information Statement and Consent Form.

Participant Name	Participant Signature	Date

Declaration by researcher: I have explained the project to the participant who has signed above, and believe that they understand the purpose, extent and possible risks of their involvement in this project.

Research Team Member Name	Research Team Member Signature	Date

Note: All parties signing the Consent Form must date their own signature.

Appendix H.2 Master Adult Non-Lymphoedema PICF

Add institution logo

HREC Project Number:	HREC/16/RCHM/136		
Research Project Title:	Skin in Primary Lymphoedema		
Principal Researcher:	Ms Jane Phillips		
Version Number:	3	Version Date:	10/10/2017

Thank you for taking the time to read this **Participant Information Statement and Consent Form (Non-Lymphoedema Group)**. We would like to invite you to participate in a research project that is explained below.

This document is 5 pages long. Please make sure you have all the pages.

What is an Information Statement?
These pages tell you about the research project. It explains to you clearly and openly all the steps and procedures of the project. The information is to help you to decide whether or not you would like to take part in the research. Please read this Information Statement carefully.

Before you decide to take part or not, you can ask us any questions you have about the project. You may want to talk about the project with your family, friends or health care worker.

Important things you need to know

- It is your choice whether or not you take part in the research. You do not have to agree if you do not want to
- If you decide you do not want to take part, it will not affect any treatment and care you get at <site >

If you would like to take part in the research project, please sign the consent form at the end of this information statement. By signing the consent form you are telling us that you:

- understand what you have read
- had a chance to ask questions and received satisfactory answers
- consent to taking part in the project

We will give you a copy of this information and consent form to keep.

1. What is the research project about?

Primary Lymphoedema is a rare condition which can occur in some children and adults. It is characterized by swelling of the limbs. We do not know how often it occurs in Australia. Currently, the standard treatment is to manage the risk of skin infection (that can happen with swelling) and reduce swelling with exercise, massage and compression. We do not know how the skin responds to compression or the best way to measure it.

This project aims to compare the skin of children, young people and adults aged between 3-40 years old, with and without lymphoedema, before and after a compression treatment is applied. This will provide information to help provide better management of lymphoedema and to help development of lymphoedema treatment guidelines for children.

It is hoped a total of 100 people will take part in this research.

2. Who is funding this research project?

The research is being conducted by Jane Phillips, as part of her PhD thesis, supported by Flinders University, South Australia. The project will take place at hospitals in Melbourne, Adelaide and Sydney.

3. Why am I being asked to be in this research project?

We are asking you because you do NOT have lymphoedema and are aged between 3 and 40 years of age.

4. What do I need to do in this research project?

We would like you to attend an appointment at <insert site name>. This appointment will take up to three hours to complete and will be organised at a time that suits you best. The appointment involves some assessment procedures and a compression treatment.

Assessment Procedures

We will complete the following procedures before you are given the compression treatment:

- Ask you to visit the bathroom to empty your bladder before we complete any procedures
- Measure your height, weight and skin temperature
- Ask you to lie on a bed for 20 minutes to allow your body to adjust before taking some ultrasound pictures. We will cover your arm or leg with a towel, except while measuring
- Ask you some questions during this 20-minute adjustment time, including what is your preferred hand or leg

- Take ultrasound pictures of three different places on your arms or legs
- Following the ultrasound, we will measure:
    1. Around your arm or leg using a tape measure
    2. How much the skin pushes back using a small machine called an Indurometer. This rests on your arm or leg and presses on the skin
    3. How stretchy your skin is by using an elasticity measure, which has a tiny cup that sucks on the skin
    4. How much moisture or water there is in the skin using a moisture meter
    5. The amount of water in the arm or leg by doing a Bioimpedance test. This involves placing some sticky patches in different places on your arms and legs.

<u>Compression Treatment</u>
We will massage (called manual lymphatic drainage) your armpits, the top of your legs and your trunk for approximately five minutes.

We will use an Intermittent Pneumatic Compression (IPC) device on your arm or leg. This treatment will take about 50 minutes to complete. This is a standard treatment for lymphoedema and is sometimes used to treat swelling related to sports injuries. An air-filled sleeve will apply waves of compression, in the same pattern as lymphatic massage is given. This will feel like gentle pressing on the leg or arm, starting at the top (thigh or arm) and gradually will include the hand or foot. The pressing is usually on for about 30 seconds, and then off for about 10 seconds. This will continue for about 40 minutes.

Following the compression treatment, we will repeat all the assessment procedures, as described above.

<u>Other important information</u>
Before the appointment, please ensure you:
- Do not exercise for two hours
- Do not drink any caffeine (coffee or tea, sports drinks such as Red Bull or cola) for two hours
- Do not drink alcohol (if applicable) for 12 hours
- Do not apply moisturizer to your skin on the day of the appointment
- Wear light, loose-fitting clothing (such as t-shirt and tracksuit pants)
- Brings something to do while lying down, such as:
    - a book
    - an iPod with music to listen to
    - an iPad with a movie to watch.

5. Can I withdraw from the project?

If you give your consent and change your mind, you can withdraw from the project. You do not need to tell us the reason why you want to stop being in the project. If you leave the project, we will use any information already collected unless you tell us not to.

6. What are the possible benefits for me and other people in the future?

There are no clear benefits for those without lymphoedema. We will give you a copy of your results with your individual measures before and after compression treatment, which you might find interesting.

We hope the information we get will benefit others in the future, by giving therapists more information for managing lymphoedema in both children and adults as well as helping to form treatment guidelines. This project will also provide information about the skin after compression for the general community, which will be of interest to therapists who use compression for travel or sporting purposes (recovery).

7. What are the possible risks, side-effects, discomforts and/or inconveniences?

None of the measurements should hurt or cause discomfort. If you are uncomfortable, please tell us. You will have a towel to cover you and we will only uncover the areas near your ankle and knee or your arm where measurements are taken. You will be monitored during the compression treatment and a bell will be available for you to use if our attention is needed.

Measurement and treatment time will take 2 ½ -3 hours, during which time you will need to lie still for a few minutes at a time while measures are recorded. In between, while we set up each new measure, you will be able to wriggle. During the compression treatment, lying still is preferable, for approximately 40 minutes, which is when a game, book or other entertainment device could be useful. A bookstand which may assist in holding devices or books will be available.
(If you bring an electronic game, please consider if it can be played lying down, or, if you will need both hands available to play it with, as you might not have the use both arms all the time during measures and treatment. You can check this with us before you agree to participate.

Taking part in this study may cause some inconvenience as it needs approximately 2 ½ to 3 hours of your time. Appointments will be made for a time that suits you best, and out-of- business hours and weekends will be available wherever possible. We will pay for parking costs at the <insert site name>. You will be given a Hush Foundation CD as a thank you for taking part in the research project.

8. What will be done to make sure my information is confidential?

Any information we collect that can identify you will be treated as confidential. It will be used only in this project, unless otherwise specified. We can disclose the information only with your permission, except as required by law.

All information will be stored securely in the School of Health Sciences at Flinders University. The results will be kept until the youngest participant is 25 years old. The research information may be destroyed or kept indefinitely in secure storage after this time. The only people who can access this information are the research team involved with this project and members of the Human Research Ethics Committee.

The stored information will be re-identifiable. This means that we will remove identifying information such

as your name and give the information a special code number. Only the research team can match your name to your code number, if it is necessary to do so.

In accordance with relevant Australian and/or <insert applicable state name> privacy and other relevant laws, you have the right to access and correct the information we collect and store about you. Please contact us if you would like to access this information.

At the end of the study, results may be presented at conferences or published in medical journals. This will be done in such a way that you cannot be identified. The results of this research will be used by Jane Phillips as part of her PhD thesis requirements.

9. Will we be informed of the results when the research project is finished?

We will send you a summary of group results at the end of the study. The summary will be of the whole group of research study participants, not individual results.

10. Who should I contact for more information?

If you would like more information about the project or if you need to speak to a member of the research team in an emergency please contact:

Name: Jane Phillips

Email: jane.phillips@flinders.edu.au

If you have any concerns and/or complaints about the project, the way it is being conducted or your rights as a research participant, and would like to speak to someone independent of the project, please contact: Director, Research Ethics & Governance, The Royal Children's Hospital Melbourne on telephone: (03) 9345 5044.

CONSENT FORM

HREC Project Number: HREC/16/RCHM/136

Research Project Title: Skin in Primary Lymphoedema

Version Number: 3 **Version Date:** 10/10/2017

- I have read, or had read to me in my first language, the information statement version listed above and I understand its contents.
- I believe I understand the purpose, extent and possible risks of my involvement in this project.
- I voluntarily consent to take part in this research project.
- I have had an opportunity to ask questions and I am satisfied with the answers I have received.
- I understand that this project has been approved by The Royal Children's Hospital Melbourne Human Research Ethics Committee and will be carried out in line with the National Statement on Ethical Conduct in Human Research (2007) - including all updates.
- I understand I will receive a copy of this Information Statement and Consent Form.

Participant Name	Participant Signature	Date

Declaration by researcher: I have explained the project to the participant who has signed above, and believe that they understand the purpose, extent and possible risks of their involvement in this project.

Research Team Member Name	Research Team Member Signature	Date

Note: All parties signing the Consent Form must date their own signature.

Appendix H.3 Master Parent Guardian Lymphoedema PICF

Add institution logo

HREC Project Number:	HREC/16/RCHM/136		
Research Project Title:	Skin in Primary Lymphoedema (Lymphoedema Group)		
Principal Researcher:	Ms Jane Phillips		
Version Number:	2	**Version Date:**	03/10/2017

Thank you for taking the time to read this **Parent/Guardian Information Statement and Consent Form**. We would like to invite your child to participate in a research project that is explained below.

This document is 5 pages long. Please make sure you have all the pages.

What is an Information Statement?

These pages tell you about the research project. It explains to you clearly and openly all the steps and procedures of the project. The information is to help you decide whether or not you would like your child to take part in the research. Please read this Information Statement carefully.

Before you decide if you want your child to take part or not, you can ask us any questions you have about the project. You may want to talk about the project with your family, friends or health care worker.

Important things you need to know

- It is your choice whether or not your child can take part in the research. You do not have to agree if you do not want to
- If you decide you do not want your child to take part, it will not affect the treatment and care your child gets at < site >

If you would like your child to take part in the research project, please sign the consent form at the end of this information statement. By signing the consent form you are telling us that you:

- understand what you have read

- had a chance to ask questions and received satisfactory answers
- consent to your child taking part in the project

We will give you a copy of this information and consent form to keep.

1. What is the research project about?

Primary Lymphoedema is a rare condition which can occur in some children and adults. It is characterised by swelling of the limbs. We do not know how often it occurs in Australia. Currently, the standard treatment is to manage the risk of skin infection (that can happen with swelling) and reduce swelling with exercise, massage and compression. We do not know how the skin responds to compression or the best way to measure it.

This project aims to compare the skin of children, young people and adults aged between 3-40 years old, with and without lymphoedema, before and after a compression treatment is applied. This will provide information to help provide better management of lymphoedema and to help development of lymphoedema treatment guidelines for children.

It is hoped a total of 100 people will take part in this research.

2. Who is funding this research project?

The research is being conducted by Jane Phillips, as part of her PhD thesis, supported by Flinders University, South Australia. The project will take place at hospitals in Melbourne, Adelaide and Sydney.

3. Why is my child being asked to take part?

We are asking your child because they have primary lymphoedema and is aged between 3 and 18 years old. We are also looking for children and young adults without lymphoedema to take part in this study as well. This is because it is important to know what is normal. People without lymphoedema are not usually tested in this way, especially young people and children.

We are asking if your child could "bring a buddy": invite a friend to take part too. If your child has a friend of the same age, please tell them about the study and ask them to contact us for more information. (Principal Researcher: Jane Phillips, mobile 0418 104 690)

If this is not possible, we would still very much like your child to take part. However, it is important for the study to have people with no lymphoedema as well.

4. What does my child need to do in this research project?

We would like your child to attend an appointment at <insert site name>. This appointment will take up to three hours to complete and will be organised at a time that suits you best. The appointment involves some assessment procedures and a compression treatment.

Assessment Procedures

We will complete the following procedures before your child is given the compression treatment:

- Ask your child to visit the bathroom to empty their bladder before we complete any procedures
- Measure your child's height, weight and skin temperature
- Get your child to lie on a bed for 20 minutes to allow their body to adjust before taking some ultrasound pictures. Your child will need to remove any compression garment they are wearing. We will cover their affected arm or leg with a towel except while measuring
- Ask your child some questions during this 20-minute adjustment time, including what is their preferred hand or leg, and how they manage the lymphoedema on a day-to-day basis
- Take ultrasound pictures of three different places on their arms or legs
- Following the ultrasound we will measure:
    1. Around their arms or legs using a tape measure
    2. How much the skin pushes back using a small machine called an Indurometer. This rests on your child's arm or leg and presses on the skin
    3. How stretchy their skin is by using an elasticity measure, which has a tiny cup that sucks on the skin
    4. How much moisture or water there is in the skin using a moisture meter
    5. The amount of water in the arm or leg by doing a Bioimpedance test. This involves placing some sticky patches in different places on their arms and legs.

Compression Treatment

We will massage (called manual lymphatic drainage) your child's armpits, the top of their legs and their trunk for approximately five minutes.

We will use an Intermittent Pneumatic Compression (IPC) device on your child's affected arm or leg. This treatment will take about 50 minutes to complete. This is a standard treatment for lymphoedema and is sometimes used to treat swelling related to sports injuries. An air-filled sleeve will apply waves of compression, in the same pattern as lymphatic massage is given. This will feel like gentle pressing on the leg or arm, starting at the top (thigh or arm) and gradually will include the hand or foot. The pressing is usually on for about 30 seconds, and then off for about 10 seconds. This will continue for about 40 minutes.

Following the compression treatment, we will repeat all the assessment procedures, as described above.

Other important information

Before the appointment, please ensure your child:

- Does not exercise for two hours
- Does not drink any caffeine (coffee or tea, sports drinks such as Red Bull or cola) for two hours

- Does not drink alcohol (if applicable) for 12 hours
- Does not apply moisturizer to their skin on the day of the appointment
- Wears light, loose-fitting clothing (such as t-shirt and tracksuit pants)
- Brings something to do while lying down, such as:
  - a book
  - an iPod with music to listen to
  - an iPad with a movie to watch.

Bring a buddy

We are also looking for children without lymphoedema to take part in this study as well. If your child has a friend, particularly if they are of the same age, please tell them about the study and ask their parent or guardian to contact us for more information. (Principal Researcher contact details are at the end of this section).

5. Can my child withdraw from the project?

If you give your consent and change your mind, your child can withdraw from the project. You do not need to tell us the reason why you or your child want to stop being in the project. If your child leaves the project, we will use any information already collected unless you tell us not to.

6. What are the possible benefits for my child and other people in the future?

This project may give your child some benefits. We will give you a report with your child's individual measures before and after compression treatment. This information may help you and your child to manage their lymphoedema; and could be useful if you wish to share the information with other health professionals in the future.

If your child has not had compression treatment before, it may help your child's condition. If you would like more information on how to access this treatment after your child has completed the research project, please ask us.

We hope the information we get will benefit others in the future, by giving therapists more information for managing lymphoedema in both children and adults as well as helping to form treatment guidelines.

7. What are the possible risks, side-effects, discomforts and/or inconveniences?

None of the measurements should hurt or cause discomfort. If you think your child is uncomfortable, please tell us. Your child will have a towel to cover them and we will only uncover the areas near their ankle and knee or their arm where measurements are taken. Your child will be monitored during the compression treatment and a bell will be available for you to use if our attention is needed.

Measurement and treatment time will take 2 ½ -3 hours, during which time your child will need to lie still for a few minutes at a time while measures are recorded. In between, while we set up each new measure, they will be able to wriggle. During the compression treatment, lying still is preferable, for approximately 40 minutes, which is when a game, book or other entertainment device could be useful. A bookstand

which may assist in holding devices or books will be available. .

(If you bring an electronic game, please consider if it can be played lying down, or, if the child will need both hands available to play it with, as the child might not have the use of both arms all the time during measures and treatment. You can check this with us before you agree to your child participating.)

Taking part in this study may cause some inconvenience as it needs approximately 2.5 to 3 hours of your time. Appointments will be made for a time that suits you best, and out-of- business hours and weekends will be available wherever possible. We will pay for parking costs at the <insert site name>. Your child will be given a Hush Foundation CD as a thank you for taking part in the research project.

8. What will be done to make sure my child's information is confidential?

Any information we collect that can identify your child will be treated as confidential. It will be used only in this project, unless otherwise specified. We can disclose the information only with your permission, except as required by law.

All information will be stored securely in the School of Health Sciences at Flinders University. The results will be kept until the youngest participant is 25 years old. The research information may be destroyed or kept indefinitely in secure storage after this time. The only people who can access this information are the research team involved with this project and members of the Human Research Ethics Committee.

The stored information will be re-identifiable. This means that we will remove identifying information such as your child's name and give the information a special code number. Only the research team can match your child's name to their code number, if it is necessary to do so.

In accordance with relevant Australian and/or <insert applicable state name> privacy and other relevant laws, you have the right to access and correct the information we collect and store about your child. Please contact us if you would like to access this information.

At the end of the study, results may be presented at conferences or published in medical journals. This will be done in such a way that your child cannot be identified. The results of this research will be used by Jane Phillips as part of her PhD thesis requirements.

9. Will we be informed of the results when the research project is finished?

We will send you a summary of group results at the end of the study. The summary will be of the whole group of research study participants, not individual results.

10. Who should I contact for more information?

If you would like more information about the project or if you need to speak to a member of the research team in an emergency please contact:

Name: Jane Phillips

Email: jane.phillips@flinders.edu.au

If you have any concerns and/or complaints about the project, the way it is being conducted or your child's rights as a research participant, and would like to speak to someone independent of the project, please contact: Director, Research Ethics & Governance, The Royal Children's Hospital Melbourne on telephone: (03) 9345 5044.

CONSENT FORM

HREC Project Number: HREC/16/RCHM/136

Research Project Title: Skin in Primary Lymphoedema

Version Number: 2 **Version Date:** 03/10/2017

- I have read, or someone has read to me in a language that I understand, the information statement version listed above and I understand its contents.
- I believe I understand the purpose, extent and possible risks of my child's involvement in this project.
- I voluntarily consent for my child to take part in this research project.
- I have had an opportunity to ask questions and I am satisfied with the answers I have received.
- I understand that this project has been approved by The Royal Children's Hospital Melbourne Human Research Ethics Committee and will be carried out in line with the National Statement on Ethical Conduct in Human Research (2007) – including all updates.
- I understand I will receive a copy of this Information Statement and Consent Form.

_____
Child's Name

_____ _____ _____
Parent/Guardian Name Parent/Guardian Signature Date

Declaration by researcher: I have explained the project to the parent/guardian who has signed above, and believe that they understand the purpose, extent and possible risks of their child's involvement in this project.

_____ _____ _____
Research Team Member Name Research Team Member Signature Date

Note: All parties signing the Consent Form must date their own signature.

Appendix H.4 Master Parent Guardian Non-Lymphoedema PICF

Add institution logo

HREC Project Number:	HREC/16/RCHM/136		
Research Project Title:	Skin in Primary Lymphoedema		
Principal Researcher:	Ms Jane Phillips		
Version Number:	2	**Version Date:**	03/10/2017

Thank you for taking the time to read this **Parent/Guardian Information Statement and Consent Form (Non-Lymphoedema Group)**. We would like to invite your child to participate in a research project that is explained below.

This document is 5 pages long. Please make sure you have all the pages.

What is an Information Statement?

These pages tell you about the research project. It explains to you clearly and openly all the steps and procedures of the project. The information is to help you decide whether or not you would like your child to take part in the research. Please read this Information Statement carefully.

Before you decide if you want your child to take part or not, you can ask us any questions you have about the project. You may want to talk about the project with your family, friends or health care worker.

Important things you need to know

- It is your choice whether or not your child can take part in the research. You do not have to agree if you do not want to
- If you decide you do not want your child to take part, it will not affect the treatment and care your child gets at < site >

If you would like your child to take part in the research project, please sign the consent form at the end of this information statement. By signing the consent form you are telling us that you:

- understand what you have read
- had a chance to ask questions and received satisfactory answers
- consent to your child taking part in the project

We will give you a copy of this information and consent form to keep.

1. What is the research project about?

Primary Lymphoedema is a rare condition which can occur in some children and adults. It is characterised by swelling of the limbs. We do not know how often it occurs in Australia. Currently, the standard treatment is to manage the risk of skin infection (that can happen with swelling) and reduce swelling with exercise, massage and compression. We do not know how the skin responds to compression or the best way to measure it.

This project aims to compare the skin of children, young people and adults aged between 3-40 years old, with and without lymphoedema, before and after a compression treatment is applied. This will provide information to help provide better management of lymphoedema and to help development of lymphoedema treatment guidelines for children.

It is hoped a total of 100 people will take part in this research.

2. Who is funding this research project?

The research is being conducted by Jane Phillips, as part of her PhD thesis, supported by Flinders University, South Australia. The project will take place at hospitals in Melbourne, Adelaide and Sydney.

3. Why is my child being asked to take part?

We are asking your child because he/she does NOT have lymphoedema and is aged between 3 and 18 years old.

4. What does my child need to do in this research project?

We would like your child to attend an appointment at <insert site name>. This appointment will take up to three hours to complete and will be organised at a time that suits you best. The appointment involves some assessment procedures and a compression treatment.

<u>Assessment Procedures</u>

We will complete the following procedures before your child is given the compression treatment:

- Ask your child to visit the bathroom to empty their bladder before we complete any procedures
- Measure your child's height, weight and skin temperature
- Get your child to lie on a bed for 20 minutes to allow their body to adjust before taking some ultrasound pictures. We will cover their arm or leg with a towel, except while measuring
- Ask your child some questions during this 20 minute adjustment time, including 'what is their preferred hand or leg'
- Take ultrasound pictures of three different places on their arms or legs
- Following the ultrasound we will measure:
    1. Around their arms or legs using a tape measure
    2. How much the skin pushes back using a small machine called an Indurometer. This rests on your child's arm or leg and presses on the skin
    3. How stretchy their skin is by using an elasticity measure, which has a tiny cup that sucks on the skin
    4. How much moisture or water there is in the skin using a moisture meter
    5. The amount of water in the arm or leg by doing a Bioimpedance test. This involves placing some sticky patches in different places on their arms and legs.

<u>Compression Treatment</u>

We will massage (called manual lymphatic drainage) your child's armpits, the top of their legs and their trunk for approximately five minutes.

We will use an Intermittent Pneumatic Compression (IPC) device on your child's arm or leg. This treatment will take about 50 minutes to complete. This is a standard treatment for lymphoedema and is sometimes used to treat swelling related to sports injuries. An air-filled sleeve will apply waves of compression, in the same pattern as lymphatic massage is given. This will feel like gentle pressing on the leg or arm, starting at the top (thigh or arm) and gradually will include the hand or foot. The pressing is usually on for about 30 seconds, and then off for about 10 seconds. This will continue for about 40 minutes.

Following the compression treatment, we will repeat all the assessment procedures, as described above.

<u>Other important information</u>

Before the appointment, please ensure your child:

- Does not exercise for two hours
- Does not drink any caffeine (coffee or tea, sports drinks such as Red Bull or cola) for two hours
- Does not drink alcohol (if applicable) for 12 hours
- Does not apply moisturizer to their skin on the day of the appointment
- Wears light, loose-fitting clothing (such as t-shirt and tracksuit pants)
- Brings something to do while lying down, such as:

- a book
- an iPod with music to listen to
- an iPad with a movie to watch.

5. Can my child withdraw from the project?

If you give your consent and change your mind, your child can withdraw from the project. You do not need to tell us the reason why you or your child want to stop being in the project. If your child leaves the project we will use any information already collected unless you tell us not to.

6. What are the possible benefits for my child and other people in the future?

There are no clear benefits for those without lymphoedema. We will give you a copy of your child's individual measures before and after compression treatment, which you might find interesting.

We hope the information we get will benefit others in the future, by giving therapists more information for managing lymphoedema in both children and adults as well as helping to form treatment guidelines. This project will also provide information about the skin after compression for the general community, which will be of interest to therapists who use compression for travel or sporting purposes (recovery).

7. What are the possible risks, side-effects, discomforts and/or inconveniences?

None of the measurements should hurt or cause discomfort. If you think your child is uncomfortable, please tell us. Your child will have a towel to cover them and we will only uncover the areas near their ankle and knee or their arm where measurements are taken. Your child will be monitored during the compression treatment and a bell will be available for you to use if our attention is needed.

Measurement and treatment time will take 2 ½ -3 hours, during which time your child will need to lie still for a few minutes at a time while measures are recorded. In between, while we set up each new measure, they will be able to wriggle. During the compression treatment, lying still is preferable, for approximately 40 minutes, which is when a game, book or other entertainment device could be useful. A bookstand which may assist in holding devices or books will be available. (If you bring an electronic game, please consider if it can be played lying down, or, if the child will need both hands available to play it with, as the child might not have the use of both arms all the time during measures and treatment. You can check this with us before you agree to your child participating.)

Taking part in this study may cause some inconvenience as it needs approximately 2.5 to 3 hours of your time. Appointments will be made for a time that suits you best, and out-of- business hours and weekends will be available wherever possible. We will pay for parking costs at the <insert site name>. Your child will be given a Hush Foundation CD as a thank you for taking part in the research project.

8. What will be done to make sure my child's information is confidential?

Any information we collect that can identify your child will be treated as confidential. It will be used only in this project, unless otherwise specified. We can disclose the information only with your permission,

except as required by law.

All information will be stored securely in the School of Health Sciences at Flinders University. The results will be kept until the youngest participant is 25 years old. The research information may be destroyed or kept indefinitely in secure storage after this time. The only people who can access this information are the research team involved with this project and members of the Human Research Ethics Committee.

The stored information will be re-identifiable. This means that we will remove identifying information such as your child's name and give the information a special code number. Only the research team can match your child's name to their code number, if it is necessary to do so.

In accordance with relevant Australian and/or <insert applicable state name> privacy and other relevant laws, you have the right to access and correct the information we collect and store about your child. Please contact us if you would like to access this information.

At the end of the study, results may be presented at conferences or published in medical journals. This will be done in such a way that your child cannot be identified. The results of this research will be used by Jane Phillips as part of her PhD thesis requirements.

9. Will we be informed of the results when the research project is finished?

We will send you a summary of group results at the end of the study. The summary will be of the whole group of research study participants, not individual results.

10. Who should I contact for more information?

If you would like more information about the project or if you need to speak to a member of the research team in an emergency please contact:

Name: Jane Phillips

Email: jane.phillips@flinders.edu.au

If you have any concerns and/or complaints about the project, the way it is being conducted or your child's rights as a research participant, and would like to speak to someone independent of the project, please contact: Director, Research Ethics & Governance, The Royal Children's Hospital Melbourne on telephone: (03) 9345 5044.

CONSENT FORM

HREC Project Number: HREC/16/RCHM/136

Research Project Title: Skin in Primary Lymphoedema

Version Number: 2 **Version Date:** 03/10/2017

- I have read, or someone has read to me in a language that I understand, the information statement version listed above and I understand its contents.
- I believe I understand the purpose, extent and possible risks of my child's involvement in this project.
- I voluntarily consent for my child to take part in this research project.
- I have had an opportunity to ask questions and I am satisfied with the answers I have received.
- I understand that this project has been approved by The Royal Children's Hospital Melbourne Human Research Ethics Committee and will be carried out in line with the National Statement on Ethical Conduct in Human Research (2007) – including all updates.
- I understand I will receive a copy of this Information Statement and Consent Form.

_____
Child's Name

Parent/Guardian Name	Parent/Guardian Signature	Date

Declaration by researcher: I have explained the project to the parent/guardian who has signed above, and believe that they understand the purpose, extent and possible risks of their child's involvement in this project.

Research Team Member Name	Research Team Member Signature	Date

Note: All parties signing the Consent Form must date their own signature.

APPENDIX J STUDY ELIGIBILITY

Initial Contact Questionnaire all participants

Questions on first contact, to ensure potential participant has no risks for participation, and is in the appropriate category to participate (primary lymphoedema and 3 – 40 years old).

"Before we start can I please confirm your date of birth?" _____

- "You are in the age group that are able to participate in this study."

OR – "I am sorry you are not in the age group we need for this study. We are limiting the age group due to the changes to skin which occur as we get older. Thank you for contacting me."

For those with lymphoedema only: "I am interested to know about your diagnosis of primary lymphoedema and some history about your condition. Are you happy for me to ask you some questions about this?"

Consent:

Yes	No

A. Where is your lymphoedema/swelling?

_____

_____

History and diagnosis

B. i. How old were you when you or your family first noticed you had swelling?

_____

B ii. How old were you when you were first diagnosed with LO?

_____

B. iii. Who provided the diagnosis? (Did your GP or other medical professional diagnose primary LO?)

_____

_____

B. iv. Were you told that it was primary lymphoedema when it was first diagnosed? Has it been decided since then that it is primary lymphoedema?

_____

_____

B. v. Were any tests done to confirm the diagnosis? (Genetic testing? Or lymphoscintigraphy? Or other imaging/test?) When? What did it show?

_____

_____

B. vi. Do you have any other syndrome associated with primary lymphoedema?

_____

Family History/ Hereditary component

C. Have any other members of your family got primary LO?

Relative	Site	Relative	Site	
Mother		Father		
Maternal Grandmother		Paternal grandmother		
Maternal Grandfather		Paternal Grandfather		
Mother's sisters / brothers		Father's sisters / brothers		
Sister				
Brother				

_____

Outcome Lymphoedema: Included ☐ **Excluded** ☐

If the prospective participant has lymphoedema, but nothing indicating primary lymphoedema, response would be:

"Thank you for your time in answering all these questions. From your answers, it sounds like it would not be appropriate for you to be a part of this study. Thank you so much for your interest in this project."

OR if has primary lymphoedema:

"Thank you for your interest in this project. In this study, we will be using a number of devices to examine and measure your skin. Before you commence the study, I need to ask some questions to make sure that none of the measures we will be using will pose any risk to your safety. Are you happy for me to ask you some questions about this, to check that it is appropriate for you to be a part of the study?"

Consent:

Yes	No

For Primary Lymphoedema and Age-matched Controls

	Right	Left
A. i. What is your preferred hand? Which hand do you write with?	☐	☐
ii. What is your preferred leg? What foot do you kick a ball with?	☐	☐

	Exclusion from Bioimpedance only	No	Yes
1.	Do you have a pacemaker?		
2.	Are you pregnant?		
3.	Do you have any implanted metal?		
	Study Exclusion (Yes in right column)	No	Yes
4.	Do you have a deep venous thrombosis (DVT - or suspected?) Or thrombophlebitis?		
5.	Do you have active metastatic cancer or malignant lymphoedema (clarify?)		
6.	Do you have a pulmonary embolus or pulmonary oedema?		
7.	Do you have ischaemic vascular disease?		
8.	Do you have heart failure: That is NOT UNDER CONTROL by medication?		
9 a. b. c.	Have you had any inflammation or infection of the skin over the past 2 months? (Cellulitis or erysipelas): Yes No - Have you been on medication (antibiotics) for less than 5 days to treat this? Site: Arm _____ Leg _____		
10.	Do you have peripheral neuropathy?		
11.	Do you have Complex Regional Pain Syndrome? Do you have any pain (in that limb) that is always there, and affects the sensation in that arm/ leg? (Known as Complex Regional Pain Syndrome)		
12a. b.	Do you have any open wounds, ulcers, or grafts or recently healed wound on your leg/arm? Yes No Site: Top of foot/back of hand __inside leg above ankle __ or above knee inner side___Inside arm above wrist ____ or just above elbow, in inner side __		
13a. b.	Have you had any recent surgery or injury that has caused increased swelling in your arm or leg? (E.g. joint sprain or surgery; skin cancer removed) Y N Site: Top of foot/back of hand _Inside leg above ankle __ or above knee inner side_ Inside arm above wrist ___ or just above elbow, in inner side_____		
14a. b. c.	Do you have any skin conditions such as psoriasis, eczema, or dermatitis? Do you use any topical cortico-steroid lotion on your arm/leg? Yes No Type _____ Site: Top of foot/back of hand __ Inside leg above ankle _ or above knee, inner side_ Inside arm above wrist ___ or just above elbow, in inner side ___		

15.	Do you have any connective tissue disease such as Marfan's disease, Systemic Lupus Erythematosis (SLE) or Ehlers-Danlos disease? If yes, specify:_____		
16.	Do you have chronic polyarthritis or rheumatoid arthritis?		
	<u>Exclusion from /elasticity measure with tape:</u>		
17.	Do you have an allergy to adhesive tape?		

Notes: If these questions are all answered negatively, then the prospective participant may be included as a study participant.

Cautions:

- Adhesive tape allergy positive: include but not use Cutometer with tape (if double sided tape used to hold in place); use only body marker for that participant

- Those from 12, 13 and 14 with site other than those mentioned - *Local skin condition not on limb site of interest: include*

Outcome: Included ☐ **Excluded** ☐

If the prospective participant has any contraindications (4-16 above), response would be: "Thank you for your time in answering all these questions. From your answers, it sounds like it would not be appropriate for you to be a part of this study. Thank you so much for your interest in this project."

If included:

Response: "Thank you for your time in answering these questions; it sounds safe for you to be part of the project - we would be very pleased for you to be included in this study.

1. a. This will involve you coming in for about 3 hours to …… (Venue) ……. for your arm/leg to be measured: is there a time that is preferable for you, (weekends/ weekdays; or evenings vs midday)? NOTE TIME: _____

1 b. For some of the measures if your arm or leg are particularly hairy, it would be ideal if you could shave the area before you come in for your appointment. Would you be happy to do that?

(If so, provide details of sites to shave – posterior leg, posterior thigh).

Note – Likely to need to shave YES / NO Happy to shave prior: YES / NO

2. This appointment time is likely to be in approximately (MONTH); I will send you out some more information about the project, to make sure that you are happy to participate and with a definite date and time.

3. Then I will contact you again in the week before your appointment to make sure that nothing has changed, and confirm that the date and time is suitable for you to come in for the study.

4. If you have a friend of the same age and gender who could come with you to also participate in the study, on the same day, it would be helpful for the study and it might make it more enjoyable for you. They would need to contact me to be sure that they are safe to participate also, but they could come with you, on the same day. Is there someone you can think of that might like to do that? You would need to ask them to contact me also, before you come.

Do you have any questions?"

Office use only:

Name _____

Code Assigned _____

Group _____ (LO / Non-LO) If Non-LO, match with Code _____

Gender _____ (M / F) Age _____ (Yrs)

APPENDIX K ATTENDANCE QUESTIONNAIRE

Attendance Questionnaire

Skin in Primary Lymphoedema

Consent form signed YES ☐

Ask participant to visit bathroom, as part of the requirements for measurement of bioimpedance. Height and weight measured, no shoes.

1. Age _____ **(yr/mths) 2. Today's date** _____

3. Gender _____ **4 Height** _____ cm **5. Weight** _____ kg

6. Skin thermography _____

Ask participant to remove compression garments, and expose the areas where measurements will be taken (hand/foot, wrist/ankle and just above elbow or knee) lie on plinth supine one pillow, draped appropriately for modesty and warmth.

"You will be resting in this position for 20 minutes so that everything stabilises before we measure you. While you are resting, I would like to ask you some questions about things that can have an effect on your skin. Is that OK?" Yes/No

This question is about ethnicity, as ethnicity can affect your skin and this study is investigating skin:

7. Where are your ancestors from/ with what people do you identify? Please specify group from:

- Europe/ Caucasian _____
- Africa _____
- An Aboriginal people _____
- Torres Strait Island _____
- Pacific Islands _____
- Indigenous peoples from North or South America _____
- Asia _____

8. Do you moisturise the skin on your arm ☐ **leg:** ☐

A. In a usual week, how many days would you moisturise your skin? _____ Days

9. Are you taking any diuretic medications such as diuretics (for fluid), anti- inflammatory medication or antibiotics (for infection)?

If so, please specify name and dose:

_____

_____

10. Have you ever had cellulitis (infection of the skin needing antibiotics)? Y / N

a. If yes, please say for each time you have had cellulitis:

	Month/year : Episode 1	**Month/year : Episode 2**	**Month/Year Episode 3**
When was it?			
Did you miss any days of work/school due to the infection? How many?	Yes / No Days (number)	Yes / No Days (number)	Yes / No Days (number)
Were you in hospital for IV antibiotics (a drip)? For how long?	Yes / No Length of hospital stay	Yes / No Length of hospital stay	Yes / No Length of hospital stay
Where was the cellulitis? (e.g. lower leg to knee)			

b. If you have had cellulitis more than three times, please indicate how many you have had:

_____ Episodes

c. And how old you were when you had the first episode: _____

How does your affected leg feel today (you will be asked these questions again after the compression) (p4)?

Before compression:

11. On a scale of 0 -10, where 0 is the worst possible and 10 is the best possible, please rate how your leg feels today. (Please circle a number that best represents how your leg feels today)

A. How does your leg feel to move?

Tight/heavy 0 1 2 3 4 5 6 7 8 9 10 Light/easy to move

B. How comfortable does your leg feel?

Aching/ Painful 0 1 2 3 4 5 6 7 8 9 10 Comfortable/ no pain or discomfort

12. Please fill out the PedsQL quality of life page. Thank you

Following questions <u>only for those with lymphoedema</u>:

I would also like to ask you some questions about the way you manage your lymphoedema. Is that OK? Yes No

13 A. Do you **wear a compression garment**: Yes No

i. In a usual week, how many days would you wear it? _____ Days

ii. On a usual day, how many hours would you wear it? _____ Hours

When you decide not to wear your garment what is the reason?

(E.g. uncomfortable, too hard to don, impractical, going out) _____

13 B. If you wear a compression garment:

a. What type of garment(s) do you wear?

Leg: (Class 1 = 15-20 mmHg; 2=20-30 mmHg; 3 = 30-40 mmHg and class 4> 40mmHg)

- below knee ☐ Class 1 2 3 4
- toe glove ☐ Class 1 2 3 4
- thigh high ☐ Class 1 2 3 4
- full leg with belt ☐ Class 1 2 3 4
- pantyhose ☐ Class 1 2 3 4
- one and a half leg with pant attached ☐ Class 1 2 3 4

b. How old were you when you began wearing a garment? _____ Years

14. Do you do simple lymphatic drainage (SLD) or have a massage (MLD)? Yes No

A. In a usual week, how many days would you do SLD? _____ Days

B. On a usual day, how many minutes would you spend doing SLD? _____ Minutes

C. In a usual week, how many days would you have MLD? _____ Days

D. On a usual day, how many minutes would you spend having MLD? _____ Minutes

15. Do you elevate your arm/leg during the day? Yes No

A. In a usual week, how many days would you do elevate your arm/leg? _____ Days

B. On a usual day, how many minutes/hours would you spend elevating? _____Mins/hrs

16. Do you apply bandages? Yes No

A. In a usual week, how many days would you apply extra compression? _____ Days

B. On a usual day, how many minutes would you spend doing SLD? _____ Minutes

17. Do you apply an Intermittent Pneumatic Compression (IPC) device? Yes No

A. In a usual week, how many days would you apply the IPC? _____ Days

B. On a usual day, how many minutes would you spend on the IPC? _____ Minutes

At what pressure: _____ mmHg

Pre-treatment cycle: _____ Minutes

Cycle time_____ Minutes/seconds

18. Do you use any other methods to manage your lymphoedema? (Please specify)

19. Please fill out the lymphoedema specific quality of life on p.5 and 6.

After compression:

20. On a scale of 0 -10, where 0 is the worst possible and 10 is the best possible, how does your leg feel? (Please circle a number that best represents how your leg feels now, after the IPC)

A. How does your leg feel to move?

Tight/heavy 0 1 2 3 4 5 6 7 8 9 10 Light/easy to move

B. How comfortable does your leg feel?

Aching/ Painful 0 1 2 3 4 5 6 7 8 9 10 Comfortable/ no pain or discomfort

21. Please comment on the experience of the intermittent pneumatic compression if you would like to.

LYMQOL LEG

Lymphoedema Quality of Life Tool

This questionnaire has been designed and validated for patients with chronic oedema/ lymphoedema of one or both legs to measure quality of life.

Please tick the box that best describes how you feel about each of the questions.

(Q1) How much does your swollen leg affect the following activities?

If any of the items are not applicable to you, please write N/A in the relevant answer box(es)	**Not at all**	**A little**	**Quite a bit**	**A lot**
a) your walking				
b) your ability to bend, eg. to tie shoelaces or cut toenails				
c) your ability to stand.				
d) your ability to get up from a chair.				
e) your occupation				
f) your ability to do housework				

(Q2) Does the swelling affect your leisure activities/ social life?				

Please give examples of this

.....

.....

(Q3) How much do you have to depend on other people?				

	Not at all	**A little**	**Quite a bit**	**A lot**
(Q4) How much do you feel the swelling affects your appearance?				
(Q5) How much difficulty do you have finding clothes to fit?				
(Q6) How much difficulty do you have finding clothes you would like to wear?				
(Q7) Do you have difficulty finding shoes to fit?				
(Q8) Do you have difficulty finding socks/ tights/ stockings to fit?				
(Q9) Does the swelling affect how you feel about yourself?				
(Q10) Does it affect your relationships with other people?				

	Not at all	**A little**	**Quite a bit**	**A lot**
(Q11) Does your lymphoedema cause you pain?				
(Q12) Do you have any numbness in your swollen leg(s)?				
(Q13) Do you have any feelings of "pins & needles" or tingling in your swollen leg(s)				
(Q14) Does (do) your swollen leg(s) feel weak?				
(Q15) Does (do) your swollen leg(s) feel heavy?				

In the past week….	**Not at all**	**A little**	**Quite a bit**	**A lot**
(Q16) Have you had trouble sleeping?				
(Q17) Have you had difficulty concentrating on things, e.g., reading?				
(Q18) Have you felt tense				
(Q19) Have you felt worried?				
(Q20) Have you felt irritable?				
(Q21) Have you felt depressed?				

(Q22) Overall, how would you rate your quality of life at present?

Please mark your score on the following scale:

0 1 2 3 4 5 6 7 8 9 10

Poor excellent

Thank you for completing this form.

If you have any comments or queries about it, please discuss these with Dr V L Keeley, Consultant

Questions 16 to 21 have been reproduced with permission from the EORTC.
These questions are <u>only a part</u> of the QLQ-C30 Questionnaire.

APPENDIX L RESULTS

Appendix L.1 NLO at baseline

Table L.1 NLO: Baseline between sides differences in NLO

Table L.1 Baseline between sides differences in NLO			
Site	**Side 1 (Side matched with the more affected PLO limb)** Mean (SD) 95% CI	**Side 2** Mean (SD) 95% CI	**Mean Difference (95% CI) p value**
Measure (n)	**ECF / ICF (Ri/R0) (13)**		
Foot	3.702 (0.854) 3.056, 4.349	3.977 (1.055) 3.384, 4.570	−0.275 (−0.801, 0.252) 0.293
Leg	1.948 (0.367) 1.333, 2.564	1.958 (0.425) 1.480, 2.435	−0.009 (−0.406, 0.388) 0.962
Measure (n)	**LEP (15)**		
Foot	1870 (580) 1629, 2112	1916 (412) 1655, 2177	−45.3 (−240, 149) 0.638
Calf	1335 (557.3) 1078, 1591	1402 (459.7) 1142, 1662	−67.3 (−275, 141) 0.513
Measure (n)	**PWC (16)**		
Foot	33.5 (7.5) 29, 38	34.0 (6.9) 31, 37	−0.5 (−4.4, 3.4) 0.796
Calf	33.4 (7.0) 29, 38	33.6 (7.8) 30, 38	−0.3 (−3.5, 3.0) 0.866
Measure (n)	**IU (16)**		
Foot	2.6 (0.9) 2.1, 3.1	2.7 (0.7) 2.2, 3.2	−0.1 (−0.5, 0.3) 0.569
Calf	3.7 (0.5) 3.4, 4.0	3.9 (0.5) 3.6, 4.3	−0.2 (−0.5, 0) 0.054

Note: each measure is given the number of decimal places in concordance with the literature for that measure.
*Significant p-value <0.05

Appendix L.2 PLO at baseline

Appendix L.2.1 Differences between uniPLO and biPLO

Comparing the affected limb of uniPLO with the more affected limb of biPLO identified significantly higher LEP at the posterior calf of biPLO than uniPLO (**Table L.2.1**). For this reason, both uniPLO and biPLO were compared with NLO for differences in LEP. There were no significant differences between uniPLO and biPLO on either side in ECF/ICF, PWC or IU.

Table L.2.1 Baseline differences between bilateral and unilateral PLO

Table L.2.1 Baseline differences between bilateral and unilateral PLO in each clinical tool										
Side	**'Affected Side'**					**'Unaffected Side'**				
Group	**Bilateral** More affected side		**Unilateral** Affected side			**Bilateral** Less affected side		**Unilateral** Unaffected side		
	Mean (SD)	**95% CI**	**Mean (SD)**	**95% CI**	**Mean Difference (CI) p value***	**Mean (SD)**	**95% CI**	**Mean (SD)**	**95% CI**	**Mean Difference (CI) p value***
Measure (n)	**ECF/ICF (R_i/R_0) (13)**									
Foot	5.876 (.642)	4.503, 7.249	6.263 (1.679)	5.177, 7.348	−.386 (−2.136, 1.363) 0.636	5.572 (.889)	4.689, 6.455	4.494 (.902)	3.795, 5.192	1.078 (−0.048, 2.204) 0.059
Leg	2.730 (1.341)	1.244, 4.216	3.325 (1.599)	2.150, 4.500	−.595 (−2.490, 1.300) 0.504	2.860 (1.502)	1.771, 3.949	2.276 (.797)	1.415, 3.137	0.584 (−.805, 1.972) 0.375
Measure (n)	**LEP biPLO (7) uniPLO (8)**									
Foot	2863 (173)	2643, 3082	2640 (329)	2434, 2845	223 (−523, 77) 0.133	2599 (441)	2226, 2973	1919 (471)	1569, 2268	681 (169, 1192) 0.013*
Posterior Calf	1841 (421)	1560, 2121	1410 (260)	1147, 1672	431 (47, 815) 0.031*	1621 (490)	1231, 2012	1145 (469)	780, 1511	476 (−59, 1011) 0.077
Measure (n)	**PWC (16)**									
Foot	44.0 (11.0)	35.9, 52.1	44.7 (10.4)	36.6, 52.8	−0.7 (−12.2, 10.8) 0.899	41.2 (5.2)	37.1, 45.3	37.4 (5.5)	33.4, 41.5	3.8 (−2.0, 9.5) 0.183
Posterior Calf	44.3 (10.9)	37.2, 51.4	49.2 (7.4)	42.1, 56.3	4.9 (−5.1, 14.9) 0.308	45.8 (9.7)	40.0, 51.6	41.2 (4.6)	35.4, 47.0	4.6 (−3.6, 12.8) 0.249

ECF, extracellular fluid. LEP, low echogenic pixels. PWC, percent water content. IU, induration units. * Significant p value < 0.05

Table L.2.1 (continued) Baseline differences between bilateral and unilateral PLO										
Side	**Affected Side**					**Unaffected Side**				
Group	**Bilateral**		**Unilateral**			**Bilateral**		**Unilateral**		
	Mean (SD)	**95% CI**	**Mean (SD)**	**95% CI**	**Mean Difference (CI) Sig***	**Mean (SD)**	**95% CI**	**Mean (SD)**	**95% CI**	**Mean Difference (CI) Sig***
Measure (n)	**Indurometry IU (16)**									
Foot	2.4 (.86)	1.71, 3.06	3.3 (.92)	2.63, 3.98	−0.9 (−0.04, 1.87) 0.058	2.9 (1.15)	1.96, 3.80	2.8 (1.29)	1.87, 3.71	−0.1 (−1.40, 1.22) 0.883
Posterior Calf	3.4 (.71)	2.86, 3.93	3.7 (.69)	3.13, 4.19	−0.3 (−1.02, 0.49) 0.465	3.3 (.80)	2.78, 3.76	3.9 (.46)	3.38, 4.37	−0.6 (−1.31, 0.09) 0.083

ECF, extracellular fluid. LEP, low echogenic pixels. PWC, percent water content. IU, induration units. * Significant p value < 0.05

Appendix L.2.2 Differences between sides in uniPLO and in biPLO

Using HFU, both uniPLO and biPLO showed a significant difference in LEP between sides, but this was only evident in the foot, where significantly higher LEP was seen on the more affected side of biPLO and the affected side of uniPLO than their respective contralateral sides (**Table L.2.2**).

In BiPLO, there were no significant differences between sides detected by any other clinical tool. UniPLO, on the other hand, showed higher ECF/ICF and PWC on the affected than the unaffected side. Significantly higher ECF/ICF in the affected limb than the unaffected limb in uniPLO was found in both the foot and the leg while significantly higher PWC was seen only at the posterior calf of the affected side in uniPLO. The Indurometer showed a significant difference between feet in uniPLO: higher IU on the affected foot indicated less tissue resistance on the affected than the unaffected side. **(Table L.2.2).**

Table L.2.2 Baseline differences between sides in bilateral and in unilateral PLO

Table L.2.2 Baseline differences between sides in bilateral and in unilateral PLO										
Side	**Bilateral PLO**					**Unilateral PLO**				
Group	**More affected side**		**Less affected side**			**Affected side**		**Unaffected side**		
	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean Difference (CI) Sig*	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean Difference (CI) Sig*
Measure (n)	**ECF/ICF (13) R_i/R_0**									
Foot	5.876 (.642)	4.503, 7.249	5.572 (.889)	4.689, 6.455	0.304 (−0.630, 1.238) 0.489	6.263 (1.679)	5.177, 7.348	4.494 (.902)	3.795, 5.192	1.769 (1.030, 2.507) <0.001*
Leg	2.730 (1.341)	1.244, 4.216	2.860 (1.502)	1.771, 3.949	−0.130 (−0.924, 0.664) 0.726	3.325 (1.599)	2.150, 4.500	2.276 (.797)	1.415, 3.137	1.049 (0.421, 1.677) 0.004
Measure (n)	**LEP (BiPLO n=7; uniPLO n=8)**									
Foot	2863 (173)	2643, 3082	2599 (441)	2226, 2973	263 (13, 513) 0.040*	2640 (329)	2434, 2845	1919 (471)	1569, 2268	721 (487, 955) <0.001*
Posterior Calf	1841 (421)	1560, 2121	1621 (490)	1231, 2012	219 (−87, 526) 0.146	1410 (260)	1147, 1672	1145 (469)	780, 1511	264 (−22, 551) 0 .068
Measure (n)	**PWC (16)**									
Foot	44.0 (11.0)	35.9, 52.1	41.2 (5.2)	37.1, 45.3	2.8 (−5.0, 10.7) 0.451	44.7 (10.4)	36.6, 52.8	37.4 (5.5)	33.4, 41.5	7.3 (−.6, 15.1) 0.067
Posterior Calf	44.3 (10.9)	37.2, 51.4	45.8 (9.7)	40.0, 51.6	−1.5 (−7.0, 4.0) 0.566	49.2 (7.4)	42.1, 56.3	41.2 (4.6)	35.4, 47.0	8.0 (2.5, 13.5) 0.007*
Measure (n)	**IU (16)**									
Foot	2.4	1.7, 3.1	2.9	2.0, 3.8	−0.5 (−1.010, 0.025) 0.060	3.3	2.6, 4.0	2.8	1.9, 3.7	0.5 (−0.001, 1.033) 0.050*
Posterior Calf	3.4	2.9, 3.9	3.3	2.8, 3.8	.1 (−0.31, 0.57) 0.544	3.7	3.1, 4.2	3.9	3.4, 4.4	−0.2 (−0.656, 0.223) 0.309

*Significant p-value <0.05

Appendix L.3 Response to compression in PLO

There was no significant response to compression in LEP in biPLO or uniPLO.

Table L.3 Response to compression in LEP in unilateral and bilateral PLO

Table L.3 Response to compression in LEP in unilateral and bilateral PLO						
Subgroup (n)	**Bilateral (7)**			**Unilateral (6)**		
Time	**Pre**	**Post**		**Pre**	**Post**	
Low Echogenic Pixels (LEP)	**Mean (SD)**	**Mean (SD)**	**Mean Difference (95% CI) P value**	**Mean (SD)**	**Mean (SD)**	**Mean Difference (95% CI) P value**
Foot	2863 (173)	2779 (329)	84 (−98, 266) 0.335	2646 (355)	2715 (334)	−69 (−251, 113) 0.422
Calf	1841 (421)	1757 (558)	83 (−275, 442) 0.618	1435 (302)	1327 (442)	108 (−280, 495) 0.553